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PERIPHERAL NERVE COMPOUND ACTION POTENTIALS
AND FIBER DIAMETER HISTOGRAMS

by

Walter Harold Olson

A dissertation submitted in partial fulfillment
of the requirements for the degree of
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CHAPTER I

INTRODUCTION

When improved vacuum tube amplifiers and low voltage oscilloscopes became available in the early 1920's, physiologists began uncovering considerable detail in compound action potentials recorded from whole peripheral nerves. For two decades thereafter many studies sought to correlate morphological properties of peripheral nerves with conduction of electrical activity. However, since 1940, relatively little of this kind of research on whole peripheral nerve has been reported.

This dissertation describes the results of experimental and model studies of relationships between myelinated fiber diameter histograms and monophasic compound action potentials in cat saphenous nerve. The study was restricted to only myelinated mammalian A nerve fibers. The results can be partitioned into four related sections:

1. Quantitative investigation of the properties of a model for reconstructing compound action potentials from fiber diameter histograms.
2. Experimental anatomical studies to obtain fiber diameter histograms from peripheral nerve cross-sections.
3. Compound action potential reconstructions from anatomical fiber diameter histograms.
4. Prediction of fiber diameter histograms from

monophasically recorded compound action potentials.

The long range goal of this research is to develop a clinically useful nondestructive estimate of peripheral nerve structure, based on electrical stimulation and recording techniques.

The remainder of this introduction is a literature review of information pertinent to this study.

COMPOUND ACTION POTENTIAL

The first accurate recording of a nerve compound action potential (CAP) with the cathode ray oscillograph was made by Gasser and Erlanger (1922). Earlier recordings utilized a string galvanometer which had considerable inertia. They described the CAP as "starting gradually, with a steep smooth anacrotic limb and more gradual catacrotic limb. In frog and some mammalian nerves there were secondary waves on the catacrotic limb." In addition, they concluded that these secondary waves, though not caused by repeated stimulation, could be explained as either "the summed effect of nerve components of different characteristics, or the production of the current itself by more than one process."

In a subsequent paper, Erlanger and Gasser (1924) sought to explain the effect of conduction distance on the relative positions of the waves and the changes in the shape of each wave. They postulated that two types

of progressive changes might occur simultaneously: (1) the CAP may be divided into discrete waves, and (2) the form of these discrete waves may be changing. Also, they conjectured that each wave may be composed of numerous smaller waves that start together, but conduct at slightly different rates. Finally, they demonstrated that each major wave conducted independently, had a different stimulus threshold, and a different absolute refractory period.

Each wave of the CAP was observed to decrease in height and become broader as the activity proceeded along the nerve, while the area, as determined by planimeter measurements, remained relatively constant. The start of the absolute refractory period was the same as the start of the CAP only at the stimulation site. For stimuli applied at a point on the nerve where a supra-maximal CAP generated elsewhere was passing, the beginning of the absolute refractory period coincided with the start of the axon potential in the slowest conducting fiber. Also, collision experiments indicated that slower conducting fibers appeared to be less excitable. From this evidence, Erlanger, Bishop, and Gasser (1926) concluded that each wave was the summation of many smaller waves, perhaps one for each nerve fiber. Thus, the CAP was thought to be composed of single fiber waves, each conducting at its own rate.

The first reference to nerve structure in connection with CAPs appears to be in discussion by Erlanger and Gasser (1924): "it is not at all surprising in view of the varied histological composition of mixed nerves to find that the action current of such a nerve as the sciatic is compound." Apparently, no attempts had yet been made to correlate quantitatively the electrically recorded phenomena with peripheral nerve morphology.

CAP RECONSTRUCTIONS

Gasser and Erlanger (1927) sought a relationship between the morphology of different peripheral nerves and the CAP recorded from the same nerves. They made the first¹ theoretical reconstructions of CAPs based on fiber diameter histograms and the following assumptions and observations:

1. Single fiber conduction velocity was assumed to be proportional to outside fiber diameter.
2. A triangular single fiber waveform was assumed.
3. The rising phase of the triangle lasted for one-third of the total constant duration for all fibers.
4. The amplitude of the triangle was constant for every fiber.
5. They assumed the activity from each fiber af-

¹In a preliminary study by Lapicque, Gasser, and Desoille (1925) relationships between nerve morphology and CAPs were briefly discussed.

affected the recording electrode according to the square of the fiber diameter.

Then, each axon potential wave was positioned along the time axis in accordance with its conduction velocity and the assumed conduction distance. The algebraic summation of all the axon potential waves gave a reasonably good prediction of the recorded CAP waveform.

The basic Gasser and Erlanger (1927) reconstruction model was used with only a few significant changes by Erlanger (1927), Bishop and Heinbecker (1930), Erlanger and Gasser (1930), O'Leary et al. (1934), Gasser (1935), and Erlanger and Gasser (1937). In some of the reconstructions (Erlanger and Gasser, 1937), the conduction velocity was assumed to vary as the fiber diameter squared and the duration of the axon potential wave for slow fibers was increased.

The assumptions required to reconstruct CAPs from fiber diameter histograms were later refined by Gasser and Grundfest (1939). They found that axon potential waves for all fibers should have a constant duration of about 0.4 msec. The supporting evidence came from two sources: (1) recordings of single axon potentials; (2) extrapolations of CAP wave durations back to zero conduction distance, a less accurate method.

Gasser and Grundfest (1939) made the axon potential wave height proportional to the first power of the dia-

meter instead of proportional to the square of the diameter. They found that experimentally recorded single axon potential heights varied linearly with conduction velocity. Since conduction velocity and fiber diameter were linearly related, axon potential height was assumed to vary linearly with fiber diameter. Axonal resistance, thought to control axon potential height for cylindrical fibers, was assumed to be inversely proportional to axonal diameter.

In their first CAP reconstructions, Gasser and Erlanger (1927) ignored the effects of myelin thickness. However, measurements of myelin thickness on 675 fibers by Gasser and Grundfest (1939) showed that relative myelin thickness was greater for fibers smaller than 7 microns in diameter. As a result, in CAP reconstructions they assumed that conduction velocity was proportional to axon diameter rather than outside fiber diameter. They obtained very good fits between reconstructed and recorded CAPs with the above assumptions.

Casey and Blick (1969) utilized the Gasser and Grundfest (1939) assumptions in their computer simulated reconstructions for responses affected by anodal polarization. Apparently, they were the first investigators to use the digital computer to replace tedious graphical constructions of CAP waveforms.

Multiphasic CAPs recorded from the skin surface

were reconstructed by Buchthal and Rosenfalck (1966). They assumed triphasic axon potential waves and used the original Gasser and Erlanger (1927) assumptions. They were interested in characterizing human peripheral nerve CAPs evoked by electrical stimulation of digital nerves. The constant relating fiber diameter to conduction velocity and the amplitude and duration of the triphasic axon potential wave were selected to minimize the difference between the reconstructed and experimental CAPs. They disregarded differences in the distance from fibers to the recording electrode because "the electrode was at a point in the electrical field around the nerve where the action potential amplitude increased about proportionally with the number of fibers activated." Later Buchthal and Rosenfalck (1971) utilized reconstructions to evaluate median nerve CAPs recorded at the wrist in polyneuropathy.

A simple arithmetic CAP reconstruction method was proposed by Landau, Clare and Bishop (1968). Each point on the CAP had an ordinate proportional to the product of the number of fibers in a single bin of the diameter histogram and the cube of the diameter for that bin. The abscissa for each point was positioned on a scale proportional to the inverse of the fiber diameters. Their rationale for how an amplitude correction factor proportional to fiber diameter can compensate for dispersive and overlap effects of axon potentials from fibers with nearly

equal diameters was not clear. In reference to above paper, Bishop, Clare and Landau (1971) stated the "further work has not confirmed the elegant simplicity of the relations between structure and function."

RELATIONSHIP BETWEEN FIBER DIAMETER AND CONDUCTION VELOCITY

Many features of nerve fibers have been examined to explain conduction velocity variations among different nerve fibers. The following features of myelinated fibers have been studied: axon and total fiber diameter, myelin thickness, internodal length, myelin biochemistry and myelin biophysical structure. Gothlin (1917) first postulated that a large fiber would conduct faster than a small one. Indirect evidence for a linear relationship between conduction velocity and fiber diameter was given by Gasser and Erlanger (1927), Erlanger (1927) and Buchthal and Rosenfalck (1966).

Hursh (1939) measured the maximum conduction velocity in cat nerves and then measured the largest fiber diameter in each nerve. By assuming that the largest fiber in a nerve trunk conducts at the highest velocity, he found that for myelinated fibers from 2 to 20 microns in diameter conduction velocity and fiber diameter were linearly related by the constant $6.0 \text{ m/s}/\mu$. For regenerated cat tibial, peroneal and saphenous nerves, Berry, Grundfest and Hinsey (1944) found the ratio of conduction velocity to fiber diameter was 5.3. Cragg and Thomas (1964)

obtained a ratio of 4.4 for rabbit peroneal nerve. Boyd (1965) found the ratio of mean conduction velocity to mean diameter for myelinated de-afferented fibers to be 5.9 for alpha, 5.2 for slow gamma and 5.0 for fast gamma. For large diameter fibers in baboon median and ulnar nerves McLeod and Wray (1967) found the ratio varied from 4.8 to 5.7 (mean 5.3) for intact and de-efferented nerve, but 3.9 to 4.4 for de-afferented nerves. Recently, Schnepf, Schnepf and Spaan (1971) found the ratio to be 6.1 for large fibers in sciatic nerves of mouse, rat and guinea pig.

Theoretical arguments that conduction velocity is a linear function of nerve fiber diameter have been given by Rushton (1951) and Goldman and Albus (1968). Many investigators have determined the ratio of conduction velocity to fiber diameter in non-mammals and found it to be generally lower than the results for mammalian nerves given above.

RATIO OF AXON DIAMETER TO FIBER DIAMETER VERSUS FIBER DIAMETER

The ratio of axon diameter to fiber diameter was first used by Schmitt and Bear (1937) and their terminology of "g" for this ratio has been adopted by most authors. From the data of Donaldson and Hoke (1905) g was 0.7 for all fiber diameters examined. Several workers later found that g increased approximately linearly with

fiber diameter from about 0.4 for small fibers to about 0.7 for large fibers (Schmitt and Bear, 1937; Gasser and Grundfest, 1939; Taylor, 1942; Sanders, 1948; Williams and Wendell-Smith, 1971). However, more recent studies with electron microscopy indicate that a constant value for g of between 0.6 and 0.7 for all fiber diameters is probably more correct (Schwarzacher, 1954; Birren and Wall, 1956; Buchthal and Rosenfalck, 1966; Dyck, 1966; Friede and Samorjski, 1967; Bishop, Clare and Landau, 1971; Schnepf and Schnepf, 1971; Waxman and Bennett, 1972). It has been suggested by Schnepf and Schnepf (1971) that errors associated with light microscopy may account for measurements of g that decrease for small fibers. The basis for their suggestion was that their measurements with light microscopy showed g decreased for small fibers, while electron microscopic measurements on the same nerves showed that g remained constant at 0.6 for all fiber diameters.

Dyck et al. (1968) point out the effects of section thickness on quantitative measurements of peripheral nerve fibers that are not at right angles to the plane of the section. They show that in sections that are too thick, obliquely cut fibers have an enlarged outside diameter and a reduced axon diameter. This effect would cause g to decrease for obliquely cut fibers in thick sections.

On theoretical grounds Rushton (1951) showed that conduction velocity, for any fiber diameter, should be maximal for g equal to 0.6.

SINGLE FIBER ACTION POTENTIAL SHAPE, AMPLITUDE AND DURATION

Gasser and Grundfest (1939) recorded single monophasic axon potentials in fibers with different conduction velocities. The triangular approximation of the potential shape appears to be reasonable, particularly since the results that follow show that slight changes in axon potential shape do not seriously affect the CAP reconstructions. They assumed that axon potential height varied linearly with fiber diameter because single fiber recordings exhibited such a relationship. Data for frog fibers (Blair and Erlanger, 1933), and for cat saphenous nerve branches (Zotterman, 1937), also support this assumption.

Gasser and Grundfest (1936) reported the duration of the axon potential to be constant (0.40 to 0.43 msec.) for mammalian A fibers. Later, Gasser and Grundfest (1939) claimed, that for fibers with different conduction velocities, the action potential durations fell "between 0.4 and 0.5 msec., without a systematic difference with respect to velocity." Recently, Paintal (1965) reported that spike duration varied inversely with conduction velocity from about 0.35 to 0.5 msec. at 37.1°C.

The rise time of the axon potential spike was

selected by Gasser and Grundfest (1936) as one-third of the total duration. Paintal (1965) found that rise time increased slightly for slow fibers while fall time was approximately inversely proportional to conduction velocity. His data indicate that rise time was about one-quarter of the total duration.

There is surprisingly little quantitative data for extracellularly recorded monophasic action potentials from single myelinated nerve fibers.

HISTOLOGICAL PROCEDURES FOR PERIPHERAL NERVE

Procedures for processing biological tissue for microscopic investigations are discussed by Pease (1964), Hayat (1970) and Weakley (1972). A tissue fixative should preserve the structure in a condition as close to the living state as possible and stop any post mortem changes. Subsequent dehydration and embedding procedures should likewise preserve the structure of the living tissue.

Double fixation with glutaraldehyde first and osmium tetroxide second is by far the most popular method of tissue preservation and combines the advantages of both fixatives (Weakley, 1972). Weakley (1972) points out that although some workers recommend a mixture of glutaraldehyde and formaldehyde, others claim that glutaraldehyde should be used alone for optimum morphological preservation. The fixation solution should be held at physiological pH by a buffer and some osmotically active

substance should be used to maintain physiological tonicity. The fixative concentration, temperature and the duration of fixation are important factors. Hayat (1970) states that perfusion of the vascular system with fixative is essential for satisfactory fixation of neural tissue.

Since most embedding material is not miscible with water, infiltration of the tissue with ethanol is usually required. This is followed by propylene oxide if an epoxy resin embedding material is used.

In part, the above procedures were developed for electron microscopy. The same techniques are desirable for light microscopy because the detail seen in epoxy resin sections is far greater than that seen in paraffin sections of similar tissue (Weakley, 1972). In a recent comparative study, Berthold and Carlstedt (1973) demonstrated that fixation in osmium tetroxide with paraffin embedding was vastly inferior to glutaraldehyde fixation and plastic embedding to identify small myelinated fibers in newborn kittens. Their light microscopic fiber counts on 0.5 micron sections prepared with the latter method were close to the counts obtained with an electron microscope. Also, Dyck et al. (1968) have shown that section thickness can seriously affect quantitative measurements of peripheral nerve fibers that are not at right angles to the section. Since section thickness should be one

micron or less, the procedures adopted for electron microscopy are desirable. On the other hand, light microscopy becomes progressively less satisfactory as section thickness decreases below one micron because the amount of tissue available for staining is small (Weakley, 1972).

Nearly all recent studies of peripheral nerve utilized very similar histological techniques. With some minor variations most workers used about 3% glutaraldehyde in a phosphate or cacodylate buffer at pH 7.4, at room temperature. The tissue was generally post-fixed with 1% or 2% buffered osmium tetroxide, dehydrated in ethanol and embedded in an epoxy resin (Araldite, Epon or Maraglas). The following authors used this general procedure to study mammalian peripheral nerves: Buchthal and Rosenfalck (1966), Dyck et al. (1968), Matthew (1968), Gutrecht and Dyck (1970), Thomas (1970), Webster (1971), Friede (1972), Phillips et al. (1972), Shimozawa (1972), and Spencer and Thomas (1972).

A radically different rapid freezing technique was proposed by Williams (1959) for quantitative studies of mammalian nerve trunks. A segment of fresh nerve surrounded by supporting fresh liver tissue was frozen by a solid CO₂/acetone mixture and sectioned in a cryostat. The sections were photographed after they were flattened on precooled slides and irrigated with saline. The

relatively thick 5 micron sections exhibited typical properties of myelinated fibers and were similar in relative size to fibers freshly teased in physiological saline. The 5 micron section thickness will introduce significant errors for obliquely cut fibers as discussed above (Dyck et al., 1968). This procedure (Williams, 1959) apparently has not been adopted by others studying peripheral nerve.

Birren and Wall (1956) examined rat sciatic nerve both with osmic acid fixation and freezing-drying. Although they preferred the freezing-drying method their study did not show which method was better for preserving the in situ structure.

MEASUREMENTS OF FIBER DIAMETER

Histologically prepared cross-sections of peripheral nerve exhibit many noncircular myelinated nerve fibers (Sunderland and Roche, 1958; Webster and Spiro, 1960; Friede and Samorajski, 1967; Babel, Bischoff and Spoendlin, 1970). These fibers may be cut through or adjacent to nodes of Ranvier, Schmidt-Lanterman clefts, or Schwann cell nuclei. In addition, many fibers appear to be cut at least somewhat obliquely and have quasi-elliptical shapes. There is conflicting evidence regarding the magnitude of random variations in diameter along nerve fibers and the extent of nerve fiber tapering and branching (Eccles and Sherrington, 1930; Duncan, 1934;

Hursh, 1939; Rexed, 1944; Sunderland and Lavarach, 1953; Sunderland and Roche, 1958; Skoglund and Romero, 1965).

Quite a variety of methods have been used to measure fiber diameters on photomicrographs of peripheral nerve cross-sections. Adequate justification for the particular method used was rarely given. Also, references to different methods used by other investigators were scarce. A sample of the various methods that have been utilized will now be given.

Many authors claim to select only approximately circular fibers for measurement, with typically little or no discussion of possible biases introduced to the neuronal population property (usually the fiber diameter histogram) under study (Sanders, 1948; Buchthal and Rosenfalck, 1966; McLeod and Wray, 1967; Gutrecht and Dyck, 1970; Bishop, Clare and Landau, 1971; Williams and Landon, 1971). Other investigators used a series of circles on Perspex or celluloid to estimate fiber diameter. Many claim, without comment, to simply "measure fiber diameter" (Gasser and Grundfest, 1939; Rexed, 1944; Fernand and Young, 1951; Boyd and Davey, 1968). Espir and Harding (1961) described three methods for matching variable diameter circles to the fiber shapes (McLeod and Wray, 1967; Dyck et al. 1968). A Zeiss particle counter has been used by several workers (Nystrom and Skoglund, 1964; Dyck and Lambert, 1966; Gyllensten et al., 1966;

Berthold and Carlstedt, 1973).

Some authors assume that every oval or asymmetric shaped fiber was cut obliquely and therefore measure the "smallest diameter" (Ogden and Miller, 1966; Landau, Clare and Bishop, 1968; Matthews, 1968; Gutrecht and Dyck, 1970). Many authors estimated fiber diameter from what could be called the arithmetic mean of mutually perpendicular maximum and minimum diameters (Sanders, 1948; Evans and Vizoso, 1951; Wasano et al, 1962; Hutchinson, Koles and Smith, 1970; Webster, 1971; Schlaepfer and Myers, 1973). Gasser and Erlanger (1927) used the geometric mean of the mutually perpendicular maximum and minimum diameters.

Kasvand, Isnor and Romero-Sierra (1970) used a flying spot scanner to quantify black and white borders in peripheral nerve cross-sections. The nerve fibers were assumed to be circular. They used computers to recognize the fibers and to compute fiber diameters based on the mean of 24 radii in a star-shaped pattern. Their report does not explain how the center of noncircular fibers was found. The automated method failed to identify and measure about 30% of the small fibers that were identified and measured manually with a ruler and compass. Also, they measured fibers with a circular type particle size analyzer. Generally the measurements obtained with the computer were greater than measurements made with a ruler

and compass, yet less than the results from the particle size analyzer. Unfortunately their tissue was fixed in 0.5% osmic acid and embedded in paraffin (3-5 micron section thickness). These procedures caused swelling that made the fibers appear quite round.

Potts et al. (1972) abandoned fiber diameter for fiber area which they plotted on the abscissa of fiber size histograms. Friede and co-workers also abandoned the concept of fiber diameter, in favor of axon circumference or number of lamellae in the myelin sheath (Friede and Samorajski, 1967, 1968; Friede and Martinez, 1970; Friede and Miyagishi, 1972).

The above methods for measuring fiber diameter and references cited are intended to be representative rather than exhaustive. No review of this subject appears to have been published.

Measurements of fiber diameter in serial sections show that considerable variation can occur (Duncan, 1934; Hursh, 1939; Sunderland and Roche, 1958). Unfortunately, these serial section measurements were all made on medium sized fibers in 5 to 10 micron thick sections with light microscopy. Duncan measured the diameters at 5 micron intervals for three osmicated fibers teased in glycerin. Although 100% diameter variations were seen along each fiber, over one half of the measurements were within one

micron of the mean. Duncan also claimed that measurements on shorter lengths of several hundred other fibers gave similar results. Hursh measured 100 serial sections of a single fiber. The mean diameter was 6.5 ± 0.47 (s.d.) microns. Fifty-eight percent of the values were within 0.2 microns of the mean. Sunderland and Roche followed one fiber in 200 serial 10 micron thick sections and six other fibers in 100 serial sections. The standard deviations for their measurements ranged from 0.8 to 1.8 microns.

PROPERTIES OF PERIPHERAL NERVE FIBER DIAMETER HISTOGRAMS

During the course of this study a list of several hundred fiber diameter histograms published in the literature has been compiled (Appendix A). Apparently no one else has attempted to characterize the general properties of peripheral nerve fiber diameter histograms.

Authors are divided almost equally between use of the actual number of fibers for the ordinate variable and percent of the total number of fibers. One investigator (Hopkins, 1970) used fiber density per square millimeter as the ordinate variable.

Fiber diameter was always plotted on the abscissa. Some latency histograms and conduction velocity histograms are available in the literature for peripheral nerve fibers. Towe and Harding (1970) discuss the nonlinear

transformation of a fiber size distribution into a temporal distribution.

Fiber diameters less than one micron in mammalian peripheral nerve are very rare. Some mammalian central fibers are myelinated down to diameters of one third of a micron (Bishop and Smith, 1964). Myelinated mammalian fiber diameters seldom exceed twenty microns. The typical upper limit for a significant number of fiber diameters is sixteen to eighteen microns.

The bin width used to compile myelinated fiber diameter histograms varied from 0.1 microns (Erlanger, 1927; Gasser and Erlanger, 1927; Gasser and Grundfest, 1939; Matthews, 1968) to 2 microns (Fernand and Young, 1951). Since the limit of resolution with the light microscope is 0.2 microns (Weakley, 1972), the use of 0.1 micron binwidths in light microscopic studies by Erlanger (1927), Gasser and Erlanger (1927) and Gasser and Grundfest (1939) seems unreasonable. No standard bin width has been established although one micron bin widths are the most common. Apparently no consideration has been given to the effect of bin width on histogram shape.

Most fiber diameter histograms have either one or two distinct peaks. Other smaller peaks may be masked in some cases by the use of wide bin widths. The ordinate values rarely remain relatively constant for a range of

diameters greater than two or three microns. Except for ventral root diameter histograms (Eccles and Sherington, 1930), multimodal diameter histograms rarely have a gap in the histogram between peaks. Measurement method and errors, random variations of fiber diameters along the longitudinal axis of fibers, and obliquely cut fibers all tend to make the peaks in the diameter histograms appear too wide (Duncan, 1934; Gasser and Grundfest, 1939), particularly for tall narrow peaks.

Some investigators sample only a portion of the fibers present, either by measuring all fibers within randomly selected areas or by supposedly random selections of individual fibers. Friede and Miyagishi (1972) used a sampling method that was biased in favor of large fibers (a grid of equi-distant parallel lines is drawn on photomicrographs and every fiber touching a line is measured.) Van Crevel and Verhaart (1963) and Donovan (1967) found that cat optic nerve fiber diameters were not randomly distributed. Donovan found a greater percentage of smaller fibers in peripheral areas of the nerve. She also found statistically significant differences in the fiber diameter distribution within smaller areas. Birren and Wall (1956) found considerable variation in fiber diameter histograms compiled from different regions of a rat sciatic nerve cross-section. Ap-

parently, for peripheral nerve, no studies have sought to examine quantitatively the homogeneity of fiber size distributions within nerve cross-sections.

CHAPTER II

METHODS

THE BASIC EXPERIMENTAL PROCEDURE

The experimental procedure is summarized first before the specific details are given. One objective was to record supramaximal monophasic compound action potentials in response to brief electrical stimuli applied directly to the cat saphenous nerve some distance away. Immediately after the recording session the cat was perfused through the heart with fixative. The same nerve was removed and prepared histologically. Light photomicrographs were made from cross-sections of the nerve. Computer analysis of measurements taken from the photomicrographs were used to compile fiber diameter histograms for the entire nerve cross-section. Relationships between the recorded compound action potential and the anatomically derived fiber diameter histogram were studied with digital computer models. A principal objective was the prediction of the fiber diameter histogram from the compound action potential.

SELECTION OF THE CAT SAPHENOUS NERVE

The cat saphenous nerve was chosen for the following reasons:

1. Six to seven centimeters of nerve trunk were available with no cutaneous branches.
2. The total number of myelinated nerve fibers was large though manageable (about 2500).

3. A large range of fiber diameters (2-16 microns) were present in significant numbers.
4. The nerve was superficial.

EXPERIMENTAL PREPARATION

Fourteen adult acute cats weighing from 2.7 to 4.2 kg. were obtained from the Unit for Laboratory Animal Medicine for the investigation. Only the physiological or only the anatomical procedures were attempted or successful for some of the cats studied. The data in the results chapters were taken from the final two cats studied (referred to as cat M and cat N).

All the animals in this study were given an initial intraperitoneal injection of sodium pentobarbital (35 mg/kg) and the left cephalic vein was cannulated. The animal core temperature, monitored by a rectal thermometer, was maintained at 37 to 38°C by an electric heating pad placed under the animal. The heating pad was unplugged during the recording session.

The animal was placed in a fixture that held the left hindlimb up to expose the medial side of the right hindlimb for surgery. An incision was made from the groin to the middle of the tibia. The skin was folded back and up where it was sutured to the fixture. This created a cavity that was later flooded with mineral oil.

The saphenous artery, vein and nerve were then

visible under the fascia. Fine glass hooks were used to tease the saphenous nerve from connective tissue for approximately one centimeter at each of three locations. The most central location was in the groin where the saphenous nerve emerges from the femoral nerve. The second location was approximately 2.5 cm. distal along the saphenous nerve. The two pairs of stimulating electrodes were later placed at these two locations. A final segment of nerve for the recording electrodes was teased out just on the proximal side of the first cutaneous nerve branch point. The exposed area was flushed with saline throughout this procedure to prevent tissue drying. (See Crouch (1969) page 246 for anatomical orientation.)

The stimulating electrode fixture was positioned near the groin and the two pairs of stimulating electrodes placed around the nerve at the first two locations described above. Next, the recording electrode pair was mounted at the most distal third location and the silver wire electrodes were wrapped completely around the saphenous nerve.

The saphenous nerve was pinched repeatedly with fine forceps just on the distal side of the recording electrodes, cut at the same location, and repeatedly pinched again. The pinching and cutting were necessary to obtain good monophasic recordings (heating the cut

nerve end, as suggested by Erlanger, Bishop, and Gasser (1926), although very effective, was abandoned because apparently it interfered with adequate perfusion of the tissue with fixative). The distal recording electrode was moved onto the crushed area of the nerve very close to the cut end. Several stimuli were applied to ensure that a good monophasic potential was available. The entire pool, including the stimulating and recording electrodes, was flooded with warm mineral oil. The mineral oil prevented tissue drying and provided a means for temperature control. A 50 cc. syringe was used to replace the cooled oil periodically. The temperature within 1 cm. of the recording electrode pair was monitored with a precision (0.1° C/div.) mercury thermometer.

The time required for the surgical procedures was minimized by planning and practice in order to obtain undistorted CAP recordings. The elapsed time from the initial leg incision to the first supramaximal recording session was reduced to approximately one hundred minutes. The time from the start of the nerve crushing and cutting operation to the first supramaximal recording session was less than five minutes.

INSTRUMENTATION

The instrumentation system utilized to record in vivo CAPs from cat saphenous nerves will be described

briefly. A block diagram of the experimental set-up is shown in figure 2-1.

Brief (30 μ sec.) square cathodal constant current electrical stimuli were applied to the stimulating electrodes at the rate of one per second. The constant voltage stimuli delivered by the stimulator (Grass S4H) were isolated from ground (Grass SIU-4B) and converted to constant current pulses (Grass CCU-1A). A precision one kilohm series resistor was used to monitor the current pulse. The leads to the stimulating electrode pairs were shielded. The stimulus could be switched to either stimulating electrode pair. Figure 2-2 is a photograph of the current pulse displayed on the oscilloscope.

The stimulating electrode fixture had two pairs of electrodes (26 ga silver wires, 1.5 mm. apart) rigidly placed 2.5 cm. apart on two arms. Each electrode pair was formed to surround approximately three-fourths of the nerve trunk when in use. The electrodes were mounted in dental acrylic.

The recording electrode fixture supported two 28 gauge silver wires that were carefully wrapped once around the nerve to form circular cuff electrodes. The distance along the longitudinal axis between the two electrodes was about 2 mm. The short wires that lead to the preamplifier were shielded.

The differential low-level preamplifier (Tektronix

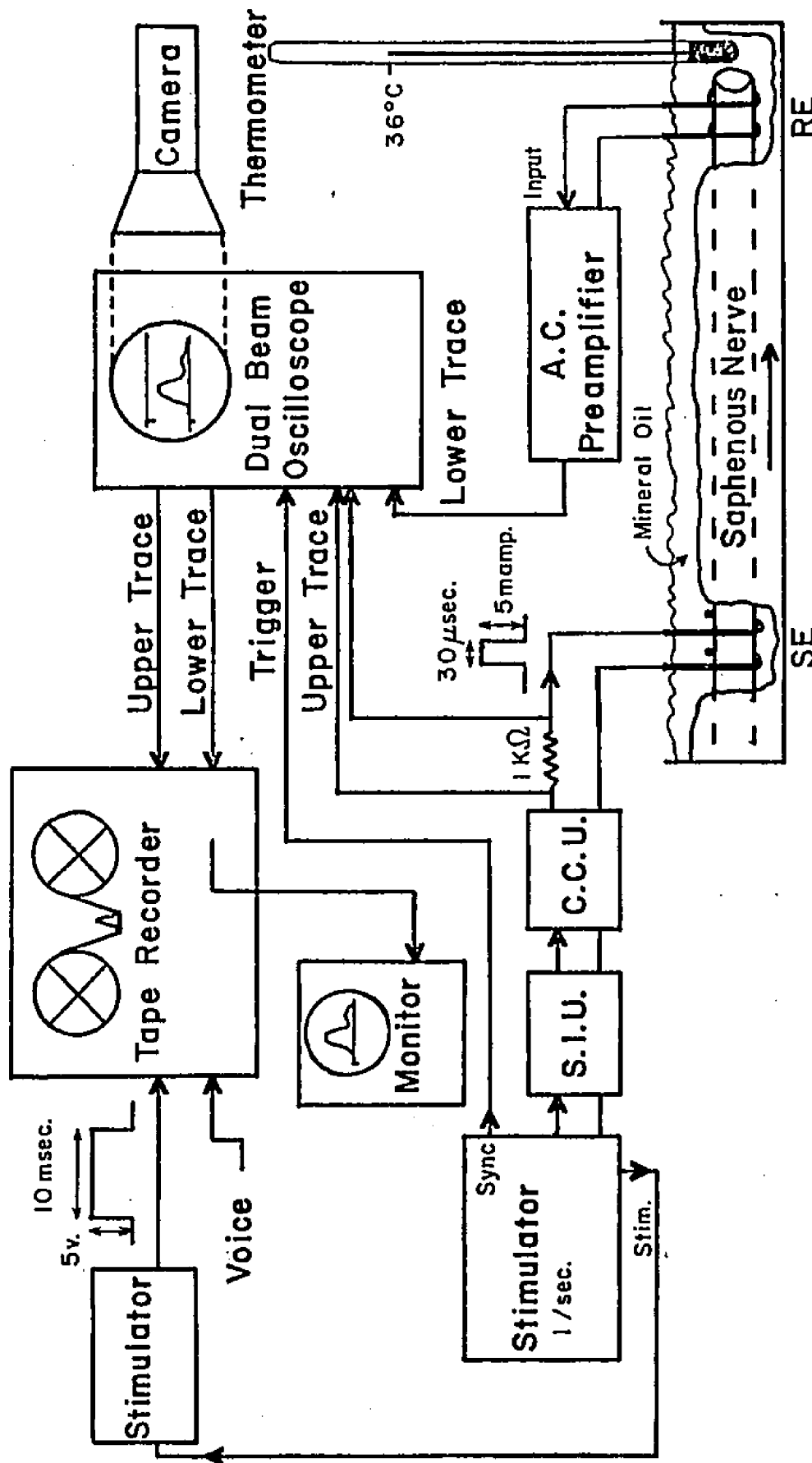


Figure 2-1. Block diagram of the experimental setup.

5 ma/div.

1 mv/div.

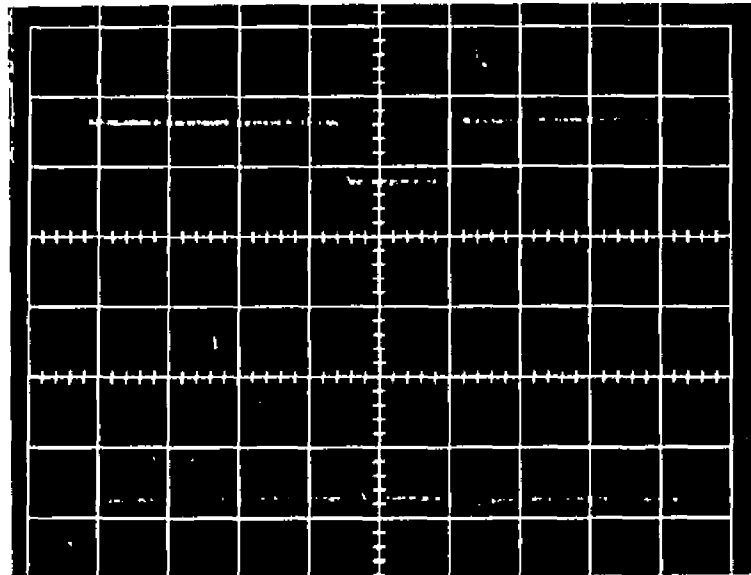
20 μ sec/div.

Figure 2-2. Photograph of the stimulus current waveform (upper) and stimulus artifact (lower).

Type 122) had a gain of 1000 with a band pass from 8 Hz. to 5 KHz. The oscilloscope (Tektronix 502A) gain was 2 or 4 with a bandwidth of 2 Hz to 1 MHz. The instrumentation recorder (Hewlett Packard 3960) FM channels had a bandwidth of DC to 5 KHz when operated at 15 IPS. The output of the instrumentation recorder was monitored during the recording session with a second oscilloscope. (Tektronix 502A).

The voltage across a one kilohm resistor in series with the constant current stimulator was used to monitor and record the stimulus current. A constant voltage, 10 msec. duration timing pulse (from Grass SD-5) was recorded on an AM channel (bandwidth of 70 Hz-60 KHz) of the recorder when each stimulus occurred.

DETAILED MEASUREMENT PROCEDURE

The CAP recording session commenced immediately after the experimental preparation was completed. The elapsed time required for the recording session was 40 to 50 minutes.

Ten segments of data were recorded sequentially on magnetic tape. On the first and last segments a one volt peak-to-peak square wave calibration signal was recorded. The eight CAP segments were recorded under four conditions of applied stimulus current, each at two conduction distances (4.2 and 6.7 cm. or 4.3 and 6.8 cm.). The four levels of stimulus current were:

1. Supramaximal for all A fibers.
2. Supramaximal for A α -fibers.
3. Slight increase above previous supramaximal for A α -fibers sufficient to give a just noticeable increased response.
4. Repeated supramaximal for all A fibers.

Each segment contained responses to approximately 50 consecutive stimuli given at the rate of one per second. During each segment a Polaroid photograph of one of the 50 responses was taken. Only the responses to stimuli that were supramaximal for all A fibers were utilized in the results reported here.

The smallest latency for the first CAP component did not decrease, for the recording techniques used in this study, as the magnitude of the stimulus current was increased from threshold to a supramaximal value.

The temperature of the mineral oil pool was adjusted to $36^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$ before each recording segment by adding warm mineral oil and allowing at least one minute for equilibration. The decrease in temperature of the mineral oil for the duration of a recording segment was usually less than 0.2°C .

ANATOMICAL PROCEDURES

Immediately following the recording session the mineral oil and electrodes were removed. The exposed tissue was rinsed several times with isotonic saline.

The animal was perfused through the heart with 0.25% glutaraldehyde in Millonig's (1962) phosphate buffer (230 cc/kg) at pH 7.4 at room temperature. At the start of the perfusion the previously crushed distal 1.0 mm. of saphenous nerve was cut off. Perfusion through the heart was continued with 3% glutaraldehyde in Millonig's phosphate buffer (1000 cc/kg) at pH 7.4 at room temperature.

At least five 3 mm. sections of the saphenous nerve adjacent to the recording electrode site were removed and placed in 5 cc. beakers filled with the buffered 3% glutaraldehyde for approximately 30 minutes. Following a ten minute wash with phosphate buffer, the tissue was post-fixed in buffered 1% osmium tetroxide for two hours at 4° C. After another ten minute wash with phosphate buffer, dehydration with ethanol in 10% steps for fifteen minutes each was begun. Four changes of 100% ethanol for fifteen minutes each were followed by three changes of propylene oxide. The tissue was immersed in a 1:1 mixture of propylene oxide and Epon for twenty minutes. Finally, pure Epon was allowed twelve hours to fully infiltrate. The tissue was put in capsules filled with fresh pure Epon and maintained at 60° C for twenty-four hours.

Approximately one micron thick transverse sections were cut on a microtome (American Optical, Spencer Model

820) with glass knives and stained with toluidine blue. A selected section was photographed with a light microscope, (Leitz Wetzlar). High contrast enlarged prints (Simmon Omega Type E enlarger) were pieced together to form a montage for each fascicle.

A high power light microscope equipped with a graduated reticule (calibrated with a stage micrometer) was used to measure the distance between two identifiable points on the section. The corresponding distance on the photomicrograph was divided by the distance on the slide to give the magnification. This procedure was repeated four times at different angles for each fascicle and the mean value was taken as the total magnification (1750-1850).

Every myelinated fiber was labeled arbitrarily with a number. All questionable objects were examined with a high power light microscope and were accepted as myelinated fibers only if a dark border enclosing a much lighter center could be identified.

The coordinates of a series of points around the outside of each myelin sheath were digitized with a semiautomatic magnetically coupled digital scanner (Edwin Industries Model PF40). The scanner was interfaced to a keypunch (IBM 029), that punched the coordinates on IBM cards. The origin of the coordinate system was adjustable and remained very stable for several

hours. The number of point coordinates digitized per fiber was variable depending on fiber sized and shape. The scanner resolution was 0.01 inches which corresponded to 0.144 microns at the lowest magnification used and was less than the resolving power of the light microscope. During this study, approximately 9,000 fiber perimeters (180,000 point coordinates) were digitized.

COMPUTER SIMULATION AND ANALYSIS

Most of the computer programs developed during the course of this study were written in FORTRAN IV. The Michigan Terminal System (MTS) with an IBM 360 model 67 dual-processor computer was utilized.

Extensive use was made of a CalComp 780/763 digital plotter. The point coordinates around the perimeter of each myelinated fiber were checked by plotting straight lines between sequential point coordinates for each fiber. The CalComp generated plot was overlayed on the photomicrograph and the perimeters compared. This procedure detected infrequent digital scanner operator errors and digital scanner malfunctions. Most of the graphs and histograms in this thesis were also prepared by the CalComp plotter.

The CAPs recorded on magnetic tape were digitized and averaged ($N = 30$) with a Hewlett-Packard 5610A A to D converter and a 2115A laboratory computer. The standard deviation of the signal for each sample point

(36.5 μ sec. sampling interval) was calculated. The results were punched on paper tape for processing by MTS programs.

Early CAP reconstruction models were studied with the aid of a DEC PDP8-338 graphical display connected to the IBM 360/67 via telephone.

CHAPTER III

MONOPHASIC CAP MODEL RESULTS

CAP RECONSTRUCTION ASSUMPTIONS

Apparently since Gasser and Erlanger (1927) first began reconstructing compound action potentials by adding together many overlapping triangles, no one has either formally examined all of the assumptions underlying such a procedure or expressed the summation mathematically. While in this case the mathematics are not particularly profound, a mathematical model is a useful aid to examine the various assumptions required for the reconstructions.

Consider a peripheral nerve of approximately circular cross-section completely surrounded at some point by a monopolar recording electrode. Let the nerve be immersed in a nonconductor such as air or mineral oil. A supramaximal electrical stimulus applied to the nerve some distance away will cause a CAP to pass by the recording electrode. Let the supramaximal CAP, $V_{\max}(t)$, be expressed as,

$$V_{\max}(t) = \sum_{i=1}^N v_i(t-t_i) \quad (3-1)$$

where N is the number of excitable myelinated fibers in the nerve; $v_i(t)$ is the voltage waveform seen by the recording electrode when an impulse in the i th fiber

passes, and t_1 is the time required for the action potential in the i th fiber to conduct from the site of stimulation to the recording electrode. This expression is valid only if superposition of the individual fiber waveforms holds. Independence of the t_1 in equation 3-1 requires the assumption that propagated activity in any myelinated fiber is not affected by activity in other fibers within the bundle.

Esplin (1962) convincingly demonstrated that for myelinated fibers, single fiber conduction velocities were unchanged when single fibers were activated alone or together with neighboring fibers. Electrophysiological studies have shown that for unmyelinated fibers, some interaction does take place (Katz and Schmitt, 1940, 1942). The principal effect was a synchronization and slowing of the impulses in the volley. Nerve fiber interaction for unmyelinated fibers has been modeled mathematically by Markin (1970) and Clark and Plonsey (1970, 1971). The latter studies concluded that the nerve trunk cross-sectional area, the interstitial specific resistivity and the membrane capacitance are particularly important in determining the degree of fiber interaction.

Note that uniform conduction velocity along the fibers need not be assumed. Only the total time delay, t_1 , is required. In general, the $v_1(t-t_1)$ may be dif-

ferent for each fiber.

All fibers with similar $v_1(t-t_1)$ can be lumped together to give:

$$V_{\max}(t) = \sum_{i=1}^M n_i v_1(t-t_1) \quad (3-2)$$

where M is the number of different $v_1(t-t_1)$ and n_i is the number of fibers with the same $v_1(t-t_1)$. Note that in equation 3-2 only similar waveforms have been combined without any reference to fiber size.

Several assumptions are required to relate the variables in equation 3-2 to nerve fiber diameter. Empirical evidence cited in the introductory chapter indicates that each $v_1(t-t_1)$ is similar for fibers of a given diameter. Therefore, all n_i fibers with the same $v_1(t-t_1)$ are assumed to have approximately the same diameter, d_i . This assumption states implicitly that the single fiber response, $v_1(t-t_1)$, does not depend on the location of the fiber within the nerve bundle. Intuitively, it would seem that fibers of a given diameter that are close to the bundle perimeter might contribute more to the CAP than fibers in the center of the bundle. This effect can be ignored if the fiber sizes are randomly distributed throughout the cross-section. It will be assumed that

$$v_1(t) = v(t) \cdot d_i^{\text{APE}} \quad (3-3)$$

where $v(t)$ is a triangular waveform that is identical for all fibers; and the axon potential exponent (APE) is

usually unity. These assumptions were first made by Gasser and Grundfest (1939). Other authors (Gasser and Erlanger, 1927; Buchthal and Rosenfalck, 1966; Landau, Clare and Bishop, 1968) have assumed that APE is two.

The time delay, t_1 , can be modeled as a simple function of d_1 if we assume that g (the ratio of axon to outside diameter) is constant for all fiber diameters:

$$t_1 = f(d_1) = \frac{\text{DIST}}{\text{CVC} \cdot d_1^{\text{CVE}} \cdot g} \quad (3-4).$$

DIST is the conduction distance and CVC is an empirically derived conduction velocity constant that relates conduction velocity to fiber diameter. The conduction velocity exponent (CVE) simulates the effect of a possible nonlinear relationship between conduction velocity and fiber diameter. Many authors find empirically that CVE should be unity (Hursh, 1939; Berry, Grundfest and Hinsey, 1944; Cragg and Thomas, 1964; Boyd, 1965; McLeod and Wray, 1967; Schnepf, Schnepf and Spaan, 1971). Blair and Erlanger (1933) give a value of 2 for CVE, while Pumphrey and Young (1938) assume a value of 0.5 for squid nerves.

If g decreases for small fibers, as Gasser and Grundfest (1939) assumed, then the following expression for t_1 can be calculated from their data;

$$t_1 = f(d_1) \left\{ \begin{array}{l} \approx \frac{\text{DIST}}{\text{CVC} \cdot d_1^{\text{CVE}} (0.514 + d_1 \cdot 0.0194)} \quad d_1 \lesssim 7\mu \\ = \frac{\text{DIST}}{\text{CVC} \cdot d_1^{\text{CVE}} (0.560 + d_1 \cdot 0.0132)} \quad d_1 \lesssim 7\mu \end{array} \right. \quad (3-5).$$

Gasser and Grundfest reasoned that conduction velocity should be related to axon diameter rather than outside fiber diameter, d_1 . Since outside fiber diameters were traditionally measured for histograms, they believed the variation in relative myelin thickness with diameter was an important consideration for CAP reconstructions.

Unless M , the number of different $v_1(t-t_1)$, is infinite, a specific diameter d_1 can occur anywhere in the range:

$$d_1 \pm \frac{\Delta d}{2} = d_1 \pm \frac{d_{\max} - d_{\min}}{2 M} \quad (3-6)$$

The bin width, Δd , of the fiber diameter histogram will represent some tolerable variation in $v_1(t)$ and t_1 that depends on the functional relationship between the d_1 and $v_1(t-t_1)$.

Equation 3-2 can now be expressed in terms of fiber diameter:

$$V_{\max}(t) = \sum_{i=1}^M n_i d_i^{\text{APE}} v(t-f(d_i)) \quad (3-7)$$

where each term in this sum is a single axon potential wave. This continuous function of time can be expressed as a discrete function of time:

$$V_{\max}(k\Delta t) = \sum_{i=1}^M n_i d_i^{\text{APE}} \cdot v(k\Delta t - f(d_i)), \quad k=0,1,\dots,L \quad (3-8).$$

This system of $L+1$ linear equations expresses the functional relationship between the fiber diameter histogram (n_i, d_i) and the CAP $(V_{\max})$. The reconstructed CAP is a unique discrete function of time for each fiber diameter histogram if the reconstruction parameters are fixed. The right side of equation 3-8 can be evaluated if values for all the parameters are given and a fiber diameter histogram is specified. A rational value for every parameter can be selected based on empirical data given in the introduction. Note that the n_i in each term of equation 3-8 appear only as simple scalars. This property of the model will be used in Chapter VI to find an unknown fiber diameter histogram when the CAP is given.

The system of equations 3-8 is evaluated on a digital computer. The major features of this superposition model are illustrated in figure 3-1. The choice of a specific fiber diameter histogram for investigations of the model properties is arbitrary. A gaussian histogram was chosen because many anatomical histograms appear to

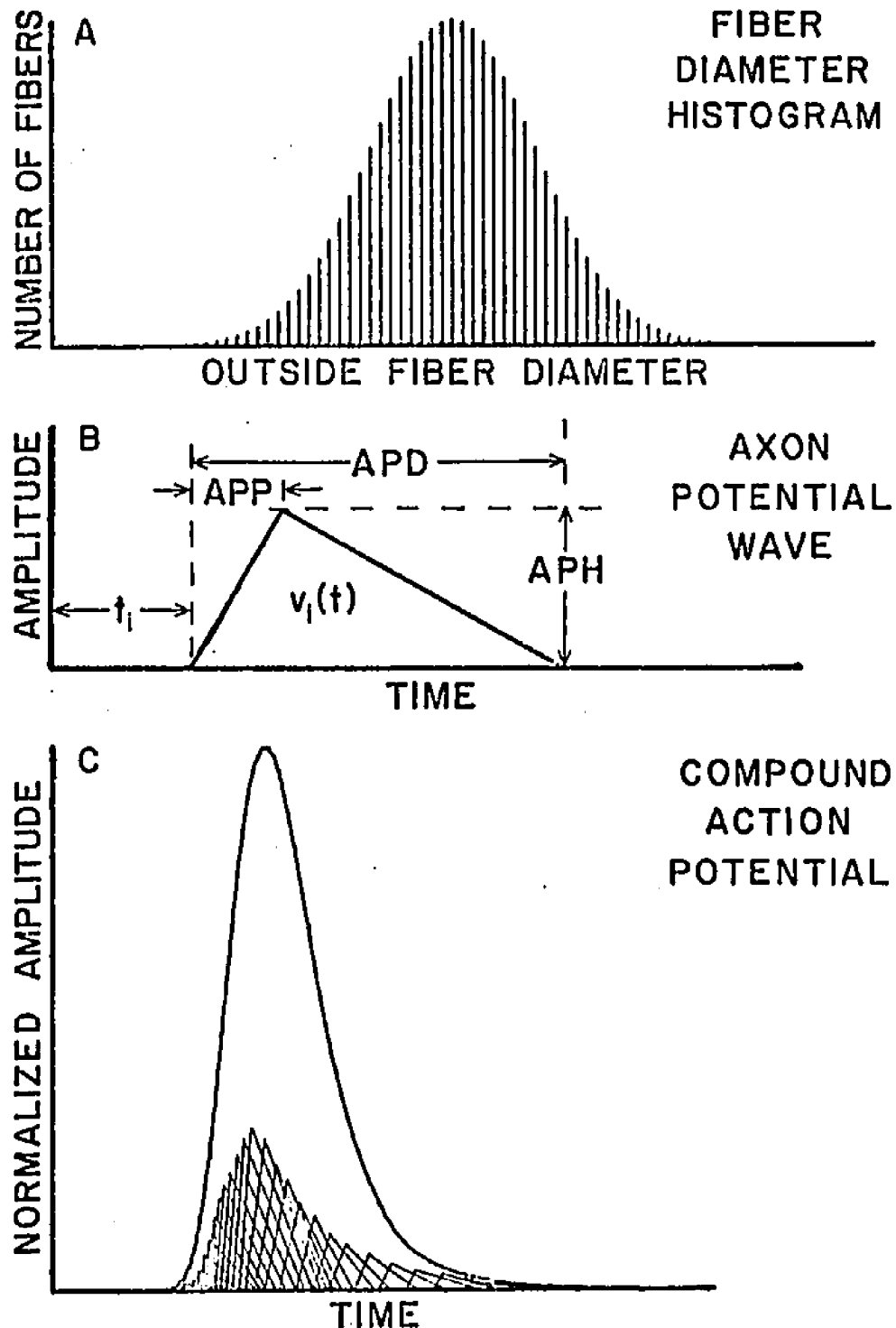


Figure 3-1. The basic CAP reconstruction model. The response for the fibers in each bin of the fiber diameter histogram (A) is modeled by a single axon potential wave (B). The reconstructed compound action potential (C) is the sum of all the axon potential waves. The triangles in (C) are illustrative only and their sum does not equal the CAP shown.

be either unimodal or bimodal gaussian in basic character (index in Appendix A). Also the mean and standard deviation can be altered systematically to assess quantitatively the effect on the CAP of changes in the histogram.

An axon potential wave (figure 3-1B) is computed for each bin (vertical line) in the fiber diameter histogram (figure 3-1A). The CAP (figure 3-1C) is reconstructed by adding together all of the axon potential waves. It is necessary to decrease the bin width, Δd , and linearly interpolate intermediate ordinate values, to reduce the jagged appearance of individual axon potential wave peaks in the reconstructed CAPs. Most reconstructed CAPs are smooth for Δd equal to 0.0625μ . Except for smoothing these jagged artifacts, the normalized reconstructed CAP shape is not affected by the bin width interpolations.

ESSENTIAL CAP PARAMETERS

The parameters required for CAP reconstructions are listed in table 3-1 where they are partitioned into three categories: fiber diameter histogram parameters, biophysical parameters, and axon potential wave parameters. The parameter symbols and default values are also given. The default values are based on published empirical data and experimental conditions used in the studies presented later. The default mean and standard deviation for the gaussian fiber diameter histogram

TABLE 3-1

ESSENTIAL MODEL PARAMETERS

I.	<u>FIBER DIAMETER HISTOGRAM PARAMETERS</u>	<u>SYMBOL</u>	<u>DEFAULT VALUE</u> ¹
	EXAMPLE: Gaussian		
	A. Mean Fiber Diameter	MFD	10.0 μ
	B. Standard Deviation	STD	2.0 μ
II.	<u>BIOPHYSICAL PARAMETERS</u>		
	A. Stimulation-Recording Conduction Distance	DIST	6.0 cm.
	B. Conduction Velocity Constant	CVC	8.8 m/s/ μ
	C. Conduction Velocity Exponent	CVE	1.
	Cond. Vel. = $CVC \cdot d_1^{CVE} \cdot g(d_1)$		
	D. Myelin Thickness Function	$g(d_1)$	For $d_1 \leq 7\mu$: $g(d_1) = 0.51 + d_1 \cdot 0.019$
		$g(d_1) = \frac{\text{Axon Diameter}}{\text{Fiber Diameter}}$	For $d_1 \geq 7\mu$: $g(d_1) = 0.56 + d_1 \cdot 0.013$
III.	<u>AXON POTENTIAL WAVE PARAMETERS</u>		
	A. Axon Potential Height Exponent	APE	1.
	$APH = d_1^{APE}$		
	B. Axon Potential Duration	APD	0.4 msec.
	C. Axon Potential Rise Time	APR	0.1 msec.

¹Default parameter values are assumed unless other values are clearly specified.

are chosen to permit parameter variations that would not require the use of unrealistic fiber diameters. All of the CAP parameters in table 3-1 will be examined in the following sections.

PARAMETER SENSITIVITY ANALYSIS PROCEDURE

Each model parameter, when varied, affects the reconstructed CAP differently. A measure of the relative effect on the CAP of different model parameters is needed to identify quantitatively the most influential parameters. Only one parameter at a time is perturbed from its default value. The effect of the altered parameter on the reconstructed CAP is quantified by a deviation measure defined as:

$$\text{Deviation} = \frac{\sum_{i=0}^L (V_{\max}(i\Delta t) - V_{\max \text{ default}}(i\Delta t))^2}{\sum_{i=0}^L (V_{\max \text{ default}}(i\Delta t))^2} \quad (3-9).$$

This measure of change in the CAP waveform is squared error divided by a constant, since the default CAP does not change. Of course information about the shape of the CAP for parameter changes is lost when deviation is computed. Therefore, both CAP waveforms and plots of deviation are given for some parameters in the following sections.

The relative effect of different parameters is studied

by perturbing each parameter by the same percentage of the default parameter value. Although the default CAP is normalized, the CAPs reconstructed for any variation in a parameter are scaled relative to the default CAP. Thus, for all parameter variations, relative CAP magnitudes are preserved.

FTBER DIAMETER HISTOGRAM PARAMETER ANALYSIS

The default CAP and fiber diameter histogram are shown in figure 3-2A. The effects of decreasing the mean fiber diameter (MFD) by 20% and 40% are also shown in figure 3-2B and 3-2C. Note that CAPs B and C are scaled with respect to the default CAP, A. The mean fiber diameter is apparently quite influential in determining both the size and shape of the CAP. The start of the CAP, the peak, and the tail are all delayed in time as MFD decreases. The total CAP duration, though difficult to quantify, does increase as MFD decreases.

The CAPs for similar percentage variations in the standard deviation (STD) of the gaussian fiber diameter histogram are illustrated in figure 3-3. Note that the maximum number of fibers in histograms B and C is decreased to keep the total number of fibers in the histogram constant. Increasing STD by 20% and 40% broadens the fiber diameter histogram and the CAP duration increases. The latency to the CAP peak decreases slightly

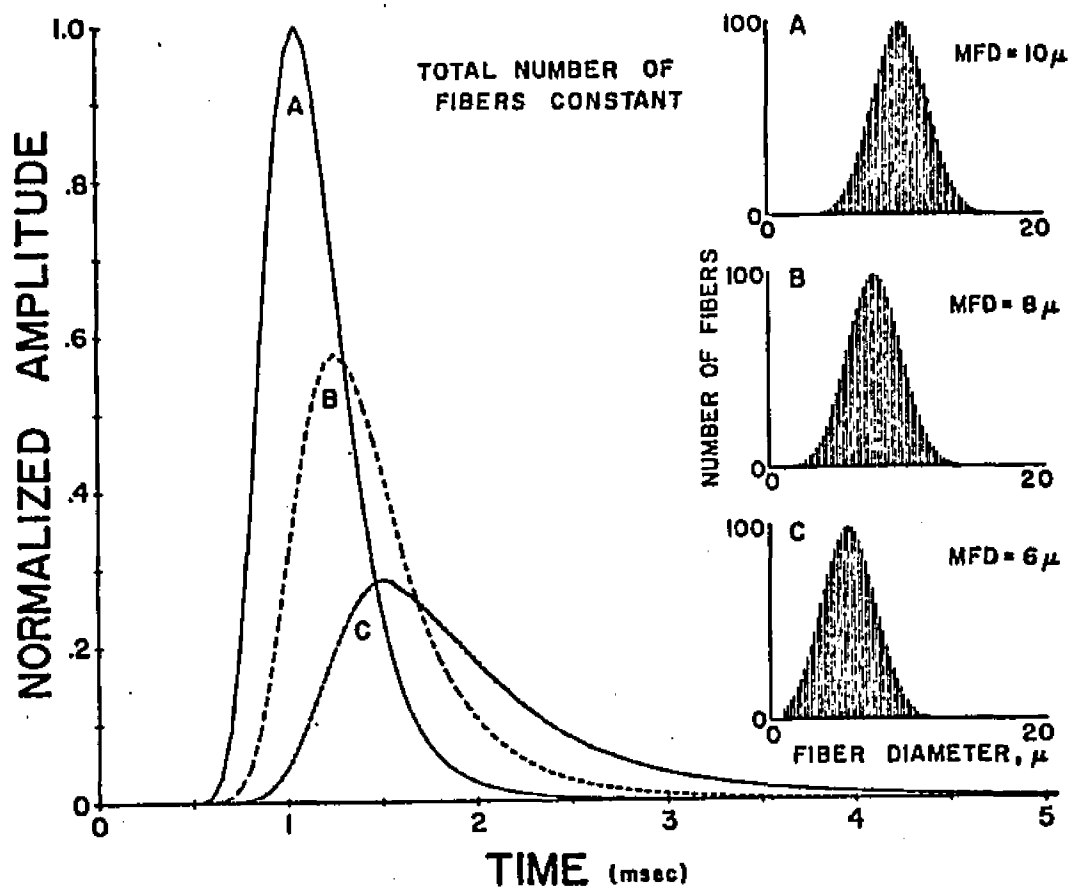


Figure 3-2. Reconstructed CAPs for three mean fiber diameters (MFD) for a gaussian fiber diameter histogram. A. CAP for the default parameters in table 3-1. B. CAP for MFD reduced to 8 microns (20%). C. CAP for MFD reduced to 6 microns (40%). The CAPs labeled B and C are scaled with respect to the default CAP in A.

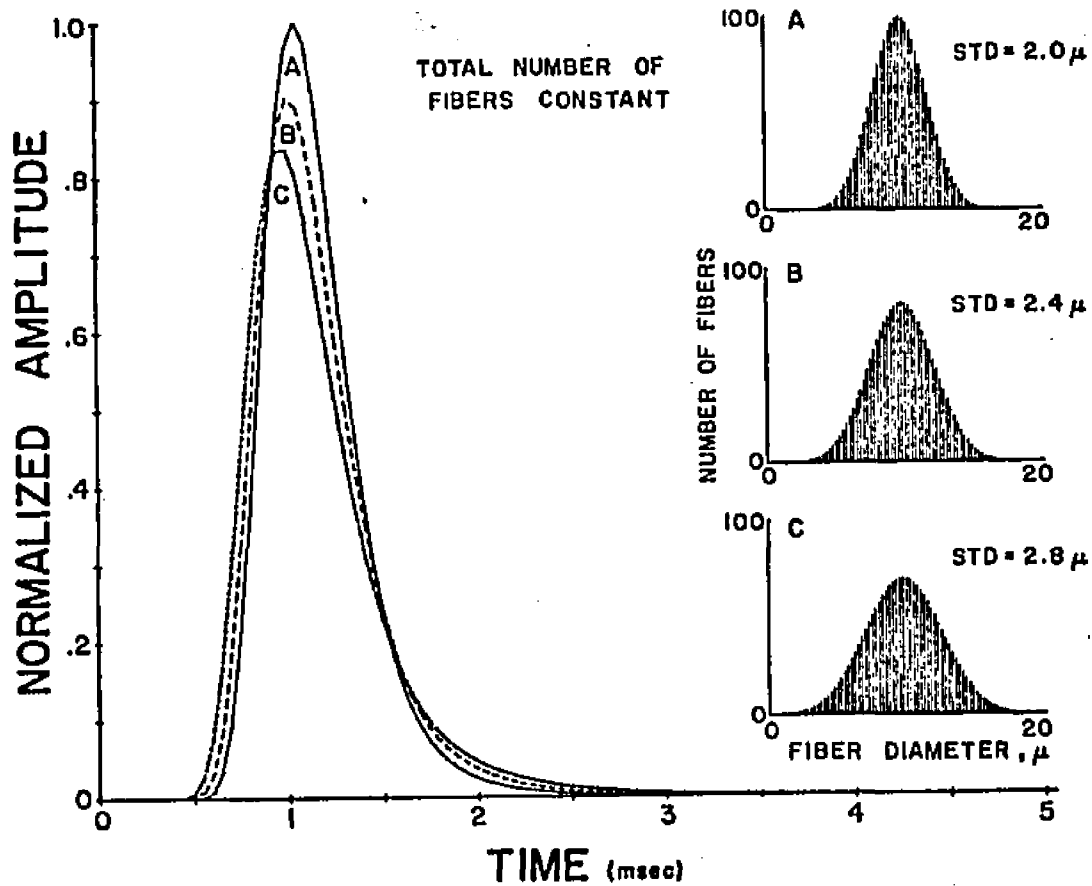


Figure 3-3. Reconstructed CAPs for three standard deviations (STD) for a gaussian fiber diameter histogram. A. CAP for the default parameters in table 3-1. B. CAP for STD increased to 2.4 microns (20%). C. CAP for STD increased to 2.8 microns (40%). Note that the total number of fibers is the same for all three fiber diameter histograms.

even though the mean fiber diameter (MFD) in the histogram is unchanged.

Inspection of figures 3-2 and 3-3 reveals that the CAP is more sensitive to changes in MFD than to proportionally similar changes in STD. The relative effect of these parameters on the deviation defined by equation 3-9 is illustrated in figure 3-4. Also the effects of changes in STD under the condition that the maximum number of fibers in the largest bin is held constant (with the total number of fibers allowed to vary) are shown. The actual values for the parameters are given on the separate axes below the graph. Also the points on the graph that correspond to the CAPs in figures 3-2 and 3-3 are labeled.

Inspection of figure 3-4 confirms that indeed MFD is a more influential CAP parameter than STD. Also note that increases in MFD cause considerably larger deviations than decreases in MFD. Similar asymmetry for STD, when the total number of fibers is constant, is present though inversely related to STD variation. For STD, with the maximum number of fibers constant, the deviation is nearly symmetrical.

BTOPHYSICAL PARAMETER ANALYSIS

The conduction distance (DIST) between the stimulating and recording electrodes is usually measured experimentally. The sensitivity of the CAP shape to er-

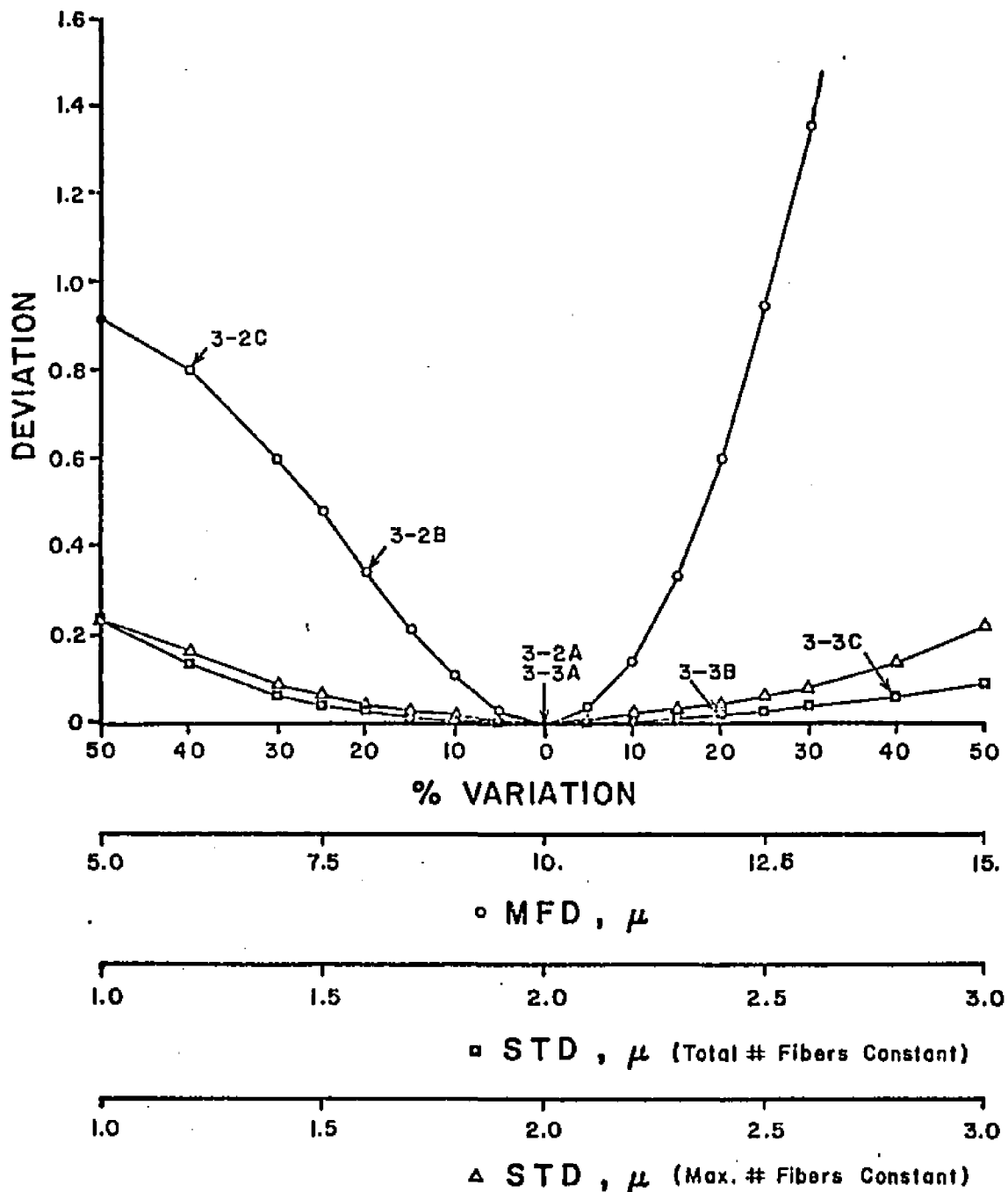


Figure 3-4. Deviation for equal percentage variations in the fiber diameter histogram parameters. Deviation is defined by equation 3-9. The actual parameter values are given on the separate axes below the graph. Deviations for changes in STD are found for two conditions: (1) Total number of fibers in the histogram constant, (2) Maximum number of fibers in the histogram constant. The points on the graph that correspond to the CAPs in figures 3-2 and 3-3 are labelled.

rors in this measurement and the dependence of the CAP waveform on the conduction distance are both of interest. Figure 3-5 demonstrates the effect of increased conduction distance on the CAP. As the distance increases by 20% and 40%, the dispersive effects cause the increased delay to the start of the CAP and the increased duration of the CAP..

In figure 3-6 the deviation is plotted for variations of conduction distance (DIST), the conduction velocity constant (CVC) and the conduction velocity exponent (CVE). These three parameters are considered together because they only affect the CAP by changing the t_1 of the component axon potential waves (equations 3-4 and 3-5). Although the CAP deviation is highly dependent on CVE, most authors agree that CVE is unity. Changes in DIST and CVC both affect the CAP deviation to a roughly similar extent. While both DIST and CVC are clearly important parameters it is interesting to observe that for proportionally equal increases in DIST and CVC, the CVC deviation is larger, while for decreases in DIST and CVC, the DIST deviation predominates over the CVC deviation. Reference to equations 3-4 and 3-5 reveals that DIST and CVC do have an inverse effect on the t_1 .

The effect on the CAP of myelin thickness, represented by $g(d_1)$, is given in figure 3-7. The default functional relationship given in figure 3-7A is

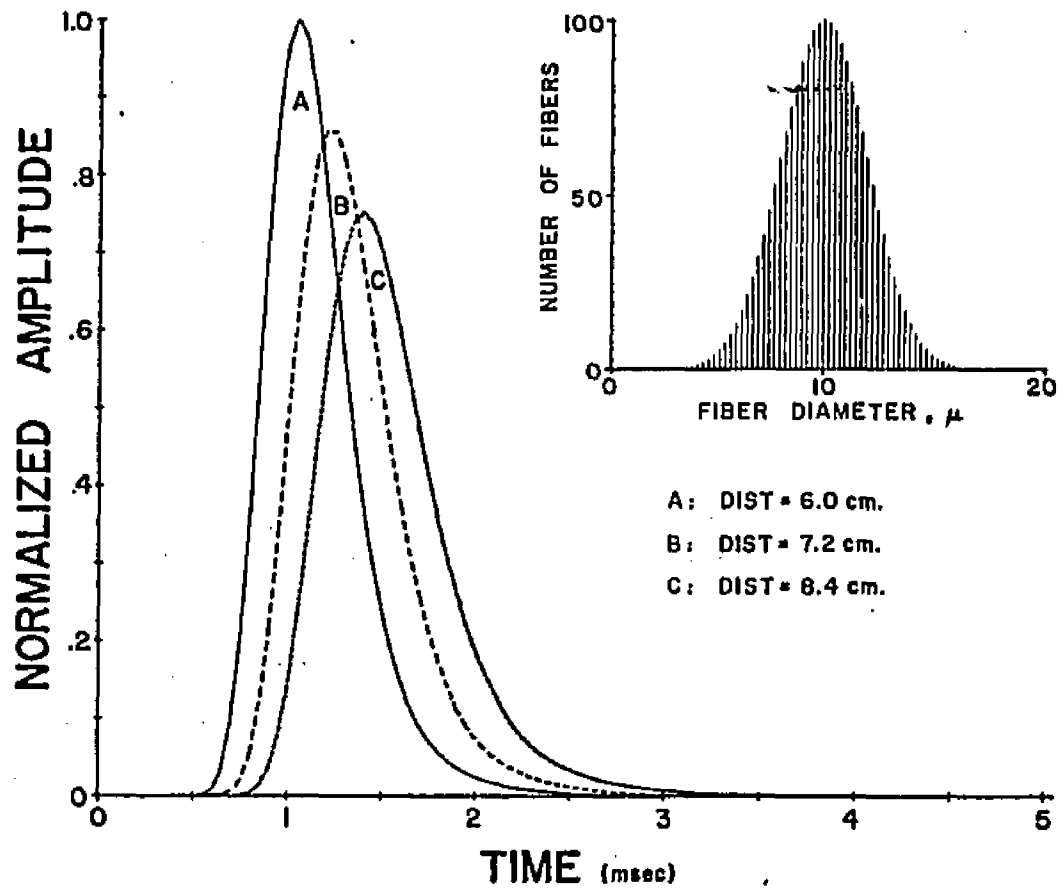


Figure 3-5. Reconstructed CAPs for three conduction distances (DIST). A. CAP for the default parameters in table 3-1. B. CAP for DIST increased to 7.2 cm. (20%). C. CAP for DIST increased to 8.4 cm. (40%).

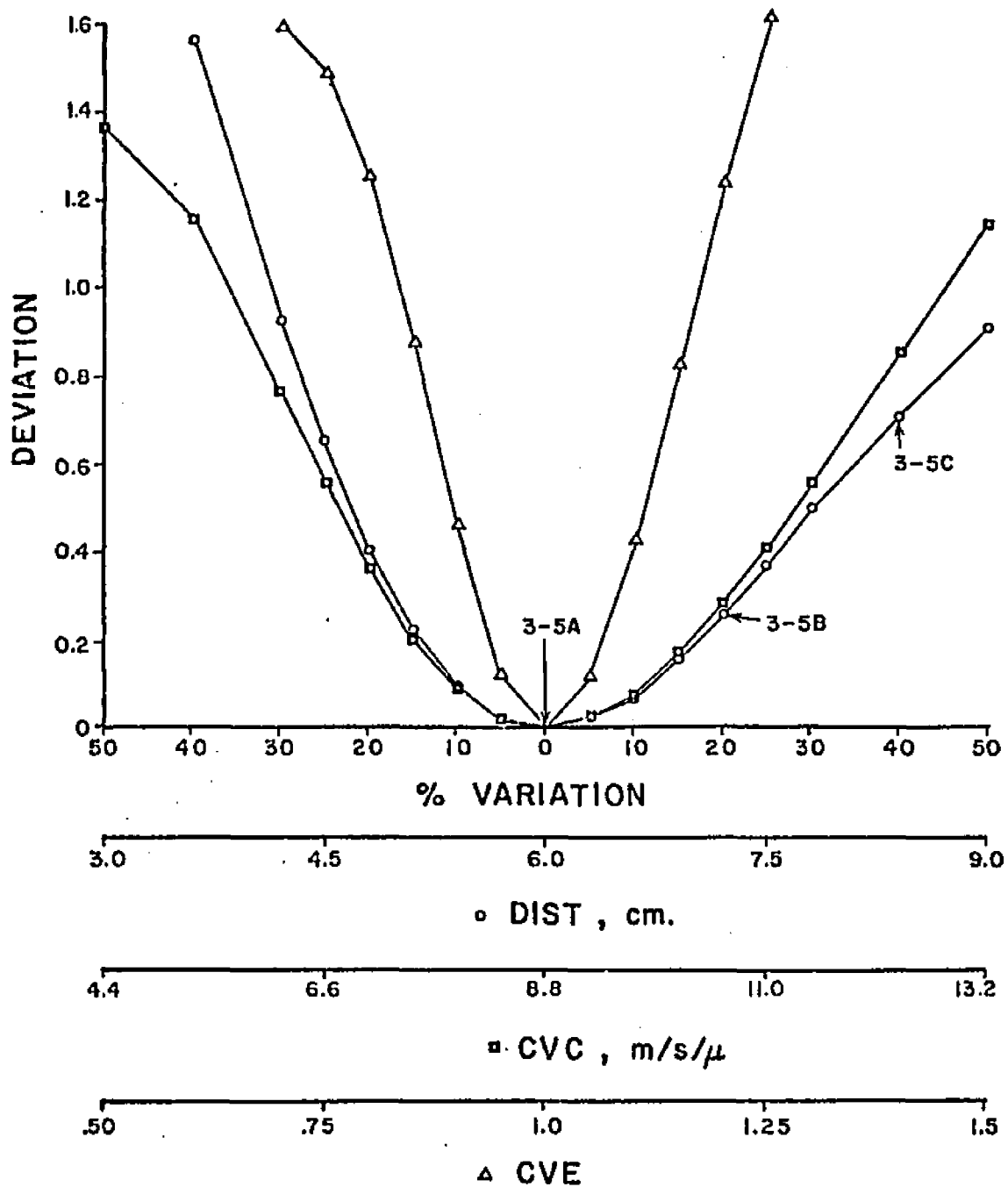


Figure 3-6. Deviation for equal percentage variations in the biophysical parameters. The actual parameter values for conduction distance (DIST), conduction velocity constant (CVC) and conduction velocity exponent (CVE) are given on the separate axes below the graph. The points on the graph that correspond to the CAPs in figure 3-5 are labelled.

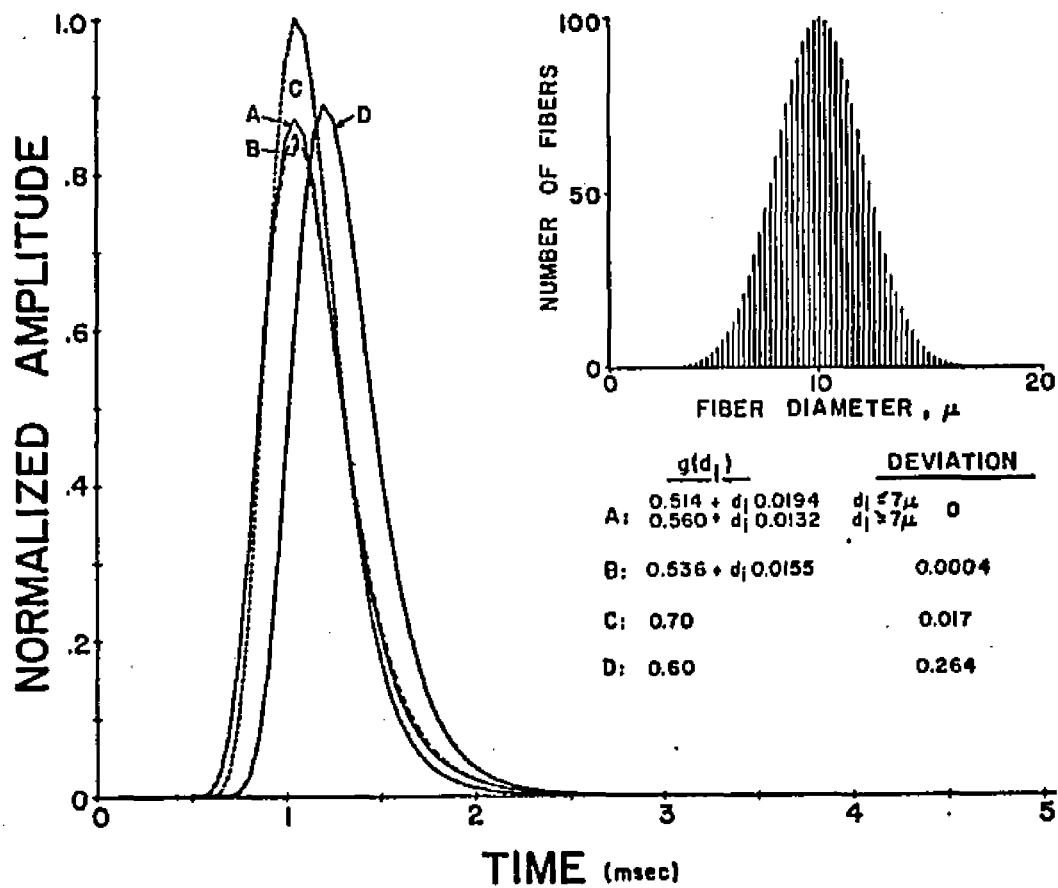


Figure 3-7. Reconstructed CAPs for several relative myelin thickness relationships ($g(d_1)$). A. CAP for the default parameters in table 3-1. B. CAP for a linear $g(d_1)$. C. and D. CAPs for two constant values for $g(d_1)$.

developed from a piece-wise linear regression of the data for the ratio of axon to fiber diameter versus fiber diameter given by Gasser and Grundfest (1939) in their figure 12. The CAP found by fitting the same data with a single regression line is given in figure 3-7B. The very small 0.0004 deviation and the close fit of the two CAPs in figure 3-7 indicate no significant differences. Recent measurements of myelin thickness with an electron microscope, particularly by Schnepf and Schnepf (1971), indicate that relative myelin thickness is nearly constant, except for random fluctuations, between 0.6 and 0.7. The CAP for a g of 0.7, labeled C in figure 3-7, deviates from A by only 0.017. The CAP denoted by D in the figure is for g equal to 0.6 (deviation of 0.264) and corresponds to the g that Rushton (1951) theoretically predicted for maximal conduction velocity.

AXON POTENTIAL WAVE PARAMETER ANALYSIS

The parameters that influence the $v_i(t)$ are studied for triangular axon potential waves. The base of the triangle is increased by 20% and 40% and the height of the triangular potential is reduced accordingly to maintain constant triangular area. The CAPs reconstructed for these conditions are shown in figure 3-8. Stretching out the axon potential wave results in only a small dispersive effect on the CAP.

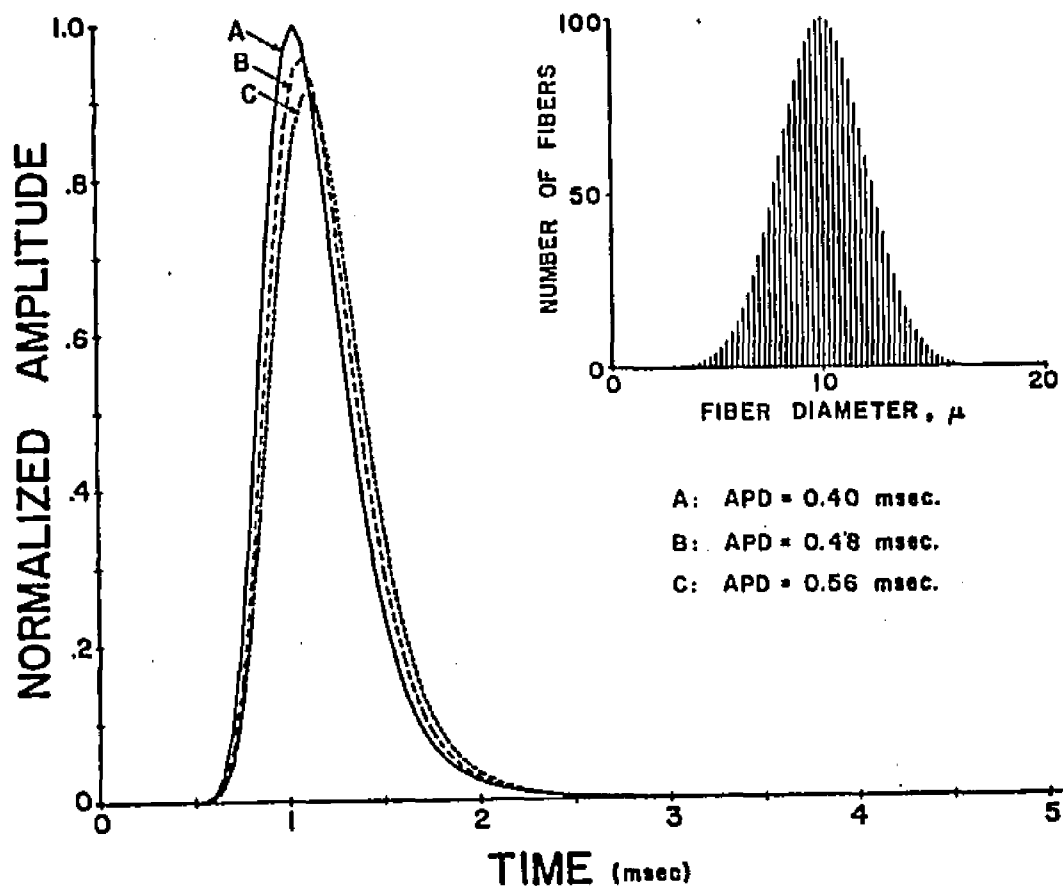


Figure 3-8. Reconstructed CAPs for three axon potential wave durations (APD). A. CAP for the default parameters in table 3-1. B. CAP for APD increased to 0.48 msec. (20%). C. CAP for APD increased to 0.56 msec. (40%).

Figure 3-9 shows the relative effects on the CAP of changes in the axon potential height exponent (APE), the axon potential duration (APD), and the axon potential rise time (APR). The APE parameter affects $v_1(t)$ according to equation 3-3 by changing the amplitude of the axon potential wave differentially so that some change in CAP shape does accompany the large change in the amplitude of the CAP. This large change in amplitude is primarily responsible for the large deviation for changes in APE.

As figures 3-8 and 3-9 show, the axon potential wave duration does not seriously affect the CAP. The points in figure 3-9 that correspond to the CAPs in figure 3-8 are labeled.

Finally, the axon potential rise time (APR) produces only a 0.004 deviation when increased or decreased by 50%. APR is the least influential CAP parameter studied.

The possible effect of the sharp angles in the triangular axon potential wave approximation is examined by assuming a smooth axon potential wave with approximately the same shape. The results shown in figure 3-10 indicate that such changes in the details of the axon potential wave have very little effect on the CAP. The deviation for the smooth axon potential is only

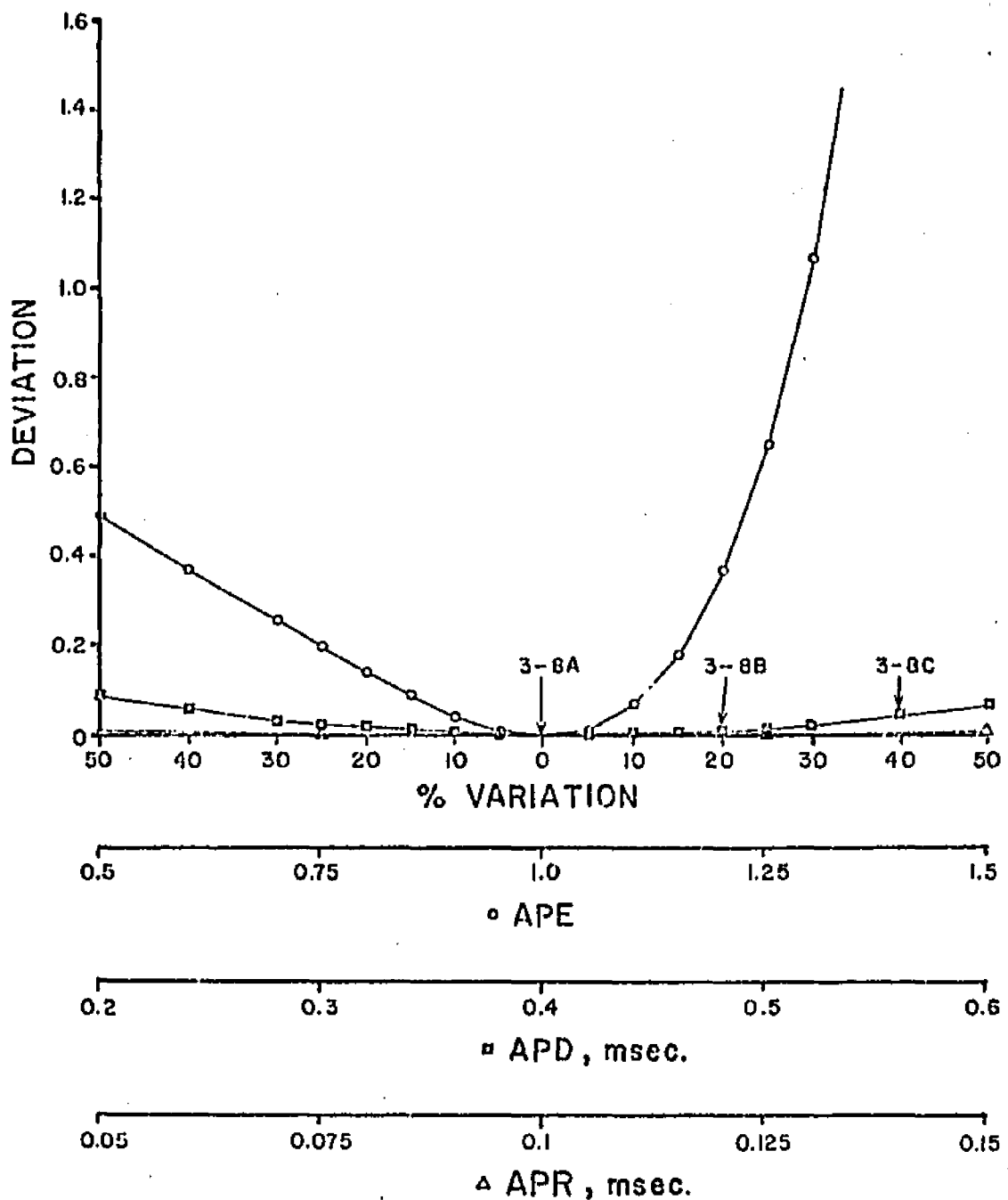


Figure 3-9. Deviation for equal percentage variations in the axon potential wave parameters. The actual values for the axon potential height exponent (APE), axon potential duration (APD) and axon potential rise time (APR) are given on the separate axes below the graph. The points on the graph that correspond to the CAPs in figure 3-8 are labelled.

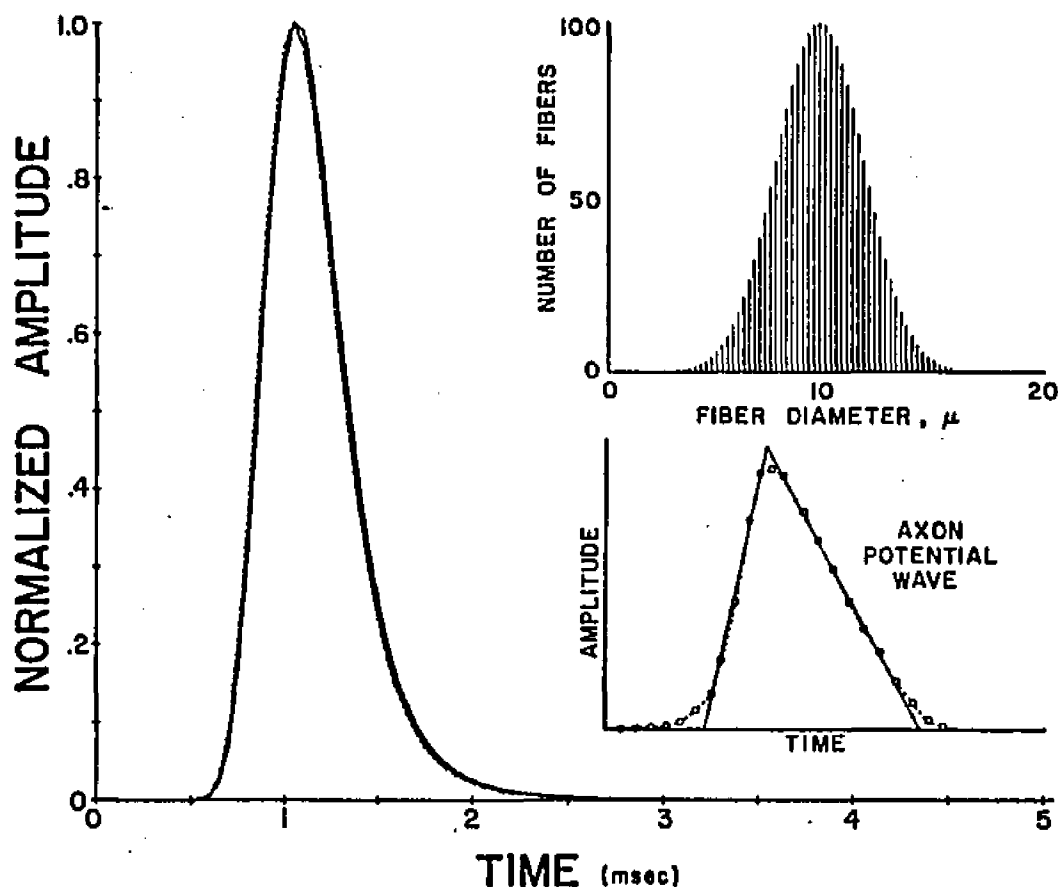


Figure 3-10. Reconstructed CAPs for triangular and smooth axon potential waves. The default reconstruction parameters in table 3-1 are used for the two CAPs shown. The CAP plotted with a solid line is reconstructed with the triangular axon potential wave. The CAP plotted with a dotted line is reconstructed with the smooth dotted line axon potential wave shown in the inset.

0.003. The triangular axon potential wave is used for all results in Chapter III; and the smooth axon potential wave is used for all results in Chapters V and VI.

PARAMETER VARIATIONS FOR NORMALIZED CAPS

Each CAP is scaled, in all the above CAP reconstructions, with respect to the default CAP; so that relative magnitudes are preserved. Although the default CAP is normalized, all the CAPs reconstructed for parameter variations are not normalized individually.

Since all the CAPs given later are self-normalized, the relative influence of the most important reconstruction parameters on the self-normalized CAPs is examined. The deviation is found again for variations in the five parameters that resulted in the largest deviations in the previous non-normalized studies (MFD, DIST, CVC, CVE and APE). The results for normalized CAPs are plotted in figure 3-11.

Decreasing the mean fiber diameter (MFD) of the gaussian diameter histogram produces a larger deviation in normalized CAPs (figure 3-11) than for scaled CAPs (figure 3-4). On the other hand, increases in MFD resulted in larger deviations for the scaled CAPs. Similar observations apply to the results for variations of the conduction velocity constant (CVC) and the conduction velocity exponent (CVE) except that differences

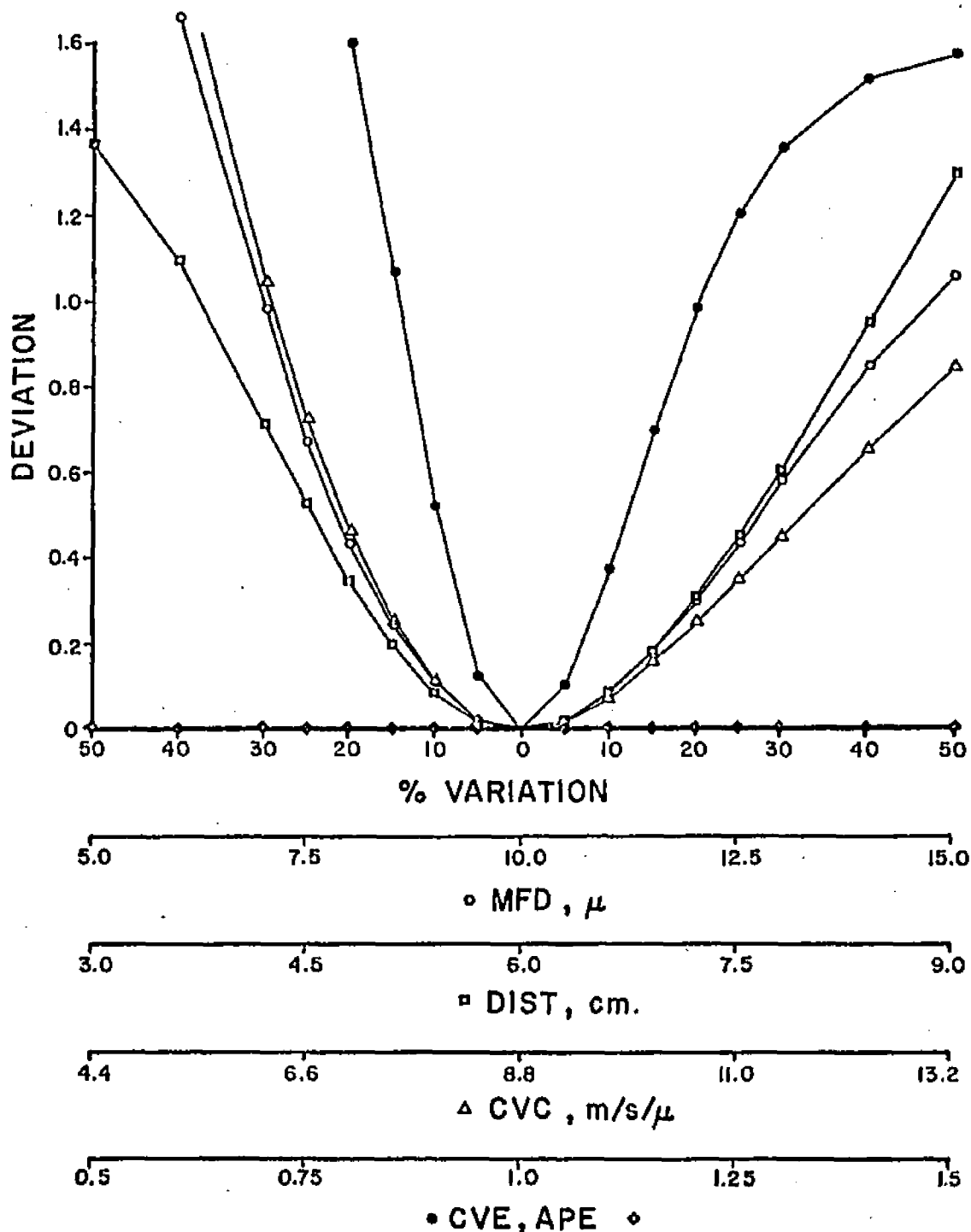


Figure 3-11. Deviation for equal percentage variations in reconstruction parameters for individually normalized CAPs. The deviations in figures 3-4, 3-6 and 3-9 are for CAPs that are scaled with respect to the default CAP. Note that deviations for the axon potential height exponent (APE) are very small.

in deviation for normalized versus scaled CAPs are considerably less than for MFD (compare figures 3-11, 3-6 and 3-4). For decreasing conduction distance (DIST) the deviation is larger for scaled CAPs than for normalized CAPs, while for increasing DIST the opposite difference is observed.

The most significant difference between the magnitude of the deviation for normalized versus scaled CAPs is observed for the axon potential wave exponent (APE) in equation 3-3:

$$v_1(t) = v(t) \cdot d_1^{\text{APE}} \quad (3-3).$$

The APE parameter affects only the amplitude of the $v_1(t)$ for each different diameter, d_1 . The exponential relationship makes the $v_1(t)$ very sensitive to changes in APE and note that because the d_1 are different, the amplitudes of the $v_1(t)$ are different unless APE is zero. The maximum deviation in the normalized CAPs for APE (figure 3-11) is 0.004 while for the scaled CAPs (figure 3-9) the deviations for APE is much larger. Apparently, at least for the gaussian diameter histogram, the relative differences in the $v_1(t)$ for changes in APE are overshadowed by the proportional increases in the $v_1(t)$ that are eliminated by normalization. Thus, for normalized CAPs, all of the axon potential wave parameter variations result in only very minor CAP deviations.

OPTIONAL MODEL FEATURES

Table 3-2 lists some optional model features that have been studied. The simulated conditions could affect correlations between experimentally recorded CAPs and anatomically derived fiber diameter histograms. The optional features given in table 3-2 are not in effect except when studied and combinations of the features are not studied simultaneously.

ALTERED FIBER DIAMETER HISTOGRAMS

The first category of optional features includes deletion of fibers from histograms and simulations of tissue shrinkage. Figure 3-12 shows the effect on the CAP of deleting all but the largest 10% and all but the smallest 10% of the fibers in the gaussian test histogram. The response for the largest fibers (figure 3-12B) is considerably larger than the response for the smallest fibers (figure 3-12C). This difference reflects the larger amplitude of the $v_1(t)$ for the large fibers that equation 3-3 requires. Figure 3-13 illustrates quantitatively, in terms of deviation, the relative effects of deleting an increasing percentage of all the largest or all the smallest fibers. It is clear that CAP reconstructions are considerably more dependent on large fibers than on small fibers. Studies with several published anatomical fiber diameter histograms that have relatively more small and large fibers show an even greater differ-

TABLE 3-2
OPTIONAL MODEL FEATURES

T.	<u>FIBER DIAMETER HISTOGRAM</u>	<u>SYMBOL</u>	<u>DEFAULT</u>
A.	Deletion of Selected Fibers	DEL	None
B.	Shrinkage	SHK	None
II. <u>NONHOMOGENEOUS NERVE</u>			
<u>BUNDLES</u>			
A.	Single Fiber Tapering	TPR	None
B.	Single Fiber Branching	BRAN	None
C.	Cross Section Diameter Distribution Bias	BIAS	None

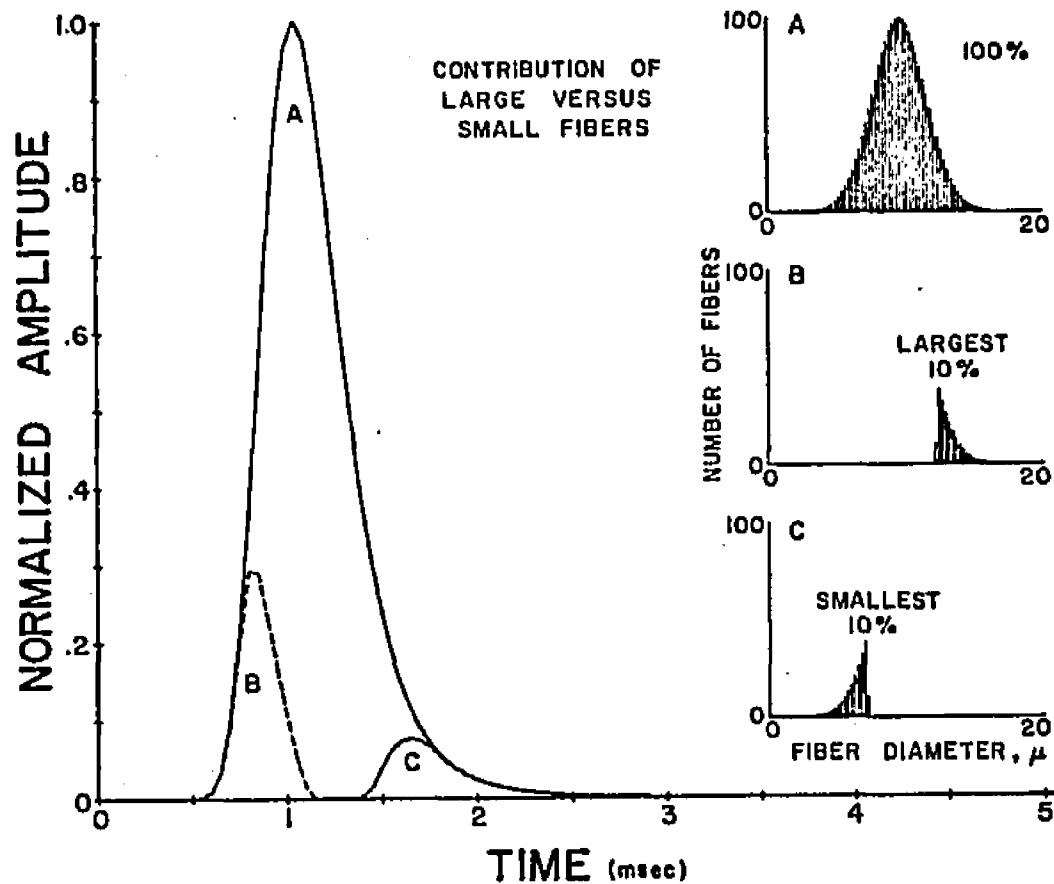
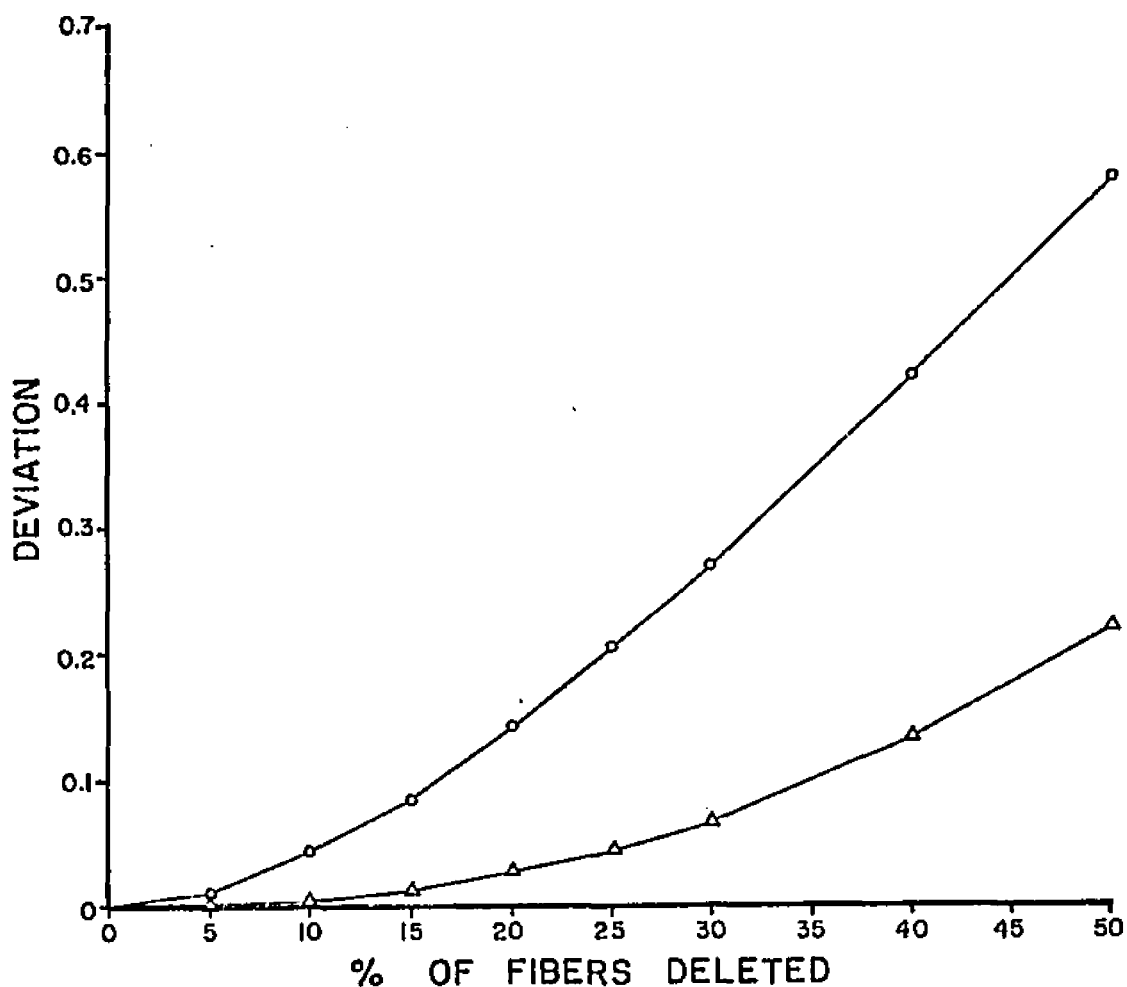


Figure 3-12. Reconstructed CAPs for large and small fibers. A. CAP for the default parameter values in table 3-1. B. CAP for the largest 10% of the fibers in A. C. CAP for the smallest 10% of the fibers in A.



◦ % OF LARGEST FIBERS DELETED

△ % OF SMALLEST FIBERS DELETED

Figure 3-13. Deviation for deletions of increasing percentages of large or small fibers. As the percentage of fibers deleted increases, all the fibers of a given diameter are deleted before progressively larger or smaller fibers are deleted. For example, 90% of the smallest fibers are deleted in the diameter histogram in figure 4-12B.

ence between the relative contribution of fibers on each side of the histogram.

Estimates of tissue shrinkage during histological procedures varied depending on the procedures used, the type of tissue studied and the method of measuring fibers. Hursh (1939) estimated overall fiber shrinkage to be 7.9%. Both Sanders (1947) and Schnepf, Schnepf and Spann (1971) estimated shrinkage at 6 to 8%. There was no evidence that shrinkage was differential. The histograms in this thesis were not corrected for shrinkage.

The effect on the CAP of 10% and 20% uniform shrinkage of all fibers is shown in figure 3-14. Although the mean fiber diameter obviously decreases by the percentage indicated, close inspection of the histograms shows that the bin width decreases also. Actually, shrinkage is similar to decreasing the mean and the bin width of the gaussian fiber diameter histogram.

Shrinkage can exert a strong influence on the CAP, as shown in figure 3-15. Comparing shrinkage in figure 3-15 with variation of histogram mean in figure 3-4 shows that shrinkage causes more deviation. For example, at 20% variation, the shrinkage deviation is 0.48 while histogram mean deviation is 0.35. This difference is not particularly apparent when the corresponding CAPs in figure 3-14C and figure 3-2B are compared.

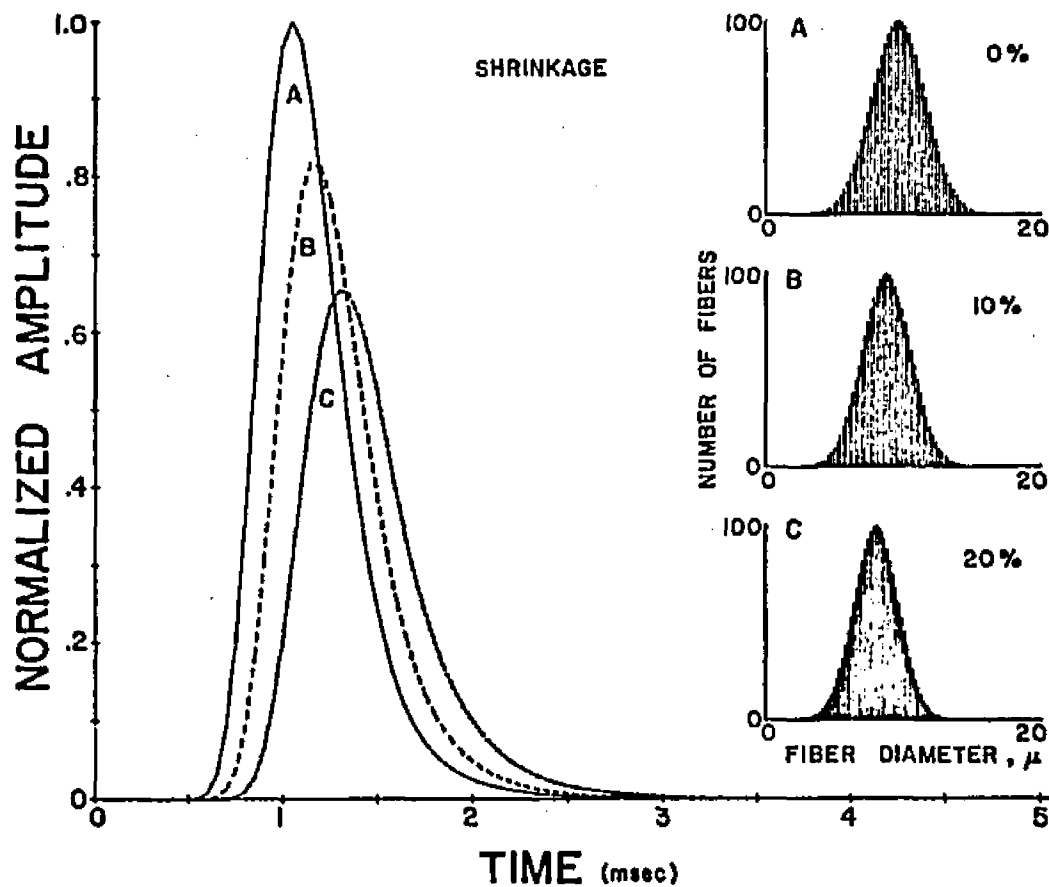


Figure 3-14. Reconstructed CAPs for fiber diameter histograms that are altered to simulate the effect of histological shrinkage. A. CAP for the default parameters in table 3-1. B. CAP for 10% uniform shrinkage. C. CAP for 20% uniform shrinkage.

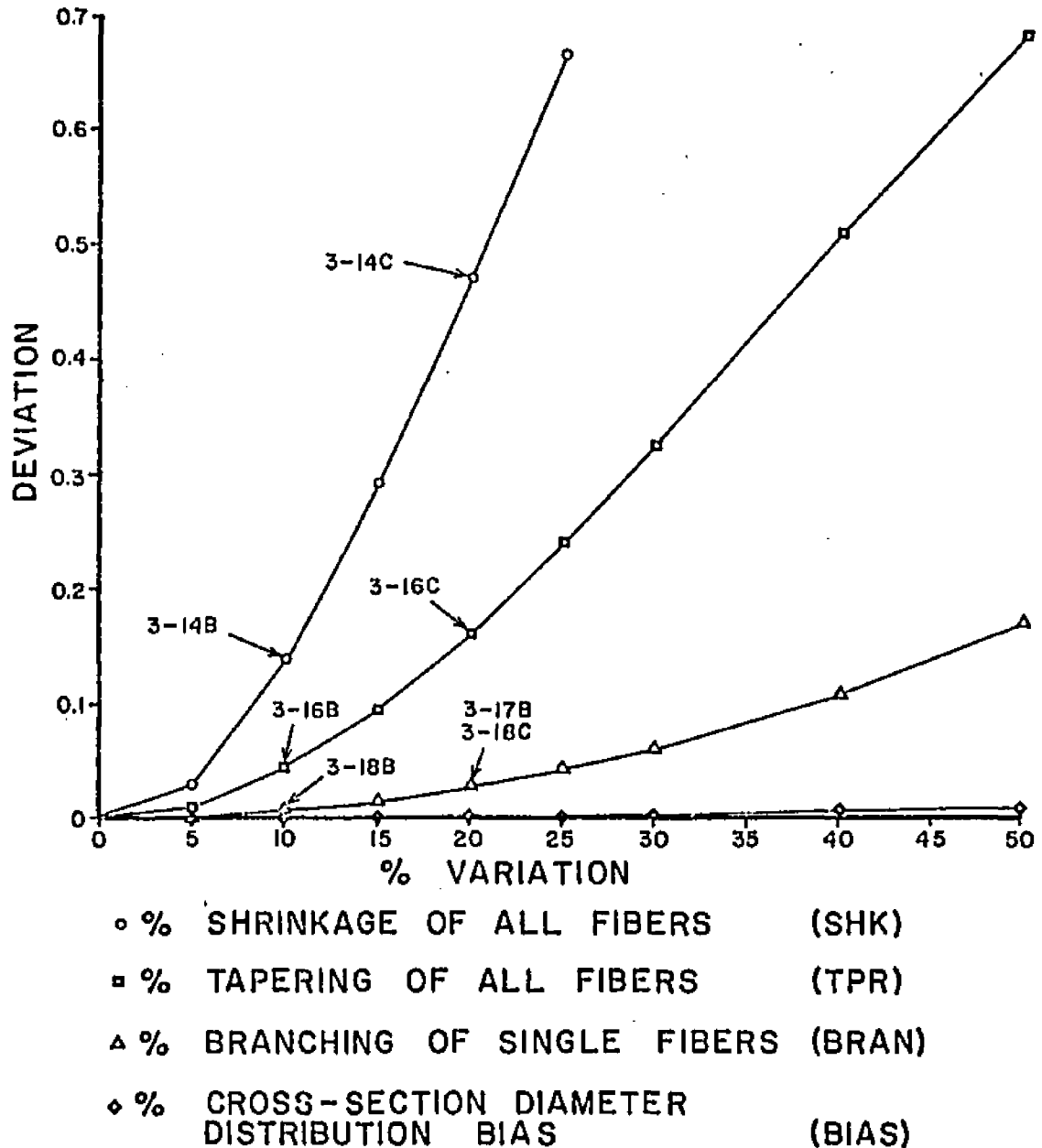


Figure 3-15. Deviation for equal percentage variations for simulated shrinkage and nonhomogeneous nerve bundle simulations. Tissue shrinkage (SHK), tapering of all fibers (TPR), branching of single fibers (BRAN) and cross-section diameter distribution bias (BIAS) are simulated. The points on the graph that correspond to the CAPs plotted in figures 3-14, 3-16, 3-17 and 3-18 are labelled.

NONHOMOGENEOUS NERVE BUNDLE SIMULATIONS

Three types of variation in the structure of individual nerve fibers and their relative spatial distribution have been simulated. The effect of all fibers tapering between the stimulation and recording sites and the effect of single fibers branching into two fibers, each with one-half the diameter of the parent fiber, have been examined. Also, the effect of nonuniform distributions of the various fiber diameters within the nerve bundle cross-section has been simulated.

Although there is little anatomical evidence to support significant systematic tapering of fibers along nerve trunks, Gasser and Erlanger (1927), Duncan (1934), Pumphrey and Young (1938), Hursh (1939) and Sunderland and Roche (1958) found size variations along single fibers without branching. While shifts in fiber diameter histograms along the course of nerves have been reported by Gasser and Erlanger (1927), Eccles and Sherrington (1930), Donaldson (1934) and Rexed (1944), it is not possible to separate the effects of fiber tapering from fiber branching. Although extensive tapering and branching does occur close to the sensory or motor end organ, it appears that relatively little tapering and branching take place in major nerve trunks.

The effect on the CAP of 10% and 20% tapering of all

the fibers is indicated in figure 3-16. The diameter histogram labeled A is assumed at the site of stimulation for all three CAPs. All the fibers then taper progressively to smaller diameters at the recording site, as indicated by the two other histograms. The three labeled histograms in figure 3-16 are the same as the three correspondingly labeled histograms shown in figure 3-14 for fiber shrinkage. The difference then is that for shrinkage each histogram represents the structure throughout the entire length of the bundle under study, whereas for tapering histogram A always represents the structure at the stimulation site and the histogram along the bundle shifts continuously toward smaller diameters, ending at the recording site in the histogram shown for each case. The CAP deviation for tapering is less than the corresponding deviation for shrinkage because although the $v_1(t)$ are identical for corresponding simulations, the t_1 for tapering increase less than for shrinkage. Thus, the CAP is affected less by tapering fibers than by shrinkage as shown in figure 3-15.

Several factors influence the CAP when some of the fibers in a nerve bundle branch into two fibers. Naturally the size of the two branches will affect both $v_1(t)$ and t_1 for each branch. Only branches that are both one-half the diameter of the parent fiber are simulated here. Also, the position of the branch point between the stimu-

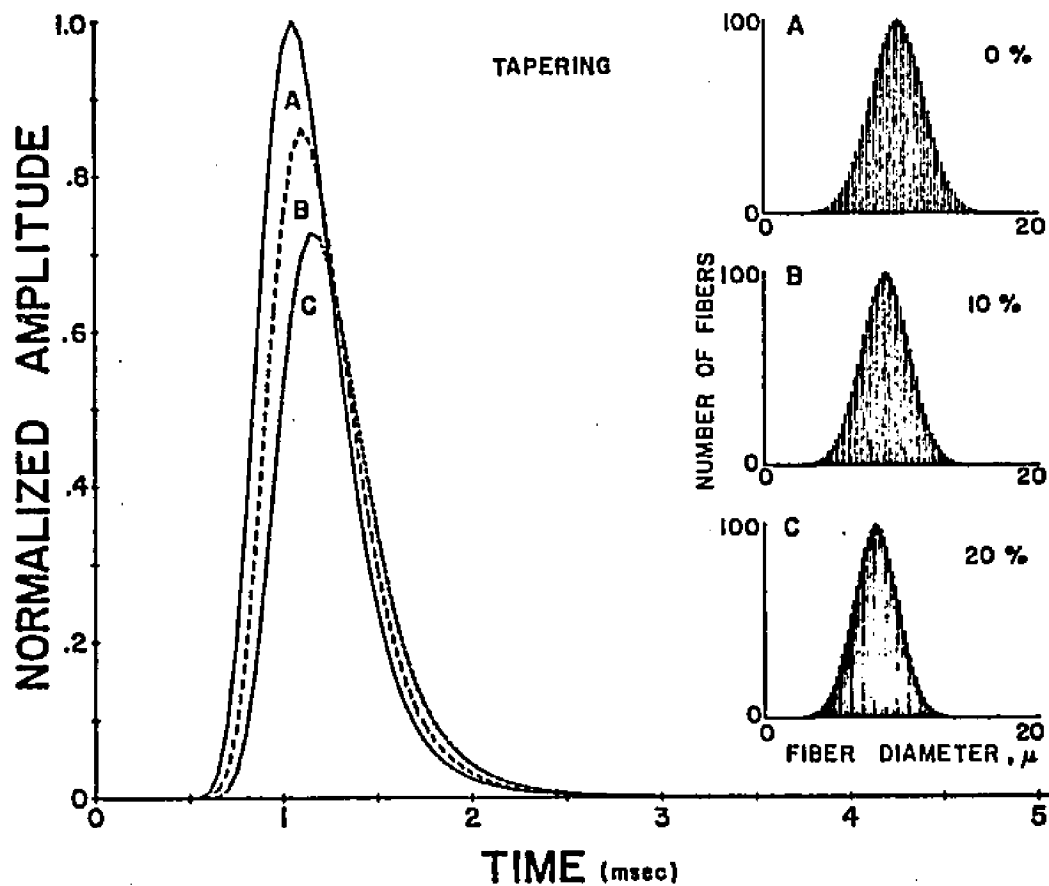


Figure 3-16. Reconstructed CAPs for simulations of tapered nerve fibers. A. CAP for default parameters in table 3-1. B. CAP for 10% tapering of all fiber diameters between the stimulation and recording sites. C. CAP for 20% tapering.

lation and recording sites will affect the t_1 . The branch point position is variable in the simulations and more than one position can be specified. Finally, the number of fibers that branch and the diameters of the fibers that branch both affect the CAP. Although any percentage of fibers can branch, only the case where all parent fiber diameters have equal probabilities of branching is considered. Fibers with diameters less than two microns are not permitted to branch because myelinated fiber diameters less than one micron are not generally found in peripheral nerves. This is not a serious constraint because most histograms for peripheral nerves have relatively few myelinated fibers less than 2 microns in diameter. Also, various compound effects such as repeated branching are not considered.

A simulation for 20% of the fibers branching at half the distance between the stimulation and recording sites is shown in figure 3-17B. Figure 3-17A shows the standard CAP and figure 3-17C simulates a nerve bundle with histogram C effective at all points along the bundle. The CAP labeled C also represents a nerve bundle with 20% branching at the stimulation site. Note the secondary wave in the CAP labeled C. The discrete peak on the left in histogram C is responsible for the secondary wave. Although curve B does not exhibit a secondary wave,

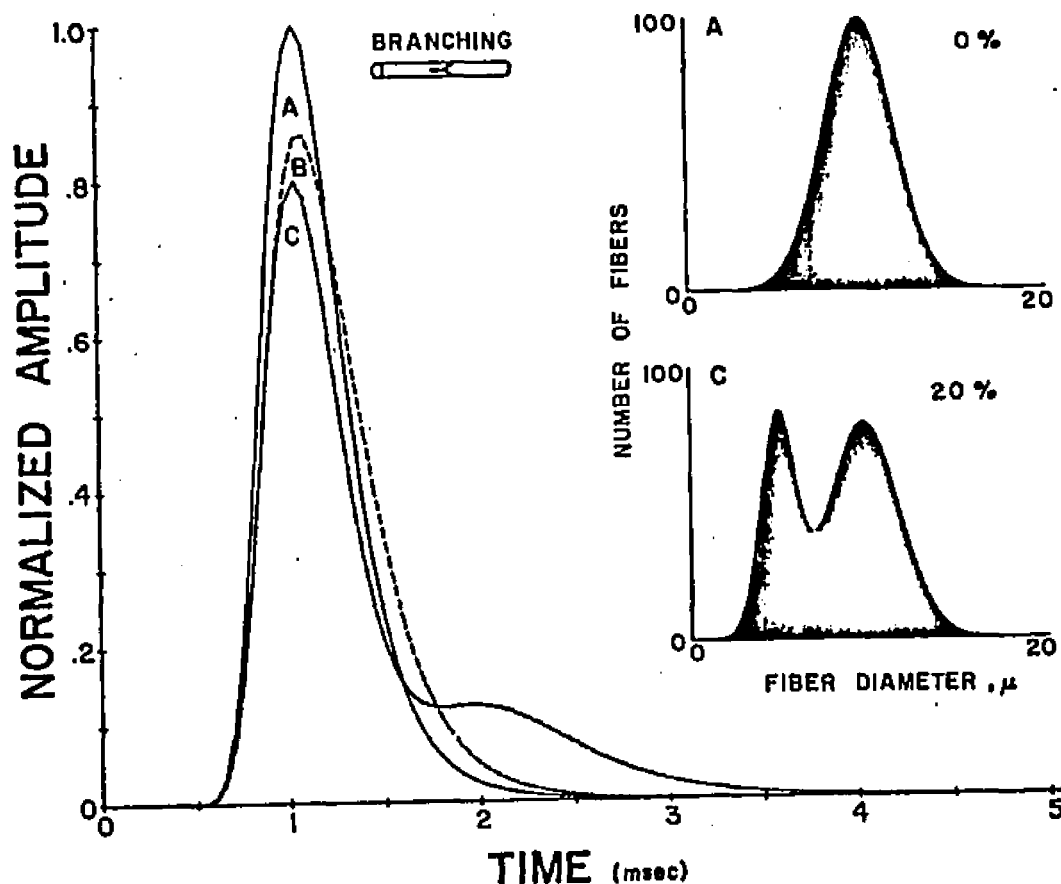


Figure 3-17. Reconstructed CAPs for simulated fiber branching. A. CAP for default parameters in table 3-1. B. CAP for 20% of the fibers branching at half the distance between the stimulation and recording sites. C. CAP for default parameters except the diameter histogram labelled C is assumed throughout the conduction distance.

as the percentage of branching is increased or the branching point is moved toward the stimulating electrode, the secondary wave would appear. Other simulations show that branching in the middle of the conduction distance is equivalent to spreading the branch points uniformly throughout the distance between the stimulating and recording sites.

The effect on the CAP of different percentages of branching at half of the conduction distance is shown in figures 3-18 and 3-15. Note that in terms of deviation, the type of branching considered here has less influence on the CAP than tapering or shrinkage.

Finally, it is possible that nonuniform spatial distributions of fiber diameters within the nerve bundle cross-section could affect the CAP recorded by an electrode on the perimeter of the bundle. For example, if all the large fibers are peripherally located within the cross-section and all the small fibers are in the center, the large fibers might influence the CAP even more than normally. Although such an extreme bias in the distribution of fiber sizes within cross-sections is not seen by anatomists, perhaps smaller biases such as Van Crevel and Verhaart (1963) and Donovan (1967) found for the cat optic nerve and Birren and Wall (1956) found for rat sciatic nerve could be important. If the CAP is very sensitive to such a bias then even small deviations from

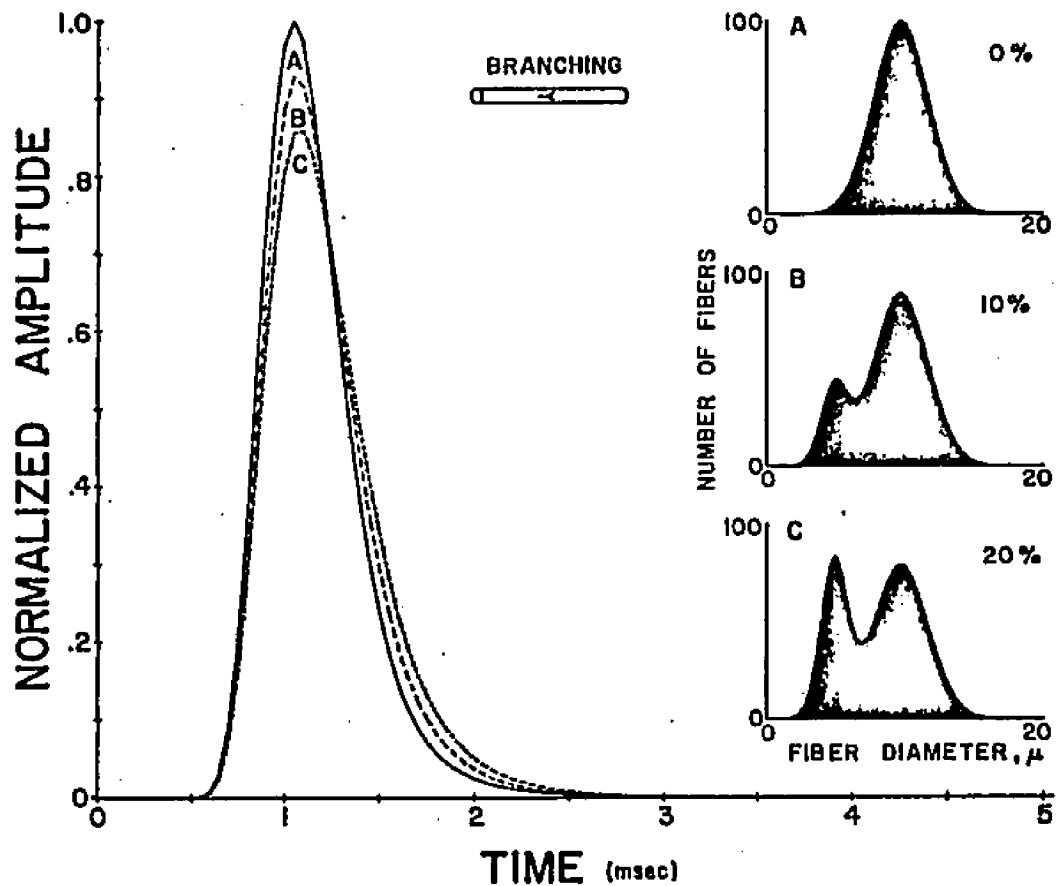


Figure 3-18. Reconstructed CAPs for simulations of branched nerve fibers. A. CAP for default parameters in table 3-1. B. CAP for 10% of all fibers branching at half the distance between the stimulation and recording sites. C. CAP for 20% of all fibers branching.

random distributions could be significant. Such a bias has been simulated by changing the diameter histogram to reflect the degree of nonuniformity in the spatial distribution of the fibers. A positive bias means that large fibers are weighted more heavily than small fibers with no change for medium size fibers. Conversely, a negative bias means that small fibers are weighted more heavily than large fibers.

Figure 3-19 demonstrates the effect of 100% positive and 100% negative bias. Essentially the gaussian diameter histograms are skewed slightly to the right in A and slightly to the left in C. The small changes in figure 3-19 for complete biasing are peculiar to this gaussian diameter histogram that has relatively few large and small fibers. Smaller percentages of positive bias for the gaussian diameter histogram gave very little deviation for the CAP as shown in figure 3-15. Positive 100% and negative 100% bias applied to a uniform diameter histogram (one containing equal numbers of all diameter fibers), shown in figure 3-20, have quite an effect on the CAP. Thus the shape of the diameter histogram is particularly important when the effects of fiber bias are considered.

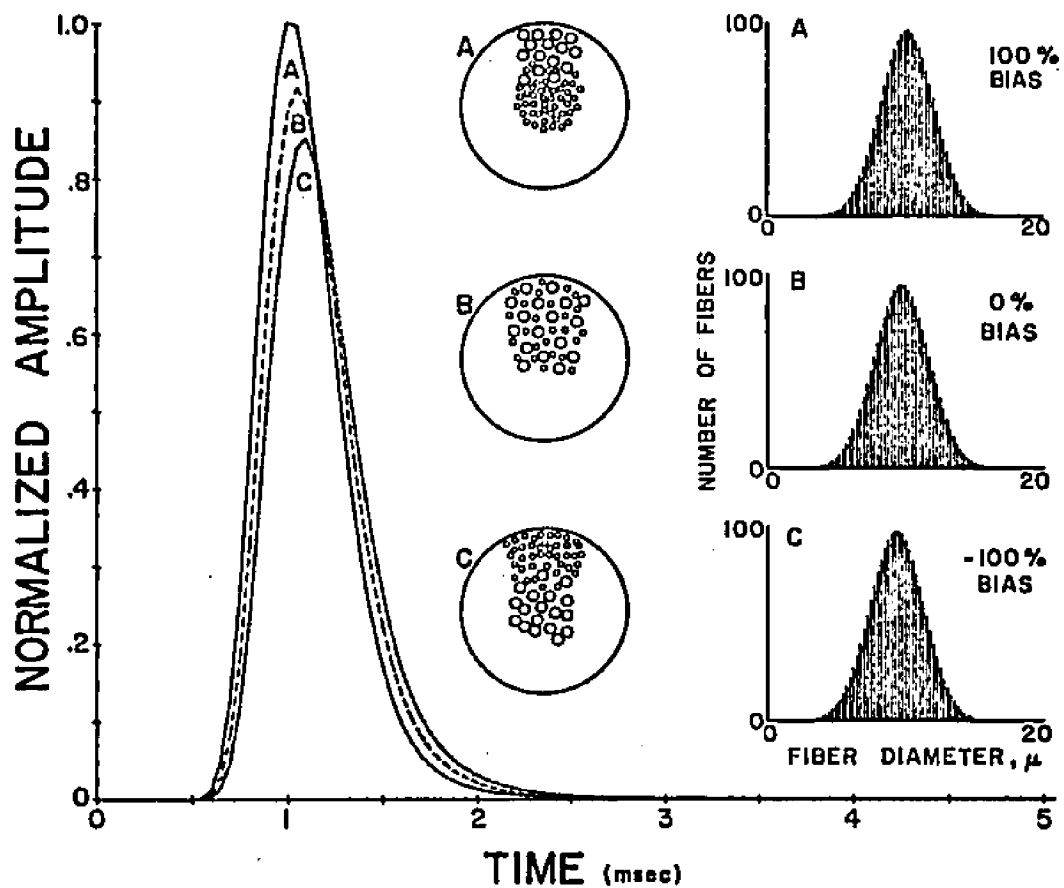


Figure 3-19. Reconstructed CAPs for non-random distributions of fiber diameters for a gaussian diameter histogram. A. CAP for 100% positive bias (large fibers weighted more). B. CAP for the default parameter values in table 3-1. C. CAP for 100% negative bias (small fibers weighted more).

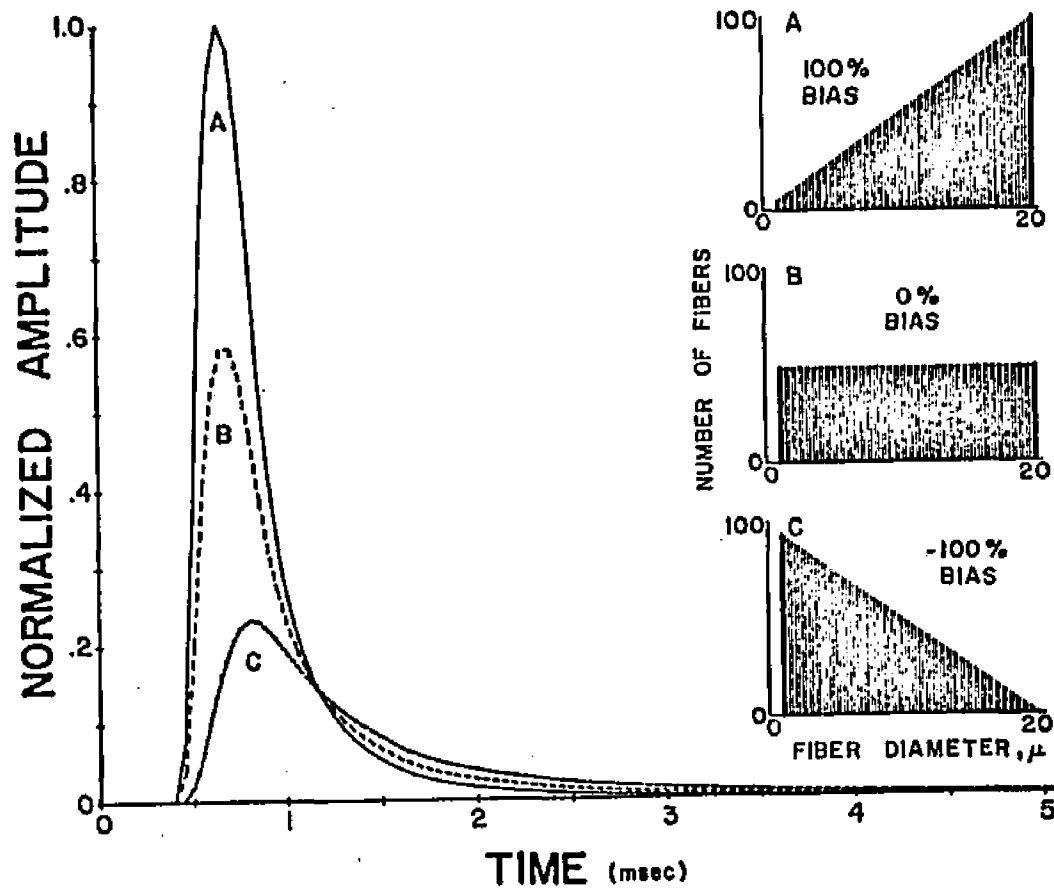


Figure 3-20. Reconstructed CAPs for non-random distributions of fiber diameters for a uniform diameter histogram. A. CAP for 100% positive bias (large fibers weighted more). B. CAP for the default parameter values in table 3-1. C. CAP for 100% negative bias (small fibers weighted more).

CHAPTER IV

ANATOMICAL FIBER DIAMETER HISTOGRAMS

Fiber diameter histograms for cat saphenous nerve are required both for CAP reconstructions and for evaluating histograms predicted from recorded CAPs. The methods used to fix and embed the tissue, cut transverse sections and prepare photomicrographs are described in Chapter II. Digital measurements of the coordinates of points on the perimeter of each fiber are utilized to compile fiber diameter histograms based on several estimates of fiber diameter.

PHOTOMICROGRAPHS AND DIGITAL MEASUREMENTS

A montage of low power photomicrographs taken from an entire cat saphenous nerve transverse section is shown in figure 4-1. Note the 100 micron bar in the lower left corner. Approximately 2500 myelinated nerve fibers are visible in the six fascicles; the smallest fascicle has 165 fibers and the largest has 574 fibers.

The epineurium surrounding the fascicles is areolar connective tissue that contains large globules of adipose tissue stained black. During the recording session, silver electrodes are wrapped completely around the outside of the epineurium.

Although most of the myelinated fibers appear to be cut transversely, there are some oblong fiber shapes in several of the fascicles. These quasi-elliptical

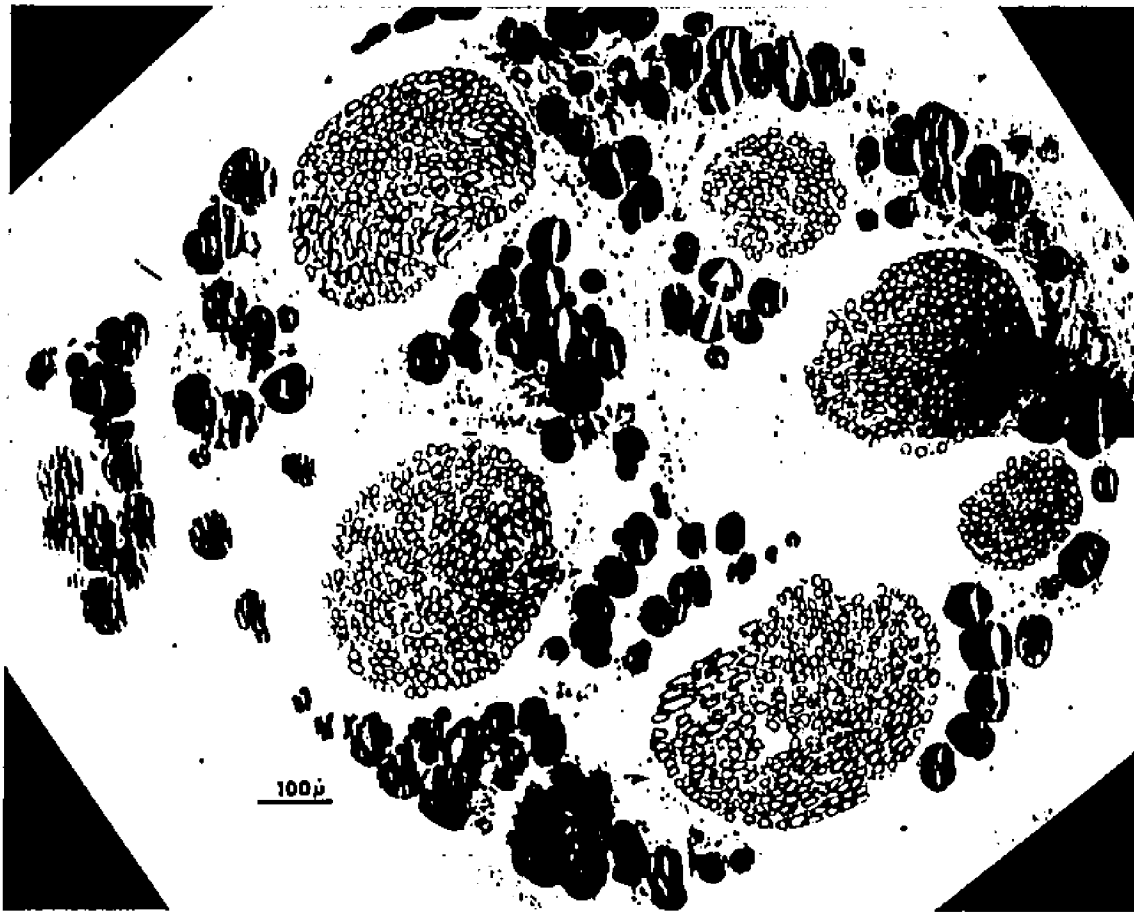


Figure 4-1. Low power photomicrograph of a 1 micron thick transverse section of cat saphenous nerve. The white arrow points to the fascicle shown at higher magnification in figure 4-2A. Cat N.

fibers appear to be cut obliquely. The sections photographed in this study are chosen for minimal obliqueness. Also note that obliquely cut fibers tend to have neighboring fibers with similar quasi-elliptical shapes. Apparently the relative positions of fibers within a fascicle change considerably over relatively short distances. Observe that a "flow" of fiber orientations can be seen within the fascicles and the direction of this flow changes considerably within the large fascicles.

The small fascicle indicated by the white arrow in figure 4-1 is shown at higher magnification in figure 4-2A. The fibers are identified arbitrarily by sequential numbers in groups of 100.

Few fibers have circular shapes. Several fibers have convoluted shapes such as number 22 (black arrow) near the upper right hand corner of the photograph. These fibers are probably cut close to a node of Ranvier. Several studies indicate that the myelin becomes fluted close to nodes of Ranvier (Webster and Spiro, 1960; Williams and Landon, 1963; Spencer and Lieberman; 1971). Williams and Landon (1963) found paranodal fibers to have typically "four ridges with intervening grooves" and "three or five ridges (are) seen occasionally." In the tissue prepared for this study convoluted fibers with five ridges are most common. Fibers cut at the level of the Schwann cell nucleus or Schmidt-Lanterman

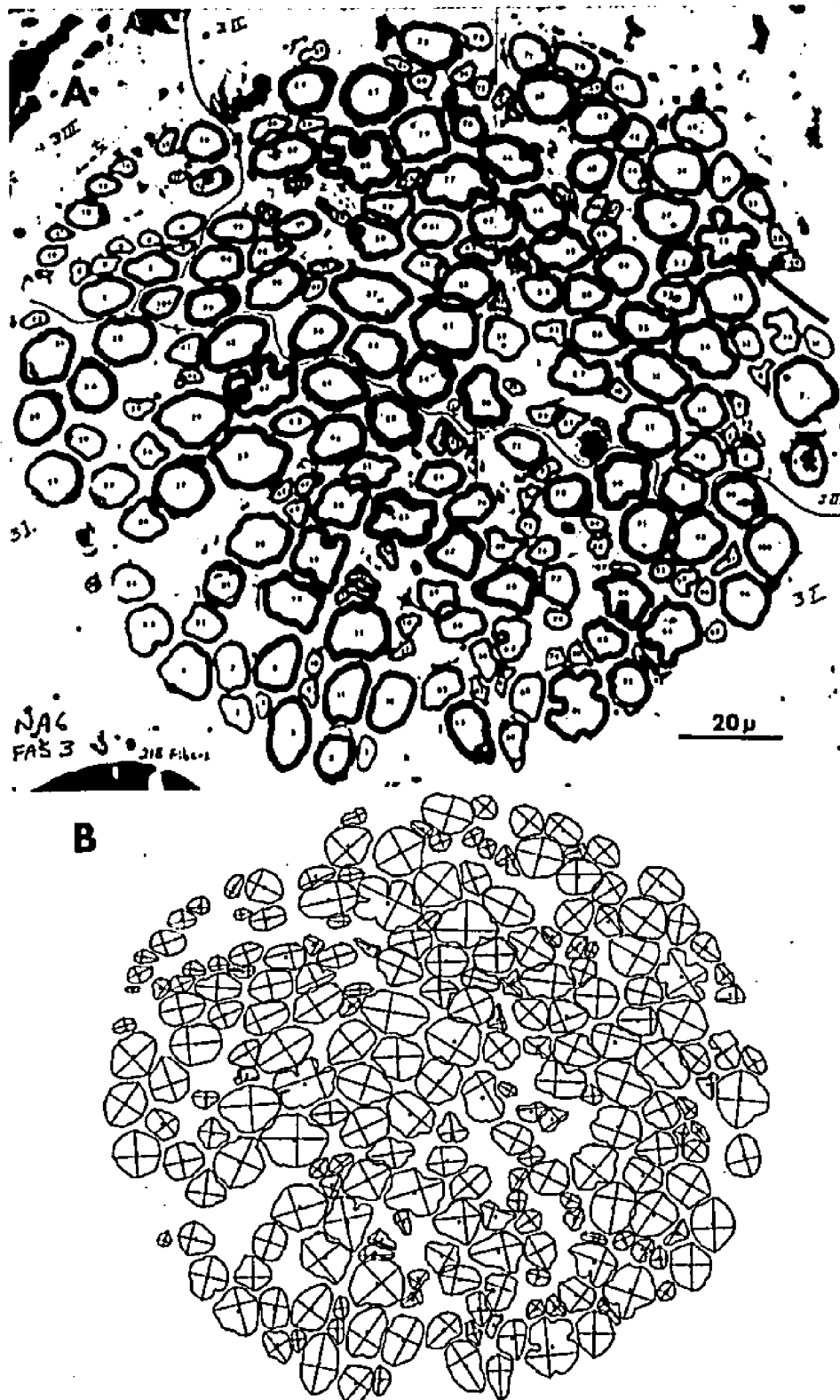


Figure 4-2. A. Photomicrograph used to make perimeter measurements. Reduced by 2.4. B. CalComp plot of perimeter data from A. Reduced by 3.0.

clefts typically have noncircular cross-sections (Sunderland and Roche, 1958; Babel, Bischoff and Spoendlin, 1970). The shape and appearance of the fibers in figure 4-2A are typical of fibers in many photomicrographs published in the literature.

Figure 4-2B is a photograph of a CalComp computer generated plot of straight lines between the coordinates of points digitized on the perimeter of each fiber. The crossed lines and symbols are described shortly. Unfortunately, the photographic magnification was less for figure 4-2B than for figure 4-2A; the actual CalComp plot does superimpose on the photomicrograph used to digitize the perimeter point coordinates.

Figures 4-3A and 4-3B show respectively a portion of a fascicular photomicrograph and the corresponding CalComp plot at the same magnification. The longest of the two mutually perpendicular lines inside each fiber in figure 4-3B is drawn between the two points on the perimeter that are the farthest apart (major chord). This major chord would be the major axis of an ellipse. The longest chord perpendicular to this major chord is computed and plotted. This minor chord would be the minor axis for an elliptical perimeter. The small triangle inside each fiber gives the location of the centroid of the fiber.

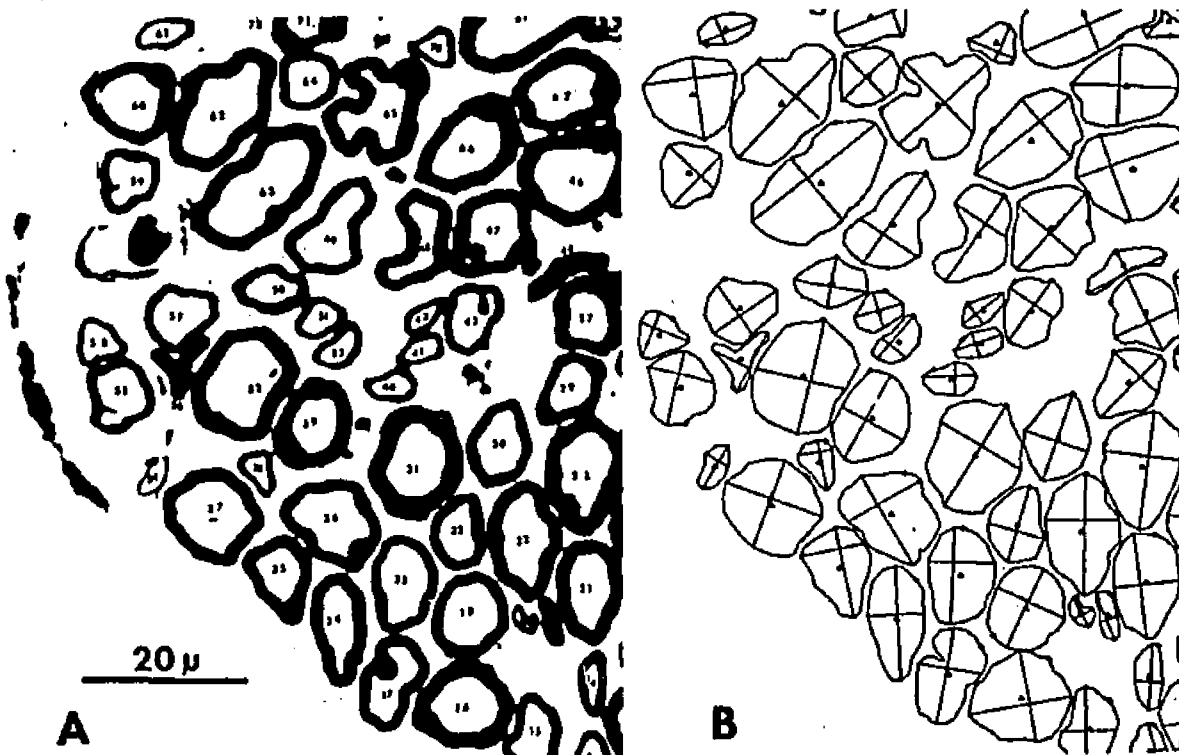


Figure 4-3. A. Photomicrograph for part of a fascicle at higher magnification. B. CalComp plot of perimeter data from A at the same magnification. The longest chord plotted inside each fiber is the major chord. The chord plotted perpendicular to the major chord is the minor chord. The triangle plotted inside each fiber gives the location of the centroid of the fiber. The small "x" plotted on each fiber perimeter designates the arbitrary starting point for the digitization. Both reduced by 1.7.

PRIMARY ESTIMATES OF FIBER DIAMETER

The word "diameter" is defined in Webster's Dictionary as "the length of a straight line through the center of an object." The term "center" is defined as "the point equidistant or at the average distance from the exterior points of a circle, sphere or other geometrical figure." Apparently the term diameter can be applied to noncircular objects; however, these definitions do not indicate how the diameter of an irregular closed figure should be measured. The problem of how to determine fiber diameter is further complicated by the existence of quasi-cylindrical fibers that are cut obliquely to give quasi-elliptical shapes.

Estimates of the fiber diameter are obtained from digital computer analysis of the series of point coordinates on the perimeter of each fiber. Four primary estimates of fiber diameter are presented in this section. Secondary estimates are considered in the next section.

Four fiber diameter histograms based on different estimates of fiber diameter for cat N are plotted in figure 4-4. Data from all the 2496 fibers shown in figure 4-1 are included. The ordinate variable is the per cent of the total number of fibers and the abscissa is the fiber diameter, given in microns. The bin width,

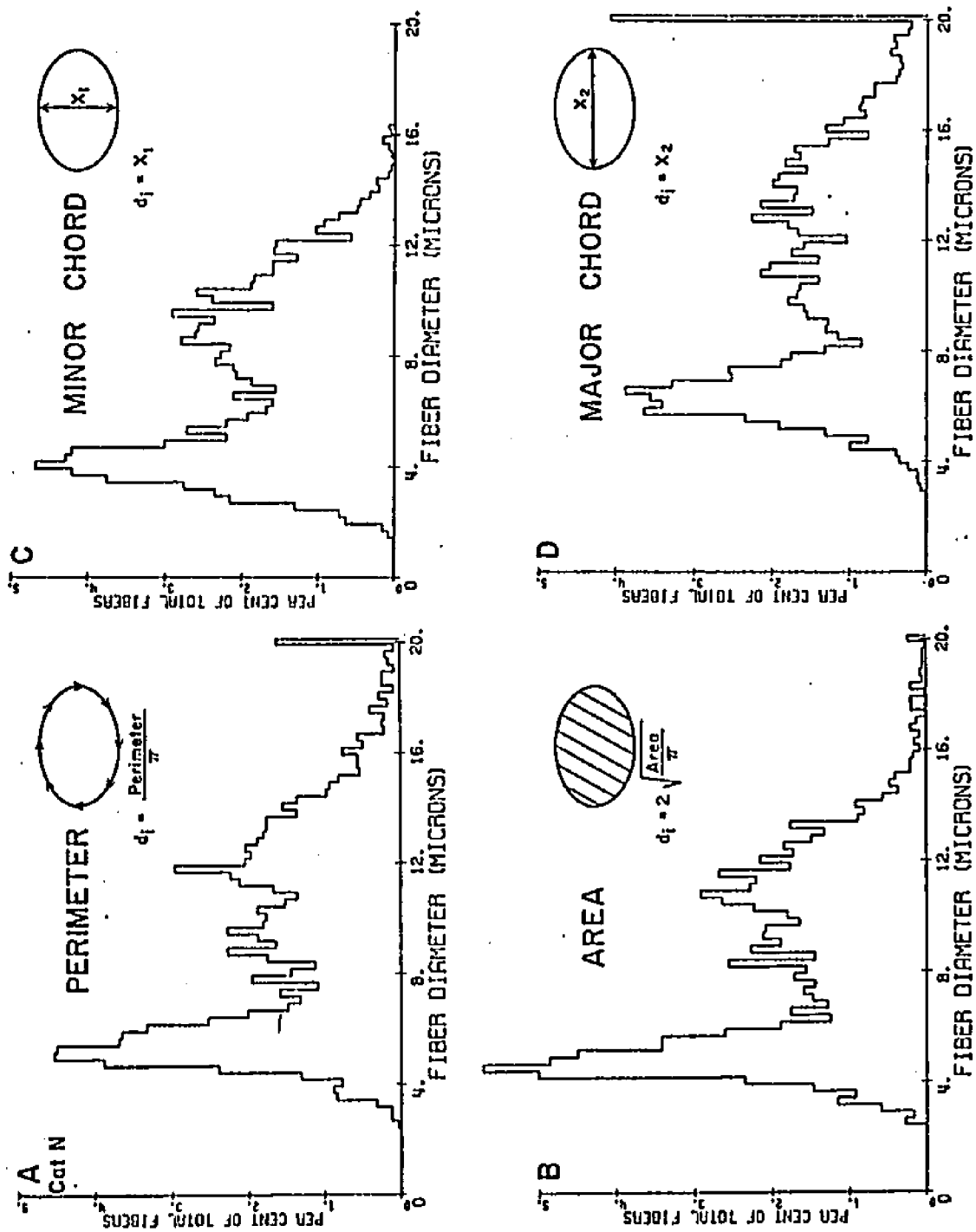


Figure 4-4. Fiber diameter histograms for the four primary estimates of fiber diameter.

Ad, is 0.25 microns. All fibers with diameters greater than 20 microns are lumped into the 20 micron bin.

The perimeter of each fiber is the sum of the distances between sequential points on the perimeter of the fiber. The diameter of a circle with equivalent perimeter is given by the perimeter/ π . The histogram in figure 4-4A is based on this estimate of fiber diameter. This histogram appears to have three major peaks. Nearly two per cent of the fiber diameters are greater than or equal to 20 microns. Since the perimeter of obliquely cut fibers and paranodal fluted fibers is larger than the corresponding internodal cylindrical perimeter, this histogram is probably biased somewhat toward large diameters.

The area bounded by the perimeter is computed with a special algorithm, that can accommodate invaginated perimeters. The diameter of a circle with equivalent area is given by two times the square root of the area/ π . The diameter histogram based on this estimate of fiber diameter is plotted in figure 4-4B. There are two distinct peaks in the histogram with perhaps a small third peak at about 9 microns. Relatively few fiber diameters greater than 16 microns are present. Like fiber perimeter, fiber area increases when the fibers are cut obliquely. However, for convoluted fibers, the area does

not increase as rapidly as the perimeter. The histogram based on area has a more narrow and taller small fiber peak than the histogram based on perimeter. Also note that this area-based histogram is not biased toward large fibers as much as the perimeter estimate histogram.

The histograms in figure 4-4C and 4-4D are based on the minor and major chords, respectively, and are quite different. The major chord, X_2 , is generally the largest of these four estimates of fiber diameter and is perhaps reasonable only for nearly round fibers. For obliquely cut fibers and fibers with irregular shapes the major chord is clearly too large an estimate of fiber diameter. The histogram based on the major chord in figure 4-4D has the smallest peaks and is skewed toward large diameters more than any other histogram in figure 4-4. Four per cent of the fibers are greater than or equal to 20 microns.

The diameter histogram in figure 4-4C, based on the minor chord, X_1 , is the estimate that should be least biased for obliquely cut fibers. The minor chord of a quasi-elliptically shaped fiber is approximately equal to the diameter of a corresponding obliquely cut cylinder. However, fibers cut at the level of the Schwann cell nucleus are generally indented by the nucleus and consequently the minor chord is less than the diameter elsewhere in the internodal region. Also, many quasi-

elliptical fiber cross-sections may result from ellipsoidal fibers that are actually cut transversely. In such cases the minor chord is an unreasonably low estimate of fiber diameter. These considerations indicate that perhaps the diameter histogram based on the minor chords is biased slightly toward small diameter fibers.

Saphenous nerve data from cat M are plotted in figure 4-5 for the same four primary estimates of fiber diameter considered above. The general form of the histograms for each estimate of fiber diameter is similar and the comments made above apply, except that only two discernable peaks are apparent in the histograms in figure 4-5.

Several general observations apply to differences between the two nerves for all four primary estimate histograms. In figure 4-6, the histograms in figures 4-4 and 4-5 are plotted together. The largest peak for small fibers is slightly taller and occurs at a slightly smaller diameter for cat M. Also, the valley between peaks is lower in all four histograms for cat M. The other main peak in each histogram for larger fibers is shifted slightly toward larger diameters for cat M. Finally, the number of fibers that are greater than or equal to 20 microns in diameter is greater for histograms from cat M.

SECONDARY ESTIMATES OF FIBER DIAMETER

Many other estimates of fiber diameter can be

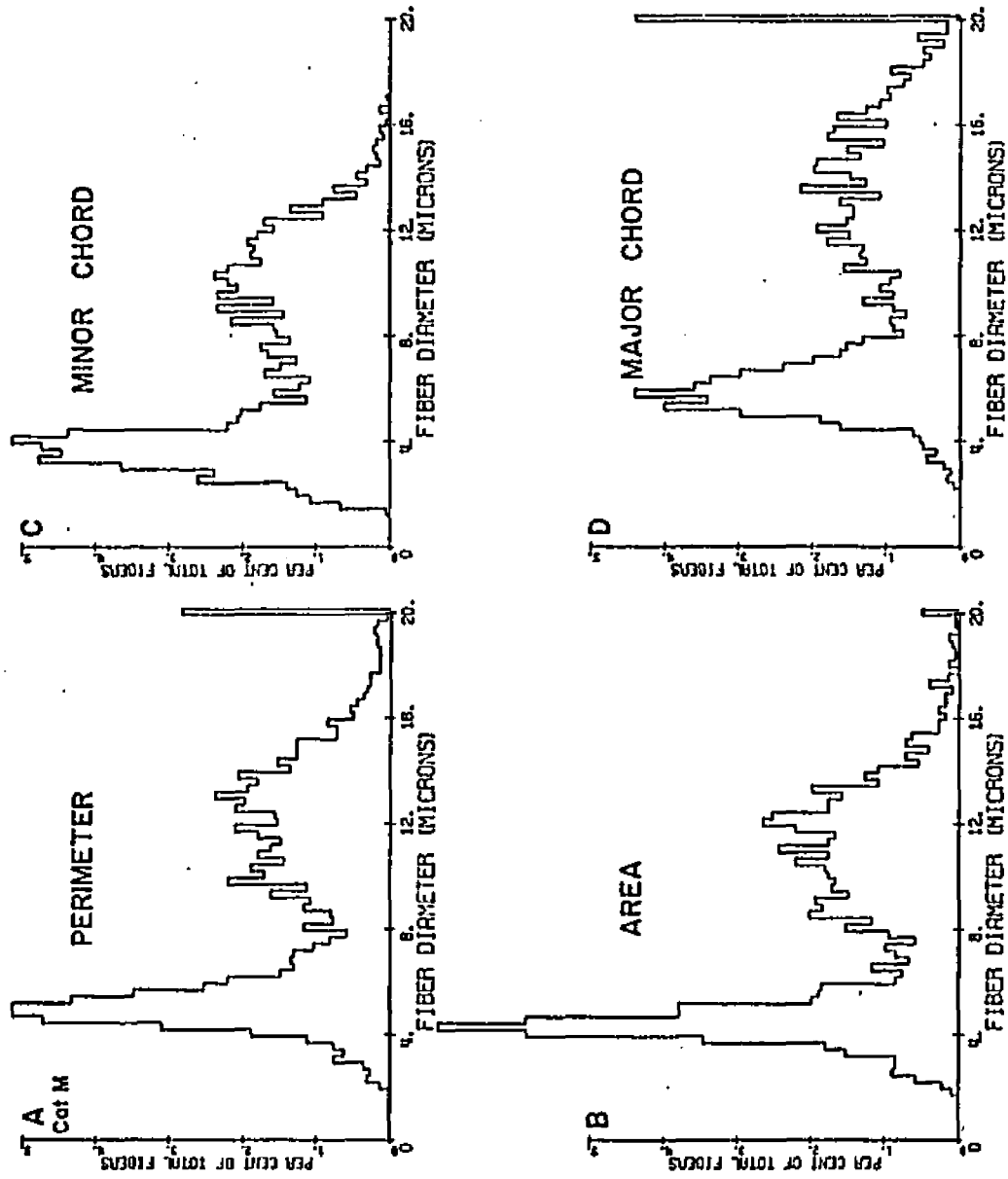


Figure 4-5. Histograms of the four primary diameter estimates for Cat M; 2217 fibers.

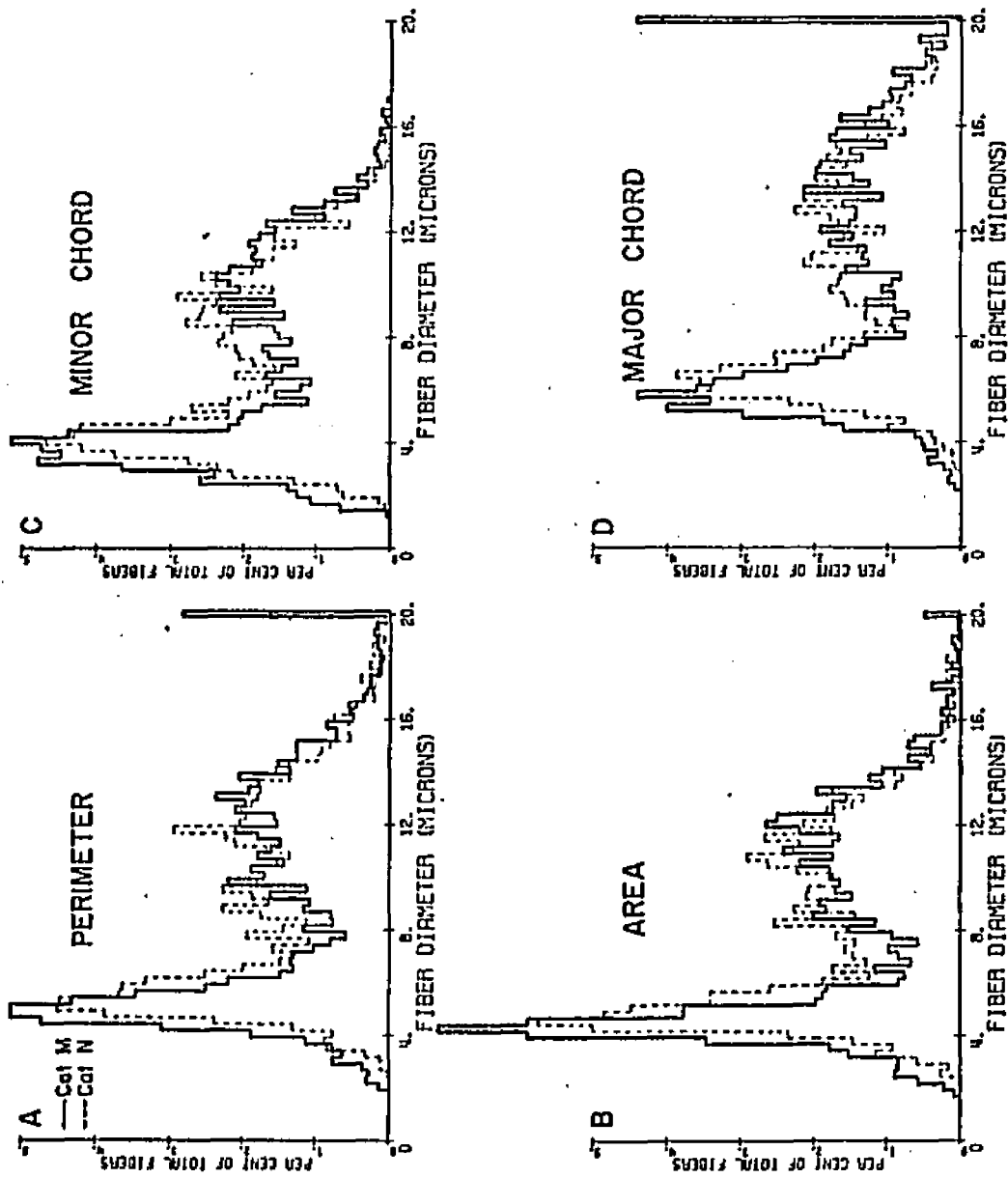


Figure 4-6. Histograms of figures 4-4 and 4-5 plotted together. Solid line for cat M; dotted line for cat N.

computed from the four basic estimates considered above and additional information, such as the position of the fiber centroid and the slope of the major chord. Several such derived estimates of fiber diameter are considered below.

Since the major chord often gives an unreasonably high estimate of fiber diameter, and the minor chord a somewhat low estimate, any function of these two chords that always gives an intermediate value may be a reasonable estimate of fiber diameter. As cited in the introduction, several authors estimate fiber diameter by the arithmetic mean of what they call the mutually perpendicular maximum and minimum diameters (Sanders, 1948; Evans and Vizoso, 1951; Wasano et al., 1962; Hutchinson, Koles and Smith, 1970; Webster, 1971; Schlaepfer and Myers, 1973). Gasser and Erlanger (1927) state they used the geometric mean of the mutually perpendicular maximum and minimum diameters.

For cat N, histograms compiled for the arithmetic, geometric and harmonic means of the major and minor chords (M_a , M_g , M_h) are plotted in figures 4-7A, 4-7B, and 4-7C. The symbol X_1 represents the minor chord and X_2 represents the major chord. Cauch's principle (Diem, 1962) states that for these three means: $M_a \geq M_g \geq M_h$, where the equality signs are valid only when $X_1 = X_2$. The three histo-

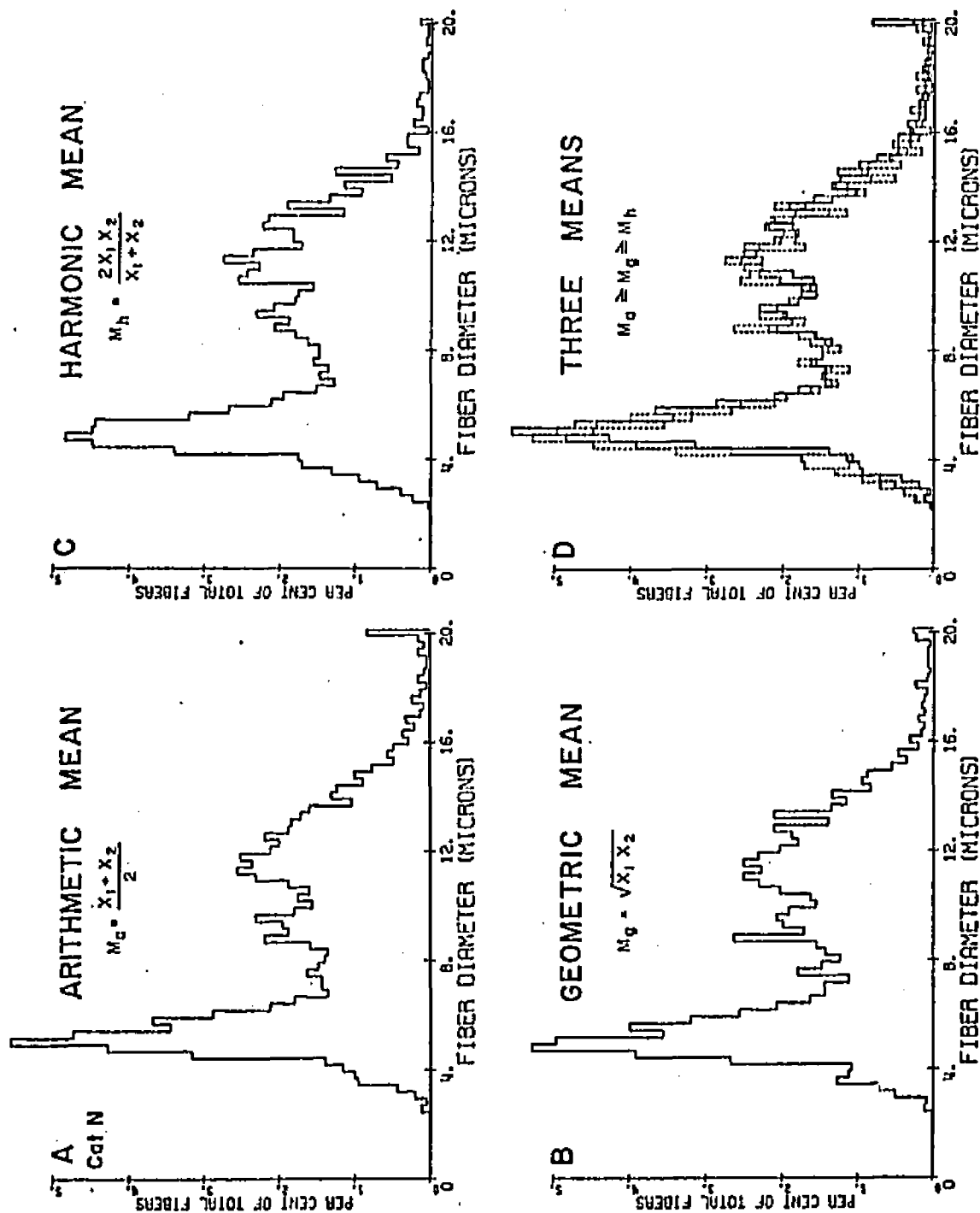


Figure 4-7. Diameter histograms for means of major and minor chords. Cat N.

grams are quite similar,¹ although for the arithmetic mean the large peak for small fibers is tallest and the possible appearance of a small intermediate peak at about 9 microns is slightly more evident. Also note that the number of fibers greater than 16 microns in diameter is largest for the arithmetic mean.

For the data from cat M, histograms for the arithmetic, geometric and harmonic means of the major and minor chords are shown in figure 4-8. The height of the tallest peak for small fibers did not become lower for the geometric and harmonic mean histograms in figure 4-8 as they did for cat N in figure 4-7. Note that the number of fibers greater than or equal to 20 microns in diameter increased for the geometric and arithmetic mean histograms. The possible emergence of a third intermediate peak at 9 to 10 microns in all three histograms of figure 4-8 is interesting because such peaks are not outstanding in the simple minor and major chord histograms in figures 4-5C and 4-5D. For all three means in both nerves, the peak in the histograms for small fibers is higher than the tallest peak in either the corresponding simple major or minor chord histograms.

A simple method of correcting the area estimate of fiber diameter for quasi-elliptical fiber cross-sections

¹ No attempt was made to distinctly identify the three curves in figures 4-7D and 4-8D.

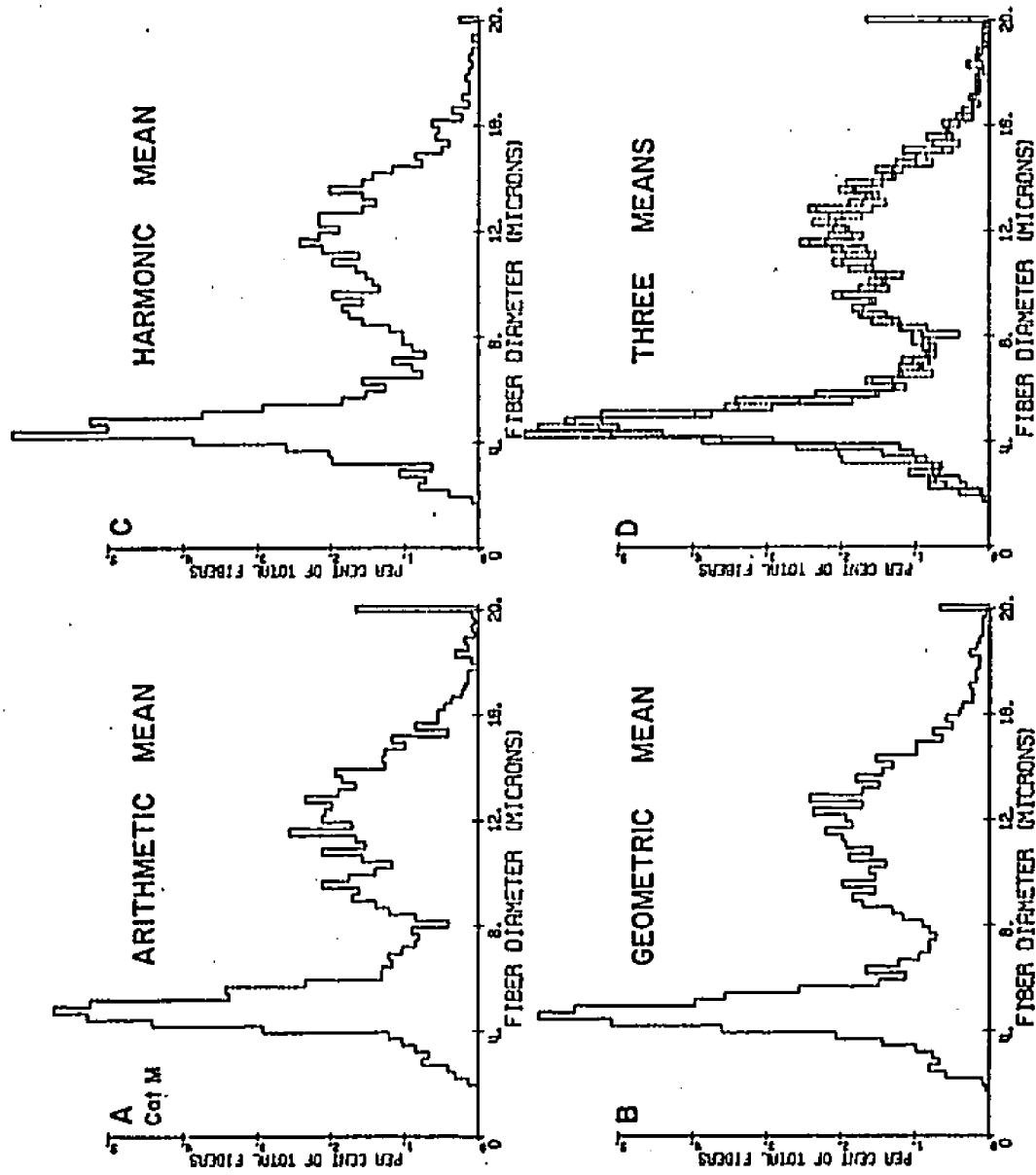


Figure 4-8. Diameter histograms for means of major and minor chords. Cat M.

is shown in figure 4-9. Note that the ordinate variable is the actual number of fibers in each bin of the histogram. This change in the ordinate variable is made to permit the identification of the number of fibers involved in changes in the histograms. The light dotted line histograms are the same as the simple area estimate histogram shown in figure 4-4B for cat N. If the ratio X_1/X_2 is less than the indicated value on each graph, the fiber area is reduced by the ratio of the area of a circle with diameter X_1 , to the area of an ellipse with minor axis X_1 and major axis X_2 . The areas of fibers with a ratio X_1/X_2 greater than or equal to the indicated value are not affected. Fiber diameter is computed in the usual way as two times the square root of adjusted area/ π . In figure 4-9 the wide solid line histograms are compiled by assuming the indicated values for the ratio, X_1/X_2 , in the analysis described above.

When X_2 is more than twice X_1 , as shown in figure 4-9A, a pile of fibers emerges at about 3.0 microns primarily at the expense of the number of fibers in the tallest peak in the histogram. Note that the central portion of the histogram is affected the least and that fewer fiber diameters greater than 16 microns are present. All the fibers in the 20 micron bin for the histogram based on simple area are adjusted to smaller diameters in

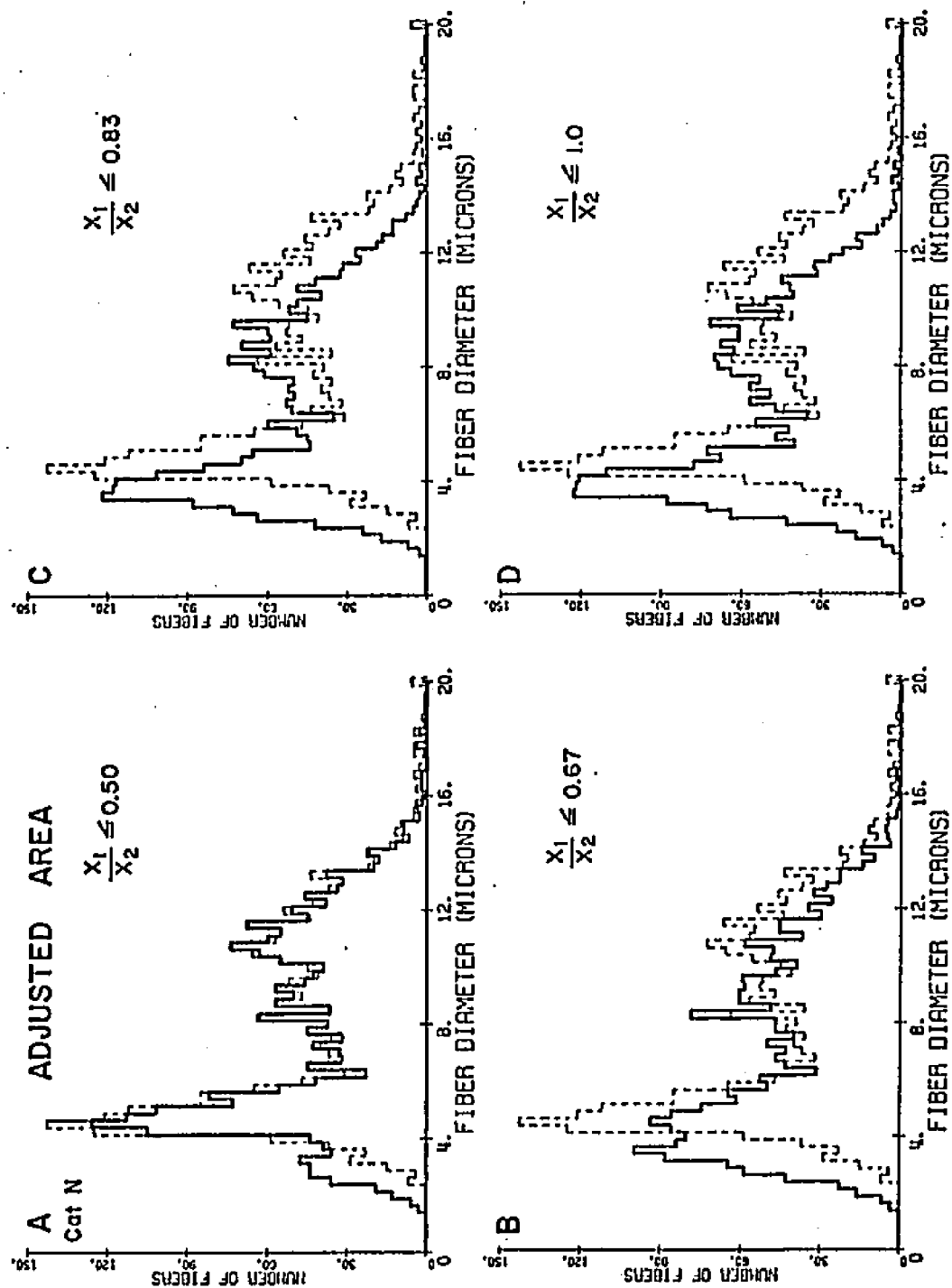


Figure 4-9. Adjusted area histograms (solid line). Only fibers with X_1/X_2 less than the value shown are adjusted. Dotted line histogram based on area. Cat N.

figure 4-9A. This indicates that these apparently very large fibers have elliptical shapes.

Several shifts in the histogram occur as the ratio X_1/X_2 increases toward unity. The new peak at about 3 microns continues to increase at the expense of larger fibers. This peak does not shift progressively to smaller diameters as the ratio increases, but grows in amplitude at approximately the same position. The peak for large fiber diameters does shift toward smaller diameters without much change in amplitude. In figure 4-9D the area of all fibers is adjusted. The adjusted histograms in figure 4-9C and 4-9D are very similar. This may result from the fact that fibers with a ratio X_1/X_2 greater than 0.83 are nearly circular and therefore are affected very little by adjustment procedure. It is also possible that very few fibers have ratios of X_1/X_2 between 0.83 and 1.0. Figure 4-15B, presented later in this chapter, does indeed show that most ratios of X_1/X_2 are less than 0.83.

The same area adjustments described above are applied to data from cat M and the results are plotted in figure 4-10. The principle features of the outcome are very similar to the results given in figure 4-9 for cat N. One difference is that the valley between the two major peaks in figure 4-10 rises more than the valley in figure 4-9, despite identical increases in the ratio X_1/X_2 used to

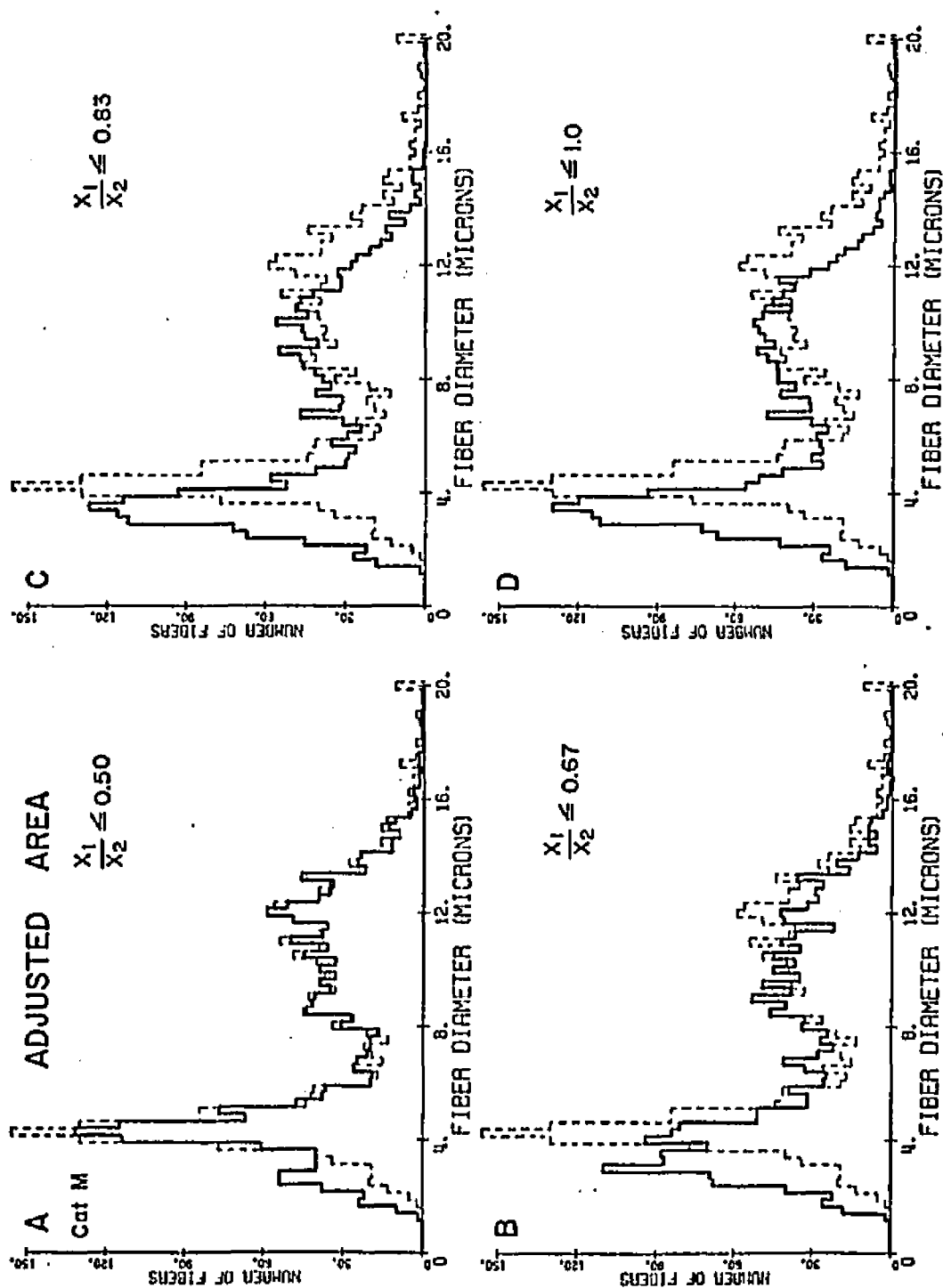


Figure 4-10. Adjusted area histograms (solid line). Only fibers with X_1/X_2 less than the value shown are adjusted. Dotted line histogram based on area. Cat M.

control the adjustments. Also, slightly less shifting of the peak for large fibers is apparent in figure 4-10B than in figure 4-9B. Furthermore, additional large fiber shifts occur in figure 4-10D that are not present in figure 4-10C.

The simple area adjustment procedure described above utilizes additional information from each individual fiber to attempt to correct for obliqueness. When data from single fibers are examined individually, it seems there is no way to differentiate between an obliquely cut cylindrical fiber and a non-cylindrical fiber that is cut transversely. The following neighbor analysis adjustments are based on the observation that obliquely cut fibers tend to have obliquely cut neighbors with some similar characteristics that do not depend on fiber diameter. Since all fiber perimeters in each fascicle are measured with respect to a common origin, information concerning the relative positions of all fibers within a fascicle is available. The positions of the fiber cross-section centroids can be used to identify neighboring fibers so that selected properties of neighboring fibers can be examined. A neighbor fiber is considered only if the distance between the two fiber centroids is less than or equal to 20 microns. The difference between the slopes of the two major chords is examined, or the difference between the

two ratios X_1/X_2 is considered. Any combination of these two differences can be used to determine the extent to which the area is adjusted for obliqueness, as described above for figures 4-9 and 4-10.

Only the slope of the major chord in neighboring fibers is examined for the histograms in figure 4-11. If the two slopes differ by more than 5° (figure 4-11A), then the weight of that neighbor is zero. If the difference is less than or equal to 5° , then a weight of one is assigned for that neighbor. The sum of all the weights is divided by the number of neighbors to determine the degree of area adjustment based on the ratio of the area of a circle with diameter X_1 , to the area of an ellipse with minor axis X_1 and major axis X_2 . If all the neighbors receive a weight of one then the adjustment for that fiber is the same as the simple area adjustment previously described. If all the neighbors receive a weight of zero then no adjustment is made.

This method has the capability to differentiate between most quasi-elliptical fibers that are cut obliquely and fibers that actually have quasi-elliptical cross-sections.

Neighbor analysis. adjustment of fiber area results in the diameter histogram shifts shown in figure 4-11, for several angular differences between neighboring

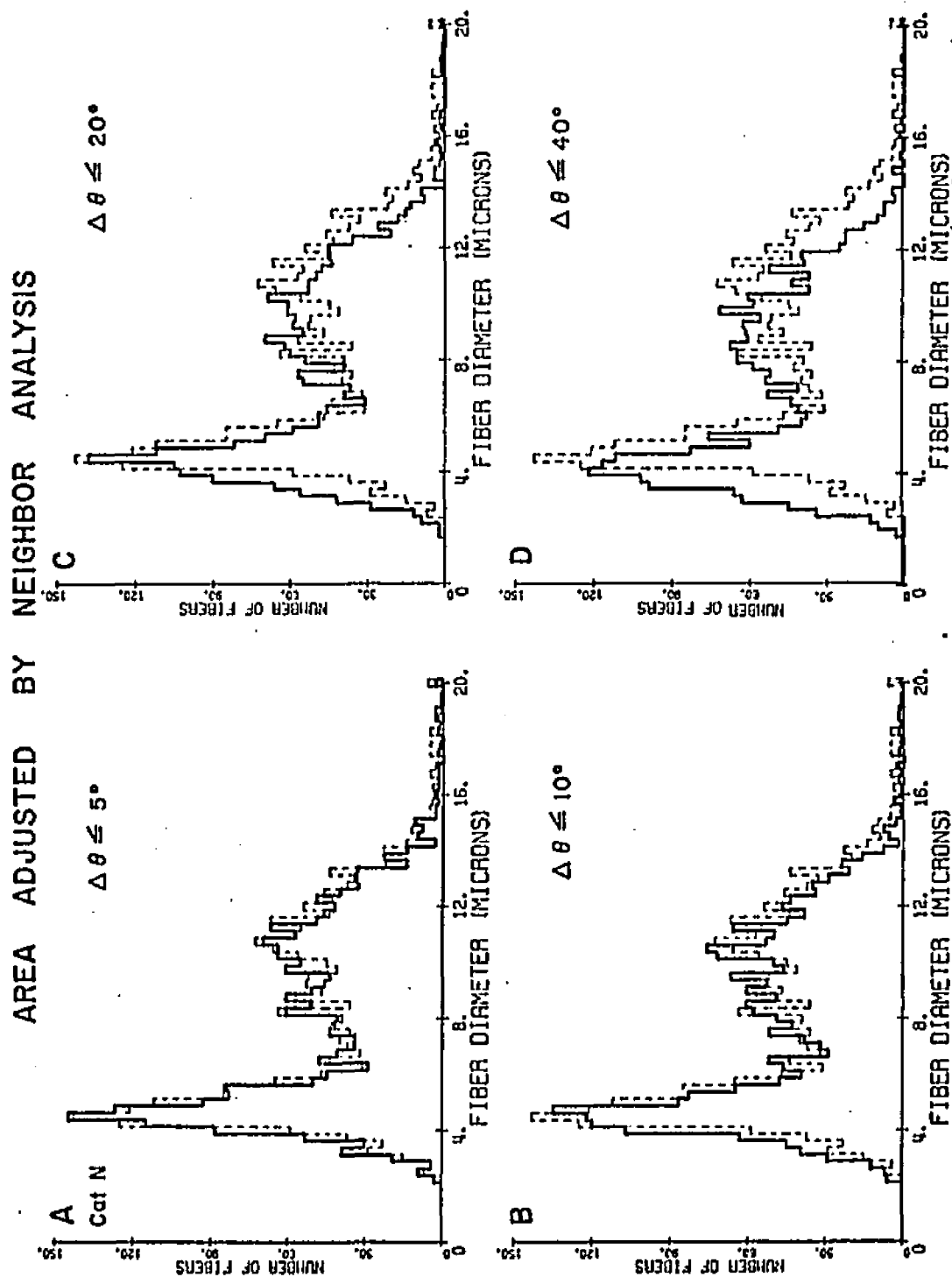


Figure 4-11. Histograms based on area adjusted by neighbor analysis (solid line). $\Delta\theta$ is the angle between neighboring major chords. The dotted line histogram is based on area. Cat.N.

major chords. The histograms for progressive doubling of the allowed angular difference in the neighbor analysis show mainly a simple shift toward smaller diameters that is remarkably similar for all fiber diameters. The apparent changes in histogram shape are a slight decline in the height and increased width of the largest peak for small diameter fibers. Also, the width of the peak for large fibers decreases.

Instead of considering only the major chord slope in neighboring fibers, the differences between the ratios X_1/X_2 in each fiber and its neighbors can be used to adjust fiber area for obliqueness. The other aspects of the neighbor analysis method are the same as described above. The allowable difference in the ratios X_1/X_2 is doubled three times to produce the shifts in the diameter histograms shown in figure 4-12. As for adjustments based on neighbor major chord slopes, the histograms in figure 4-12 shift to the left an amount that is remarkably the same for all fiber diameters in each histogram. The width of the tallest peak increases, while the width of the peak for large fibers decreases slightly.

In figure 4-13 both the slope of the major chord and the ratio X_1/X_2 are considered simultaneously with equal weight. The parameters used for the neighbor analysis adjustment in figure 4-13A for cat N are the same as the

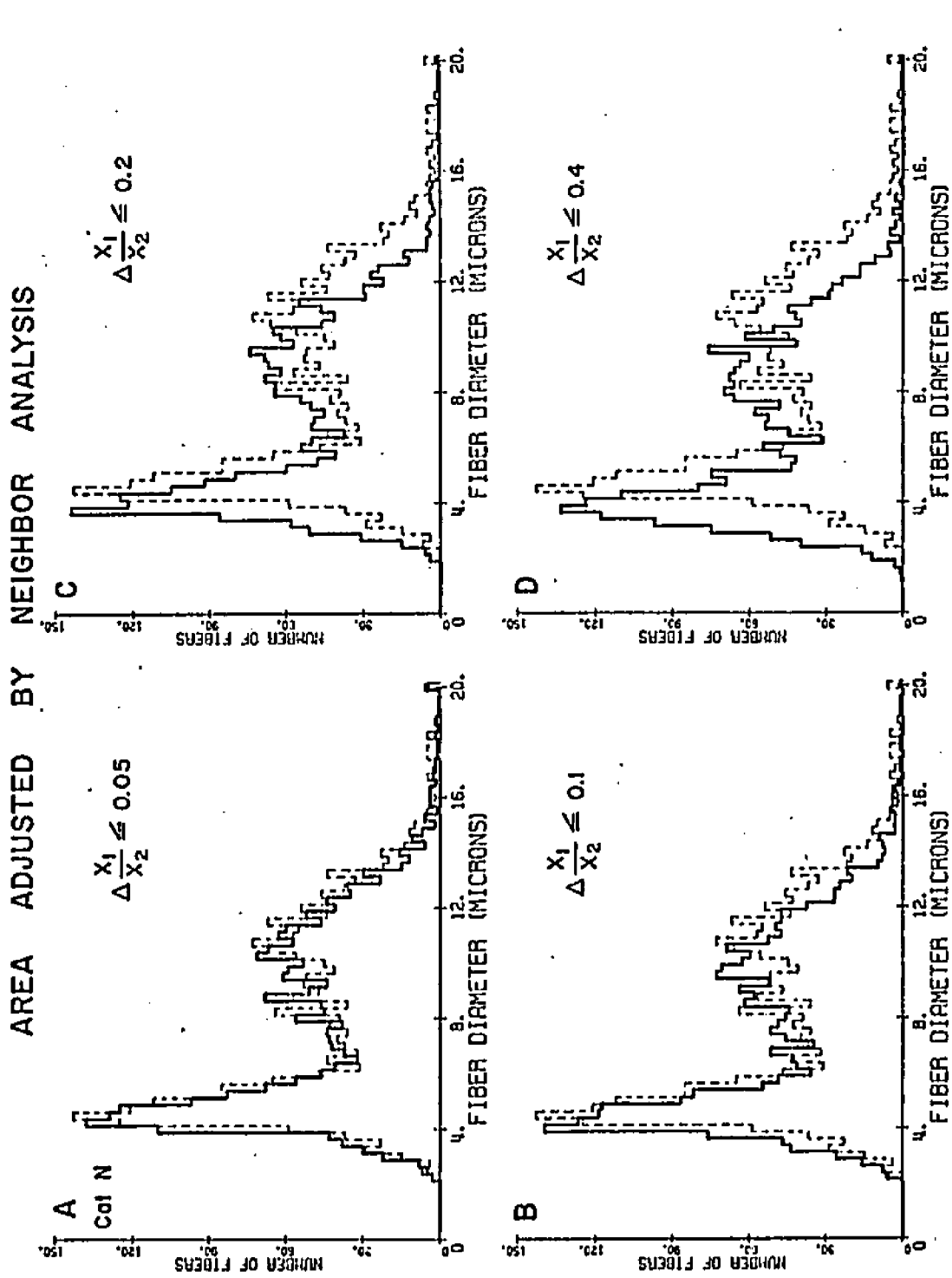


Figure 4-12. Histograms based on area adjusted by neighbor analysis (solid line). $\Delta X_1/X_2$ is the difference between neighboring ratios. The dotted line histogram is based on area. Cat N.

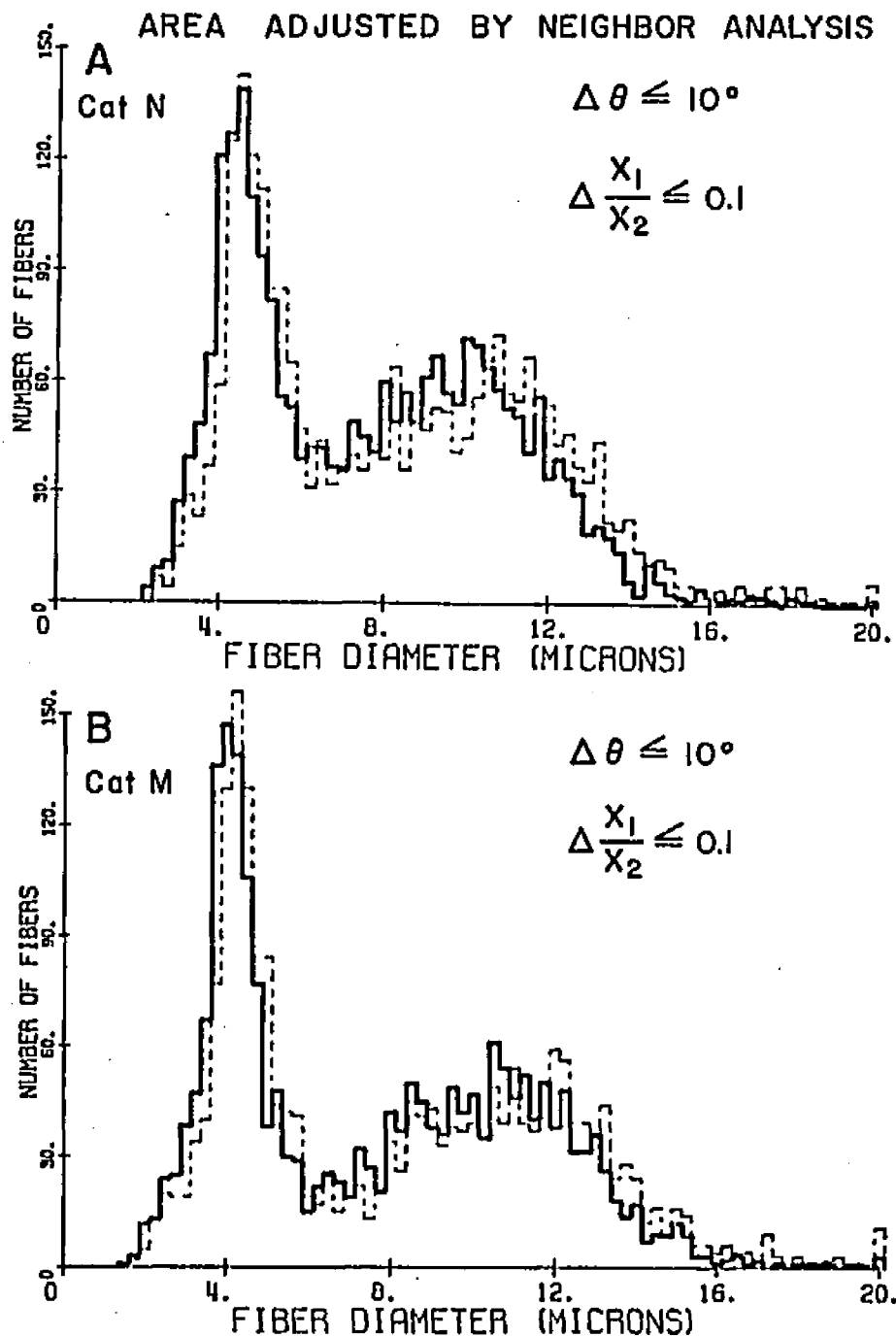


Figure 4-13. Histograms based on area adjusted by neighbor analysis (solid line). $\Delta \theta$ is the angle between neighboring major chords. $\Delta X_1/X_2$ is the difference between neighboring ratios. The dotted line histogram is based on area.

values used individually in figures 4-11B and 4-12B.

It is not surprising that the results for this combination of the two obliqueness indicators is similar to the results for each indicator used separately. The application of the same kind of neighbor analysis to data from cat M gives a similar result, as shown in figure 4-13B.

Certainly more complicated schemes could be contrived to selectively adjust the fiber diameters. The development of such schemes seems pointless in view of the scant knowledge of the sterology of families of quasi-cylindrical oriented structures. Currently, only a qualitative evaluation of the parameter values for the neighbor analysis described above is possible.

HISTOGRAM BIN WIDTH VARIATIONS

The width of the bins that authors used to compile fiber diameter histograms varied from 0.1 micron to 2 microns (Appendix A). No justification was generally given for the bin width used and the affects of bin width on the histogram shape were ignored.

Figure 4-14 shows four histograms compiled from the same basic data for 2496 fibers. The fiber diameter is based on area and the bin width is progressively halved three times starting at one micron. Note that each time the bin width is halved, the scale on the ordinate is doubled, so that relative magnitudes are preserved.

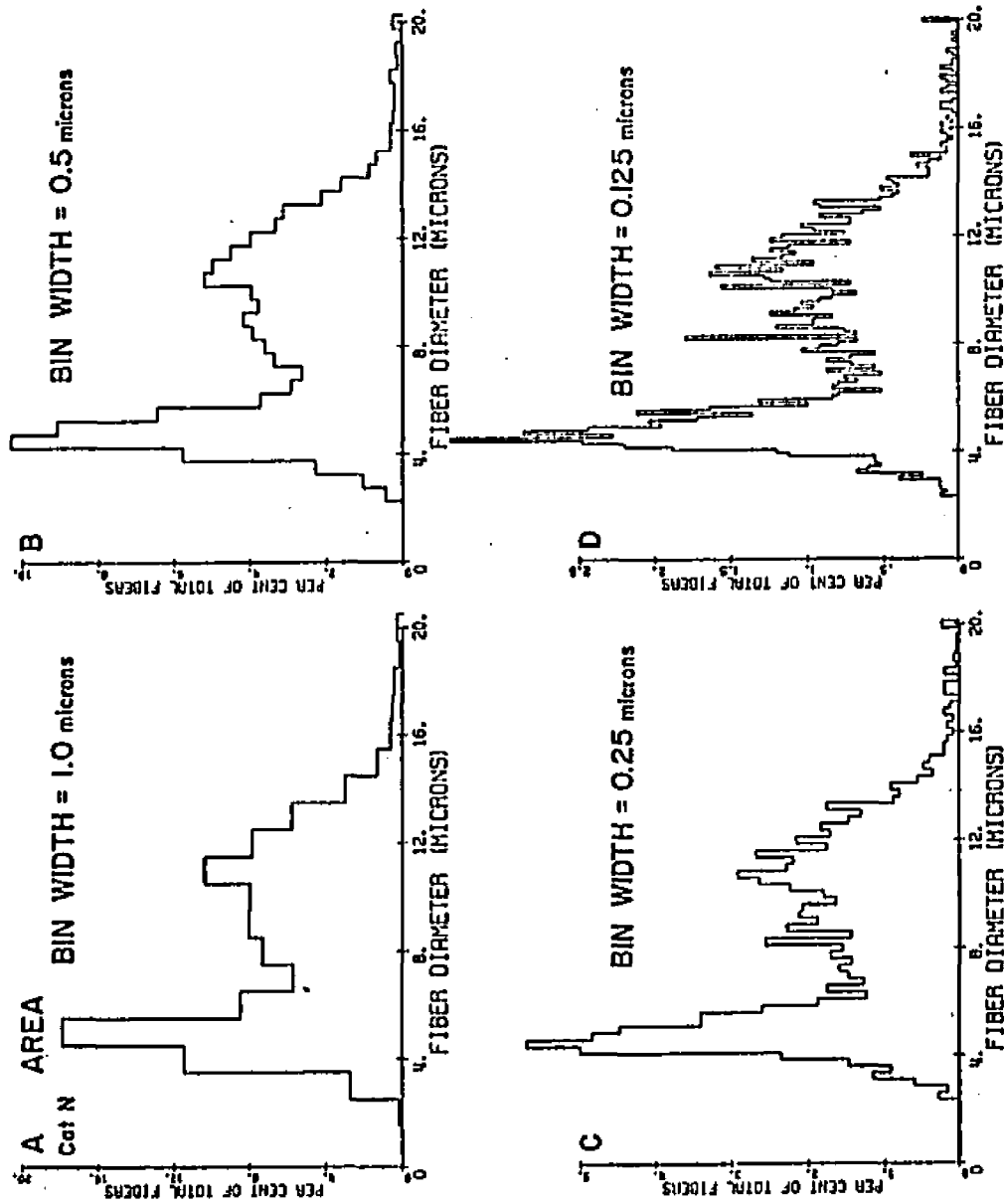


Figure 4-14. Histograms for diameter based on area compiled for different bin widths. Each histogram was compiled separately from the raw data for each fiber. Cat N.

Much of the detail present in figure 4-14B for 0.5 micron bin widths is lost when the bin width is increased to 1 micron for figure 4-14A. The histograms in figures 4-14B and 4-14C have essentially the same shape, although the histogram for 0.25 micron bin widths has some bins that appear to fluctuate randomly. As the bin width decreases further to 0.125 microns in figure 4-14D the ordinate sensitivity has increased enough to make the contribution of single fibers visible. The smallest incremental change in bin height among adjacent bins due to single fibers is particularly discernable in figure 4-14D between 16 and 20 microns. Note that the relative size of the tallest bin in the peak for small fibers tends to increase as the bin width decreases. The largest peak in the histogram appears to shift to the left as the bin width decreases (compare figures 4-14A and 4-14C). Also, the amplitude of the bin centered at 20 microns increases as the bin width decreases. Apparently, this occurs because a diameter of exactly 20 microns is assigned to all fibers that have diameters larger than 20 microns. Undoubtedly the measurement resolution is exceeded for 0.125 micron bin widths. The increasing jaggedness in the histograms, as the bin width decreases, is accentuated by the progressive increases in vertical sensitivity.

MEAN RATIOS OF MINOR TO MAJOR CHORDS

Consider the possibility that fibers in some particular size range have a greater tendency to be cut obliquely. Histograms based on estimates of fiber diameter that are affected by obliquely cut fibers, would be differentially biased if the degree of fiber obliqueness is diameter dependent.

Figure 4-15B (solid line) shows the mean ratio X_1/X_2 for the fibers in each bin of the histogram in figure 4-15A, based on fiber area. The dotted line histograms in figure 4-15B show the standard deviation of the ratios X_1/X_2 , under the assumption that the ratios are normally distributed within each bin. The diameter histogram is plotted directly above the mean ratio histogram so that comparisons can be made. When the bin amplitude in the diameter histogram falls below 1/2 of one per cent, less than a dozen fiber ratios are used to compute the mean ratio. Thus, it is not surprising that the standard deviation increases for bins below 3 microns and above 15 microns.

The mean ratio X_1/X_2 is highest at 0.7 for the pile of fibers centered at about 11 microns. The mean ratio decreases slightly to 0.65 for the large number of fiber diameters in the vicinity of 5 microns. Thus, small fibers have a slightly higher tendency to be cut obliquely

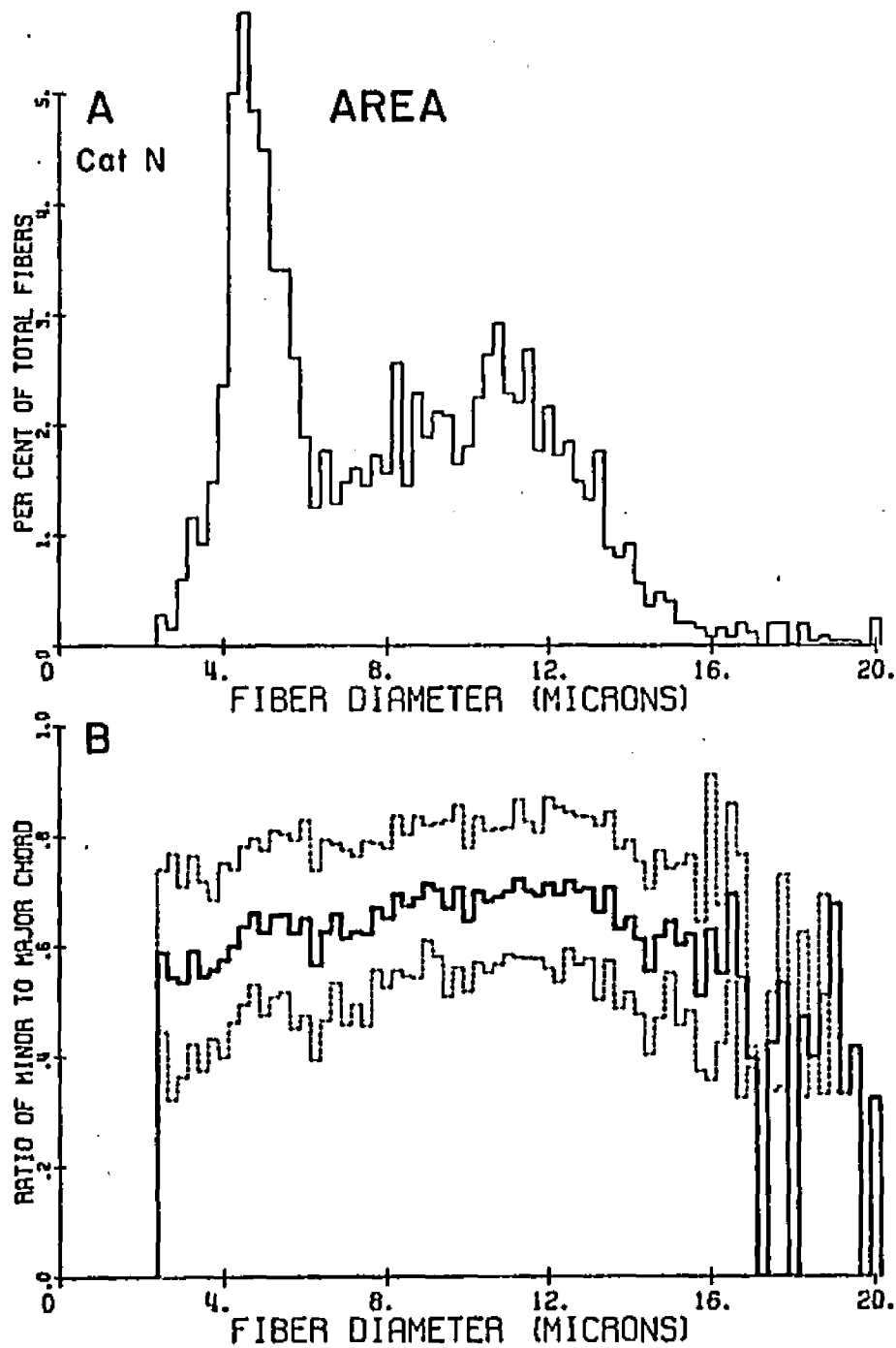


Figure 4-15. A. Diameter histogram based on area for cat N. B. mean ratio of minor to major chord for fibers in each bin of A (solid line). The standard deviation of the ratios in each bin is also plotted (dotted lines).

than do large fibers. The mean ratio X_1/X_2 decreases for the few fibers that are greater than 14 microns. This indicates that nearly all fibers greater than 15 microns in diameter are cut obliquely. There are large fluctuations in the mean and standard deviation for these fibers, probably because so few extra large fibers are present. Note also that the fibers greater than or equal to 20 microns have a particularly small mean ratio X_1/X_2 of 0.3. The standard deviation is not calculated for the 20 micron bin.

MEAN DISTANCE TO THE PERIPHERY

The possibility that fiber diameters are not randomly distributed throughout the cross-section is examined next. The distance in microns from the centroid of each fiber to the closest point on the outside perimeter of the epineurium is calculated for each fiber individually. This is an estimate of the shortest distance from each fiber centroid to the silver recording electrode. In figure 4-16B the mean distance to the periphery is plotted (solid curve) for the histogram based on the area estimate of fiber diameter in figure 4-16A. The dotted line histograms in figure 4-16B show the standard deviation of the distances to the periphery for each bin, assuming the distances are normally distributed within each bin. The standard deviation for the 20 micron bin is not computed.

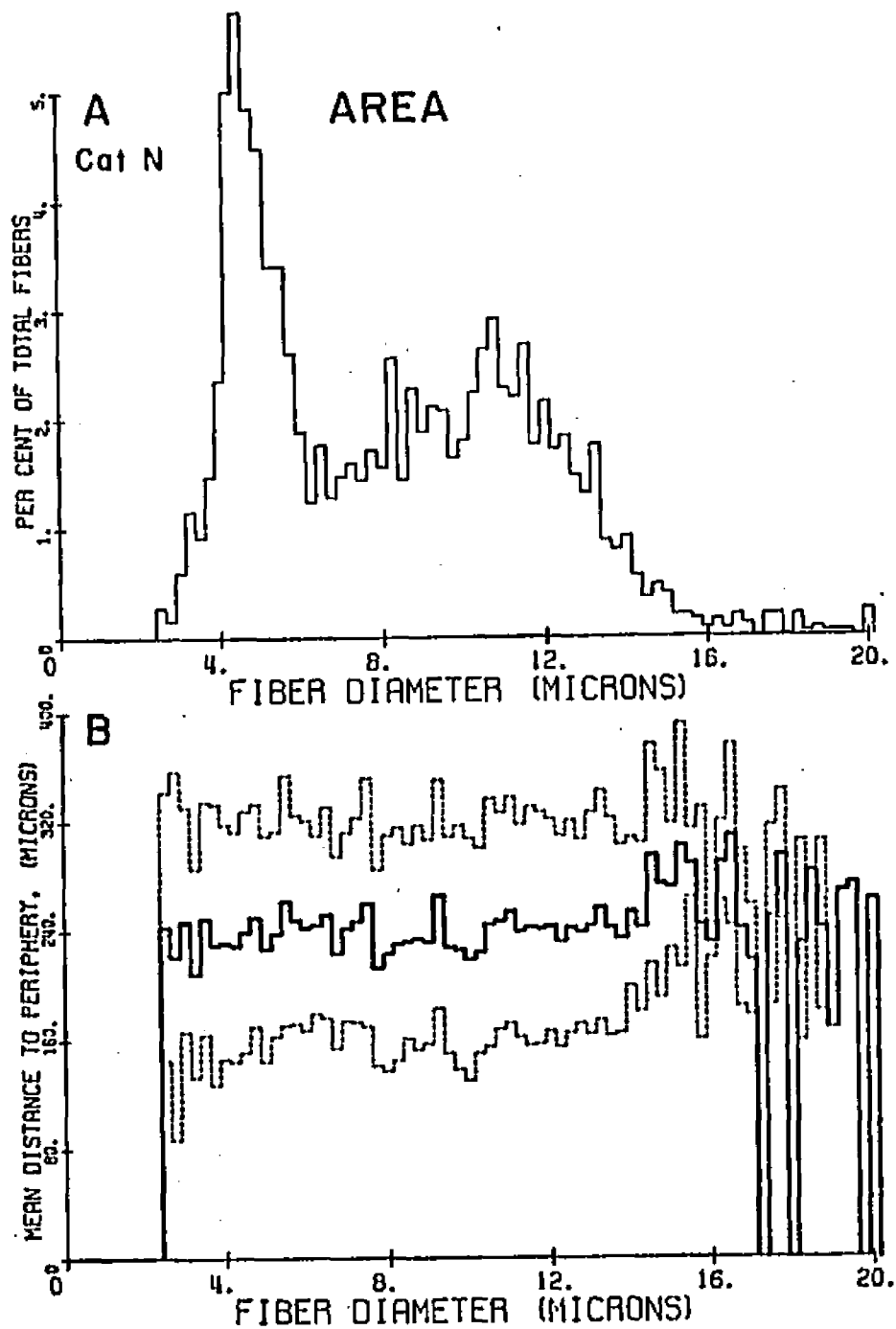


Figure 4-16. A. Diameter histogram based on area for cat N. B. Mean of the smallest distance from each fiber cross-section centroid to the whole nerve perimeter for fibers in each bin (solid line). The standard deviation of the distances in each bin is also plotted (dotted lines).

The mean and standard deviation of the distance to the periphery remain quite constant from about 3 microns to 14 microns. It is not surprising that many of the fibers larger than 14 microns have long distances to the periphery. Figure 4-1 shows that many of the very obliquely cut fibers in the three largest fascicles happen to be toward the center of the whole nerve. Also, the standard deviations for bins below 3 microns and above 14 microns are increased, probably because the number of fibers in these bins is small.

SEQUENTIAL SECTION HISTOGRAMS

Measurements from serial sections of single fibers have shown that considerable variation in diameter occur (Duncan, 1934; Hursh, 1939; Sunderland and Roche, 1958). Sunderland and Lavarack (1953) site evidence that slight shifts in diameter histograms have been found in sections taken at different levels. Apparently histograms have not been compiled for serial sections or sections taken at short intervals.

A small fascicle is sectioned nine times at 0.1 mm. intervals to provide some data for the study of fiber diameter histogram variation. The number of fibers identified and measured in the nine sections varies from 162 to 167. This very small fascicle contains only about 6.5% of the myelinated fibers present in the whole

saphenous nerve. The previous histograms are compiled from measurements on a total of 2496 or 2217 fibers in a single section.

The solid curve in figure 4-17A is the mean histogram based on the major chord for the nine sections described above. The relatively small numbers on the ordinate scale indicate that the number of fibers in any bin is small. The bin width is increased to 0.5 microns for these histograms. The two dashed curves in figure 4-17A represent the standard deviation of the nine values available for each bin, assuming the values are normally distributed. In figure 4-17B the same mean histogram (solid line) and the standard error of the mean (dashed curves) for the major chord are plotted. Both the standard deviation (STD) and the standard error of the mean (SEM) increase as the diameter decreases. Although considerable variation occurs, the number of fibers in any bin is small and the bin width is close to the resolution of the light microscope. When the mean minus the STD or SEM is negative, the ordinate is set equal to zero.

Figures 4-17C and 4-17D are similar to figures 4-17A and 4-17B respectively, except that fiber diameter is based on the minor chord. The STD and SEM both increase as the diameter decreases. Also note that the STD and SEM decrease as the mean value decreases for small fibers.

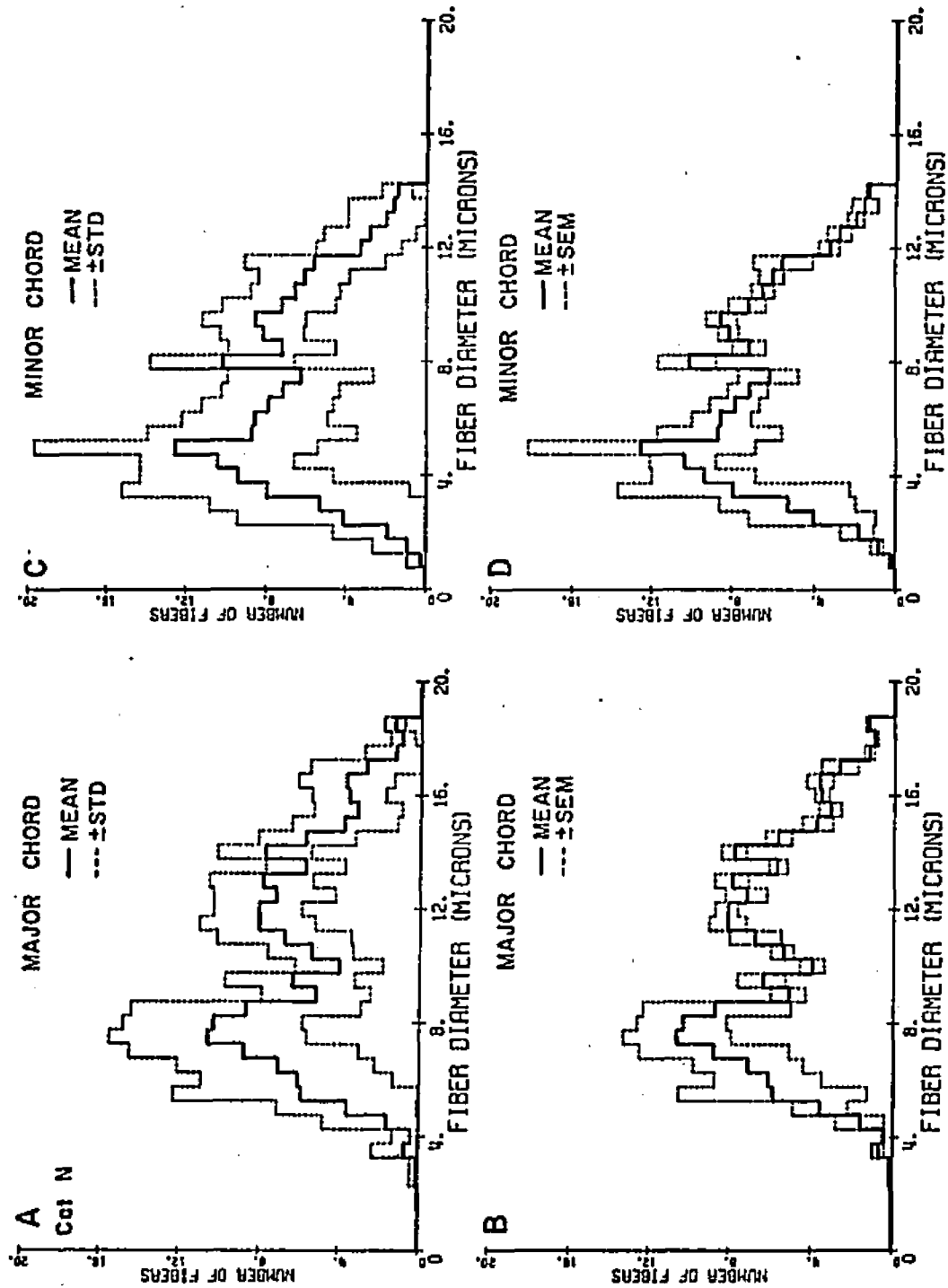


Figure 4-17. Statistics for histograms compiled for 9 sections cut 0.1 mm. apart. Major and minor chords. Only approximately 165 fibers are measured in each section.

For example, the variation near 4 microns in figure 4-17A and 4-17B is much less than the variation near 8 microns. The slightly larger maximum STDs and SEMs for the minor chord may simply be related to larger mean ordinate values.

The variations for histograms based on area and area adjusted by neighbor analysis are given in figure 4-18. The parameters used for the neighbor analysis are the same as for figure 4-13. The degree of variation seen in figure 4-18 is similar to that observed in figure 4-17 for different estimates of fiber diameter. Note that the variation for adjusted area from neighbor analysis is not substantially less than the variation in histograms based on simple area. Though not shown, the variation in the histograms for these data based on perimeter is similar to the variation in figure 4-17 and 4-18.

REPEATABILITY OF DIGITAL PERIMETER MEASUREMENTS

The repeatability of fiber diameter measurements is never discussed in any of the papers listed in the introductory section that deals with techniques for measuring fiber diameters.

Figure 4-19 illustrates qualitatively the repeatability of the measurement procedure used for this study. Data from two independent sets of measurements on each fiber made by the author eight months apart are plotted

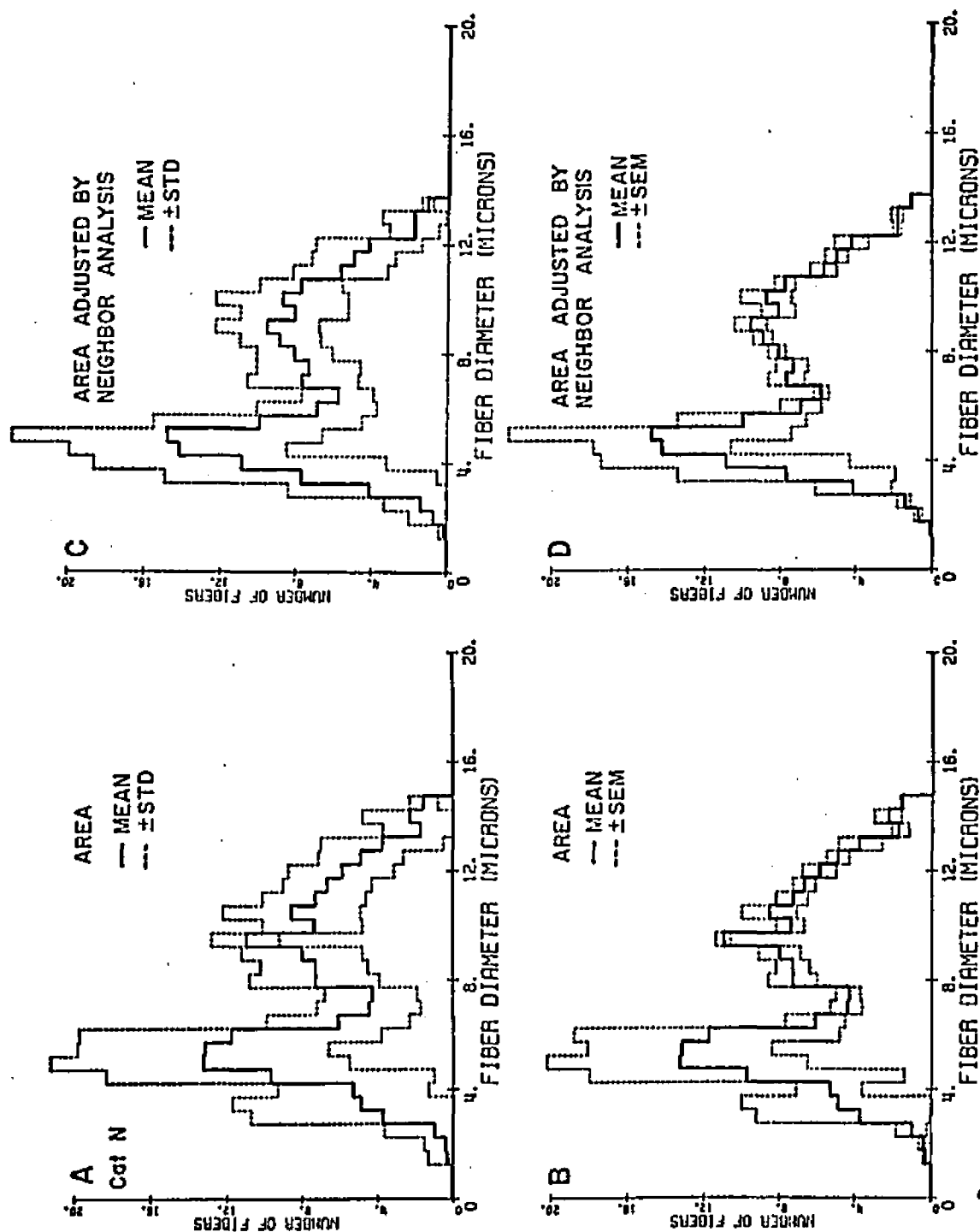


Figure 4-18. Statistics for histograms compiled for 9 sections cut 0.1 mm. apart. Area adjusted by neighbor analysis. Only approximately 165 fibers per section.

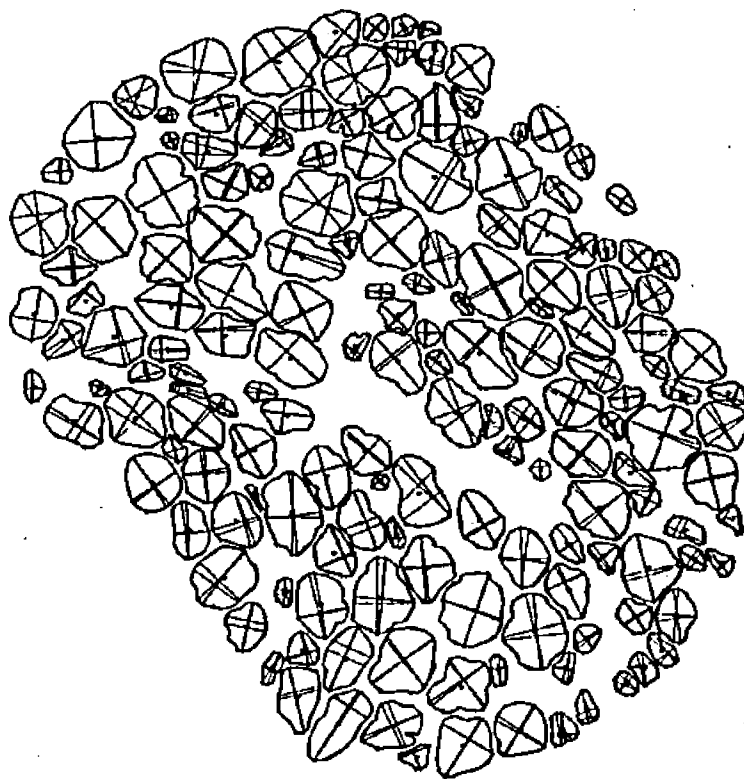


Figure 4-19. CalComp plot of fiber perimeters, major and minor chords, and centroids for two independent series of perimeter measurements made on the same photomicrograph. 163 fibers.

together. The perimeter point coordinate resolution is ± 0.01 inches for both orthogonal coordinates. The incremental CalComp plotter step size is less than 0.005 inches and the line width is approximately 0.02 inches.

The plot shows that, although the perimeters and symbols match closely, some major and minor chords are clearly different. Since the major chord is drawn between the two points on the perimeter that are the farthest apart, the two major chords will coincide only if the same two points on the perimeter are digitized both times. A small change in the major chord can make quite a difference in the minor chord. Many fibers that exhibit two very different major and minor chords are nearly round and thus, any small difference in the actual points could appreciably affect the chord positions. The length of the chords for nearly round fibers, however, should not be vastly different.

Statistics are given in table 4-1 for the differences in diameter computed (for each of the four basic estimates of fiber diameter) from two measurements on each fiber in figure 4-19. The differences in diameter for all 163 fibers in figure 4-19 are used to compute the mean difference in diameter, the standard deviation of the difference (assuming the differences are normally distributed) and the maximum difference. It is clear that

TABLE 4-1

Statistics for the differences in diameter for two measurements on each fiber in figure 4-19. Differences are given in microns.

	Mean Difference	Standard Deviation of Difference	Maximum Difference
Perimeter	0.101	0.199	0.822
Major Chord	0.143	0.262	1.082
Minor Chord	0.180	0.332	1.659
Area	0.070	0.123	0.548

diameters based on area are least subject to variation while diameters based on the minor chord vary the most.

CHAPTER V

COMPOUND ACTION POTENTIAL RECONSTRUCTIONS

Supramaximal monophasic compound action potentials (CAPs) recorded from cat saphenous nerve are used to evaluate CAPs reconstructed from fiber diameter histograms. This chapter is divided into three sections in which (1) Recorded CAPs are presented, (2) CAP reconstructions from the diameter histograms in Chapter IV are compared with recorded CAPs, and (3) CAP model parameters are optimized to reduce differences between reconstructed and recorded CAPs.

RECORDED CAPS

The methods used to record monophasic CAP's on magnetic tape are described at length in Chapter II. A photograph of a single supramaximal monophasic CAP recorded for a conduction distance of 6.7 cm. from cat N is shown in figure 5-1. The voltage and time scales indicate that the CAP is a low-level short duration biopotential. At the time marked "S" a 30 μ sec. current stimulus is applied. Following a 0.75 msec. delay, the response occurs with two major peaks. For this particular nerve and conduction distance, a third small peak is present on the declining portion of the first major peak. These peaks are labeled by Gasser and Grundfest (1939) in order of occurrence, as α , β and δ .

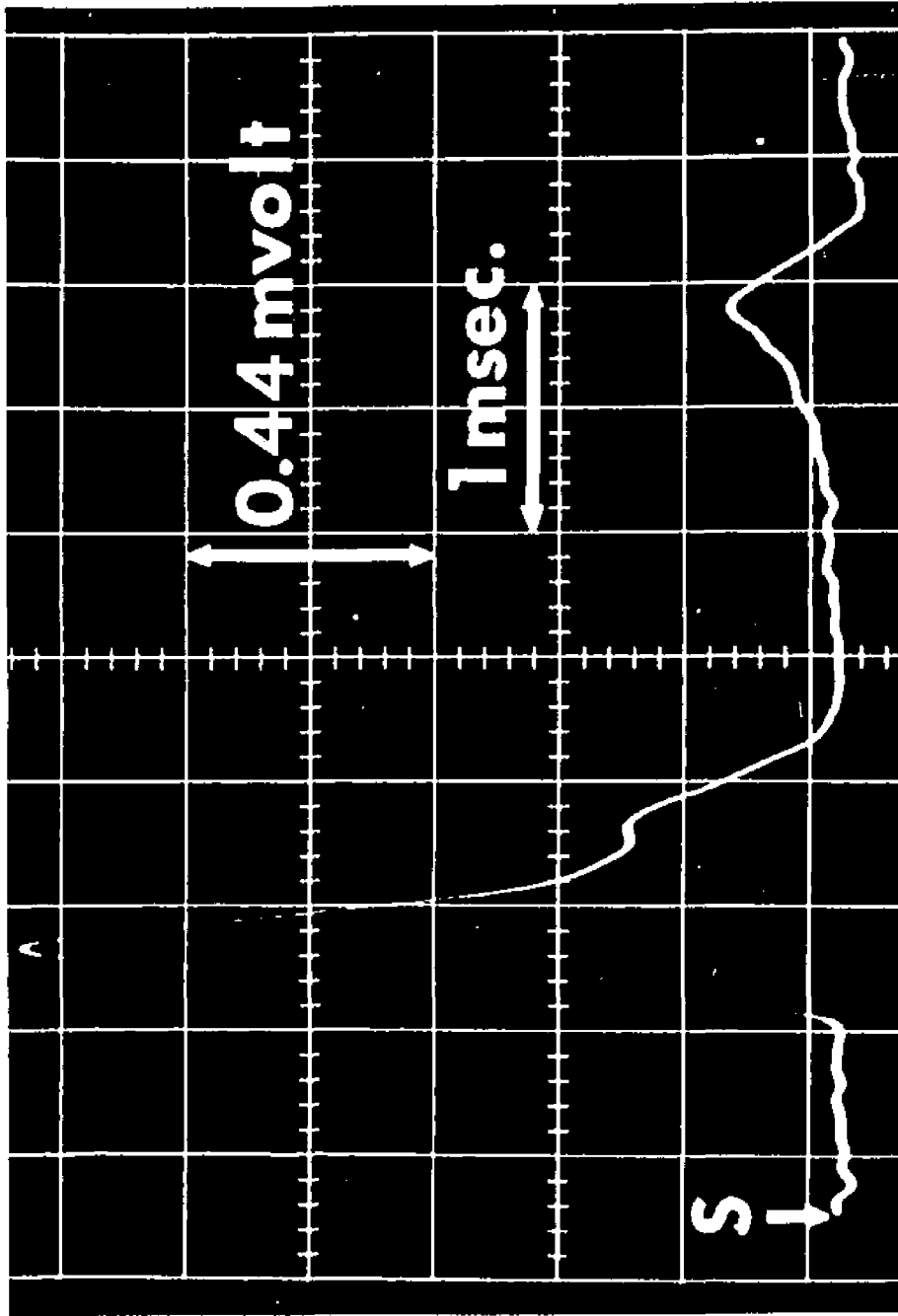


Figure 5-1. Photograph of a single recorded compound action potential from cat N. "S" marks the time when the $30 \mu\text{sec.}$ constant current electrical stimulus is applied to the nerve 6.7 cm. proximal to the recording electrode.

Thirty consecutive CAPs recorded at a rate of one per second are averaged and plotted in figure 5-2 for conduction distances of 4.2 and 6.7 cm. (cat N). The relative magnitudes are preserved for these two recordings. Note that the inflection on the decline of the first major peak in the CAP recorded at 6.7 cm. is not discernible in the CAP recorded at 4.2 cm. of conduction. The small deflection below the time axis near the origin is part of the stimulus artifact. The CAPs do fall slightly below the baseline following major peaks in the waveforms. These deflections below the baseline could not be eliminated by: (1) Additional pinching of the nerve between the two recording electrodes, (2) Application of heat to the cut end of the nerve or (3) Application of isotonic KCl to the cut end of the nerve. Thus, these waveforms are assumed to be essentially monophasic.

The CAPs in figure 5-3 for cat M are recorded and averaged for conduction distances of 4.3 and 6.8 cm. The 2.5 cm. distance between the two stimulating electrode pairs is identical for recordings from cats M and N because the same rigid electrode fixture is used in both experiments. Although relative magnitudes are preserved within each figure for the two waveforms shown, the largest CAP in each figure is self-normalized.

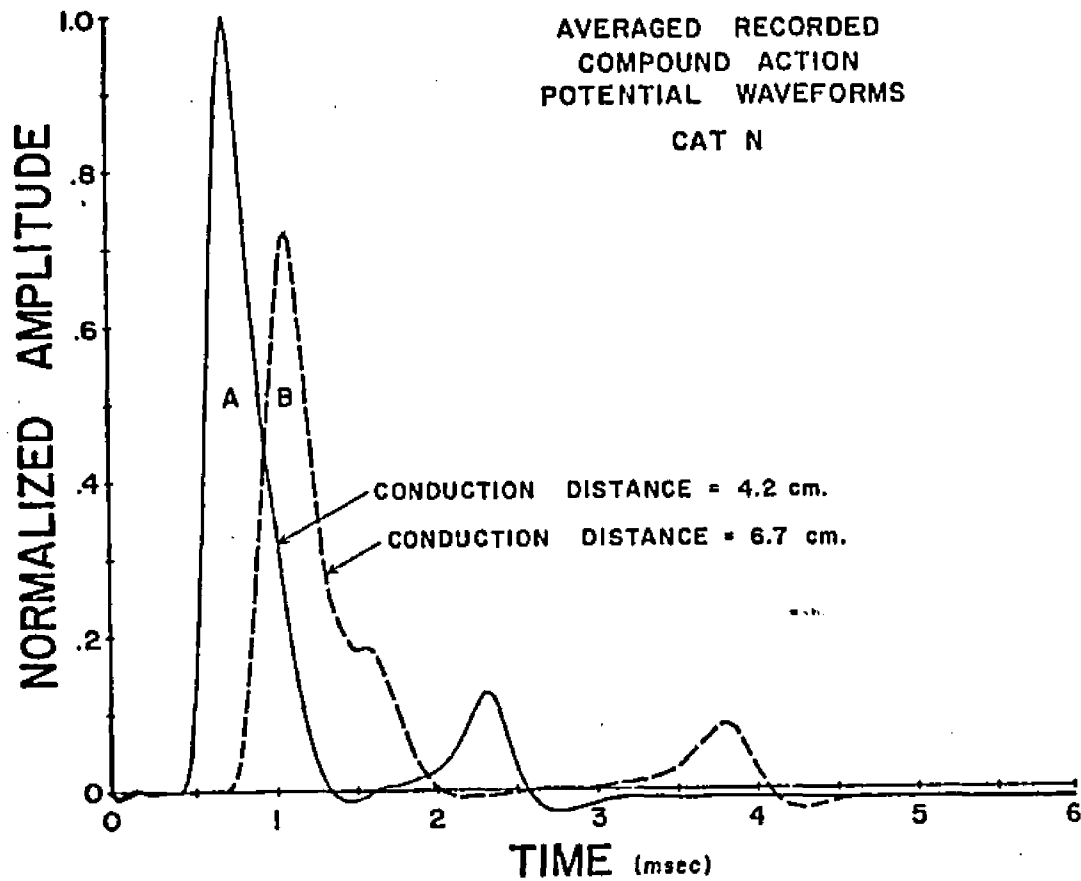


Figure 5-2. Average waveforms for compound action potentials recorded at two conduction distances from cat N. These curves are the average of 30 individual CAPs. The relative magnitudes for the two curves are preserved.

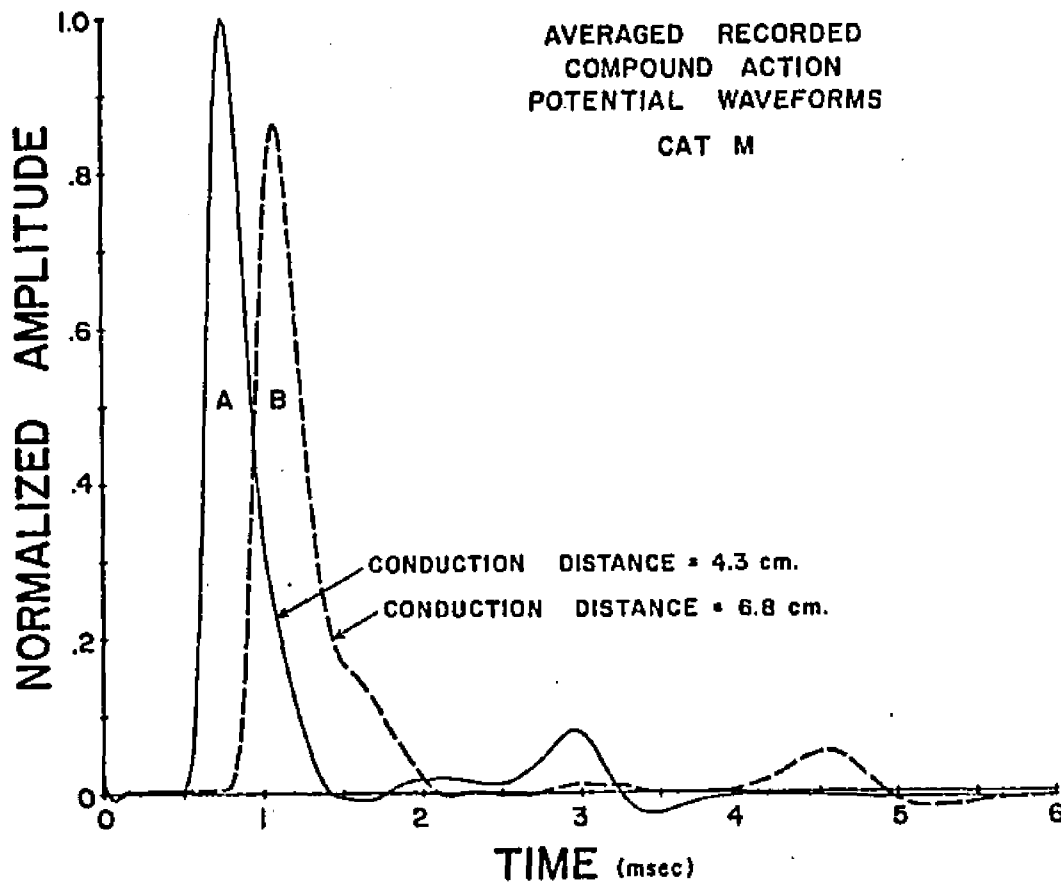


Figure 5-3. Average waveforms for compound action potentials recorded at two conduction distances from cat M. These curves are the average of 30 individual CAPs. The relative magnitudes for the two curves are preserved.

There are several interesting differences between the CAPs recorded from the two animals under nearly identical conditions. The slightly longer conduction distance for the recordings from cat M explains at least part of the larger latency in figure 5-3. Also, it is not surprising that the latency difference is greater for the last small peak that results from the small fibers.

The relative amplitude of the first peak in the waveforms for the longer conduction distance is less in figure 5-2 than in figure 5-3. Recall that the difference in the conduction distance is the same for the two experiments. This difference in relative amplitude could result from a broader distribution of the velocities (and also the diameters) for the fibers that contribute to the first peak in figure 5-2 than in 5-3. This explanation is supported by the observation that the first peak for curve A in figure 5-2 is broader than the corresponding peak in figure 5-3, despite the longer conduction distance in the latter.

The inflection on the decline of the first peak for curve B in figure 5-2 is more pronounced than the inflection for the corresponding curve in figure 5-3. This occurs in spite of the longer conduction distance for the curve with less inflection (figure 5-3).

The smaller amplitude of the last peak for small fibers in figure 5-3 may result from two effects. First,

the longer conduction distance for cat M tends to decrease the amplitude and spread out the contribution of any pile of fibers. Second, the distribution of the large fibers determines the amplitude of the tallest peak and therefore, because the CAPs are normalized, the amplitude of the small peak for each nerve is quite dependent on the composition of the large fibers.

The recorded CAP waveforms plotted in figures 5-2 and 5-3 are the mean for thirty consecutive responses to the same stimulus applied at a rate of one per second. The variability of the response is illustrated in figures 5-4 and 5-5 for CAPs from cats N and M respectively. The mean CAP and standard deviation of the mean are plotted. A normal distribution of the thirty samples at each point in time is assumed. The standard deviation is small for all values of time in both figures. The variation is slightly greater for ordinate values near zero. Inspection of figure 5-1 shows that on a single CAP recording some apparently random noise is present for small ordinate values. The small variation in the CAP waveform shape also indicates that the response is stationary for at least thirty seconds.

Equation 3-9 is the definition of the deviation for reconstructed CAPs with respect to the reconstructed CAP for the default parameter values given in table 3-1.

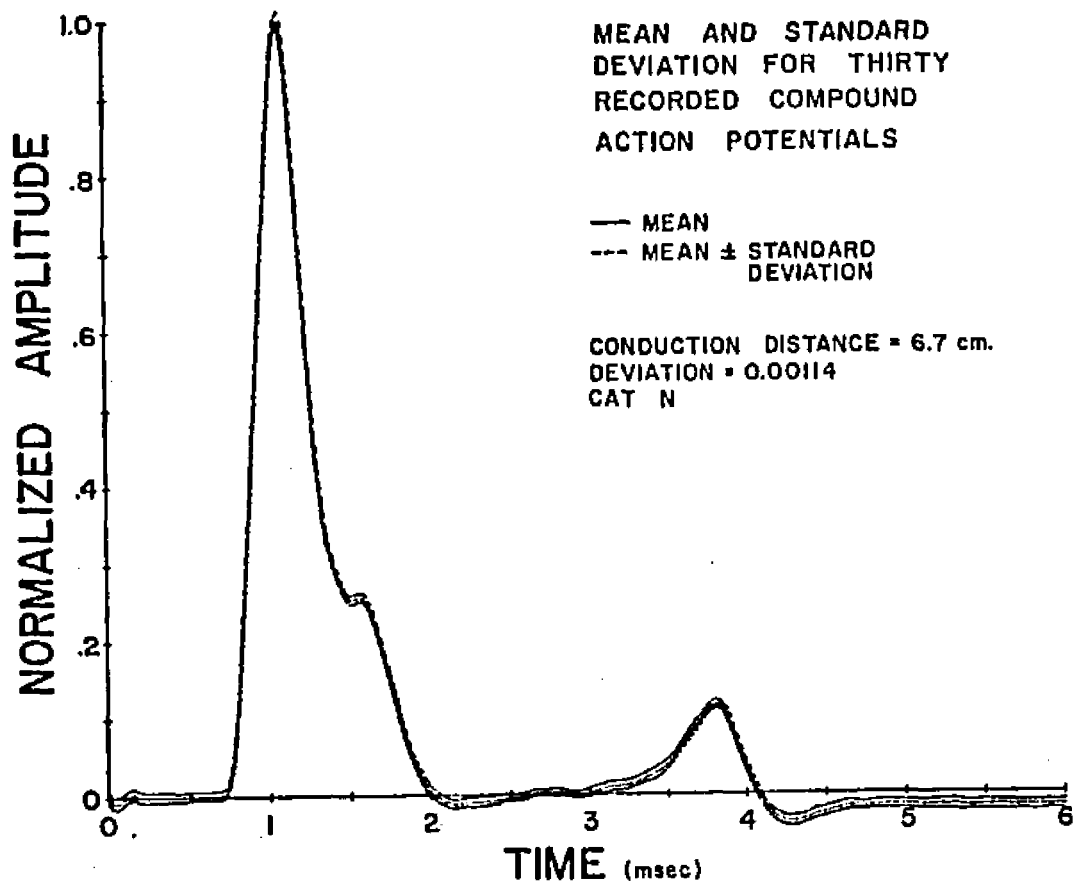


Figure 5-4. Variability of recorded CAP waveforms for cat N. The mean and standard deviation for thirty CAPs recorded one per second are plotted for the long conduction distance (6.7 cm.).

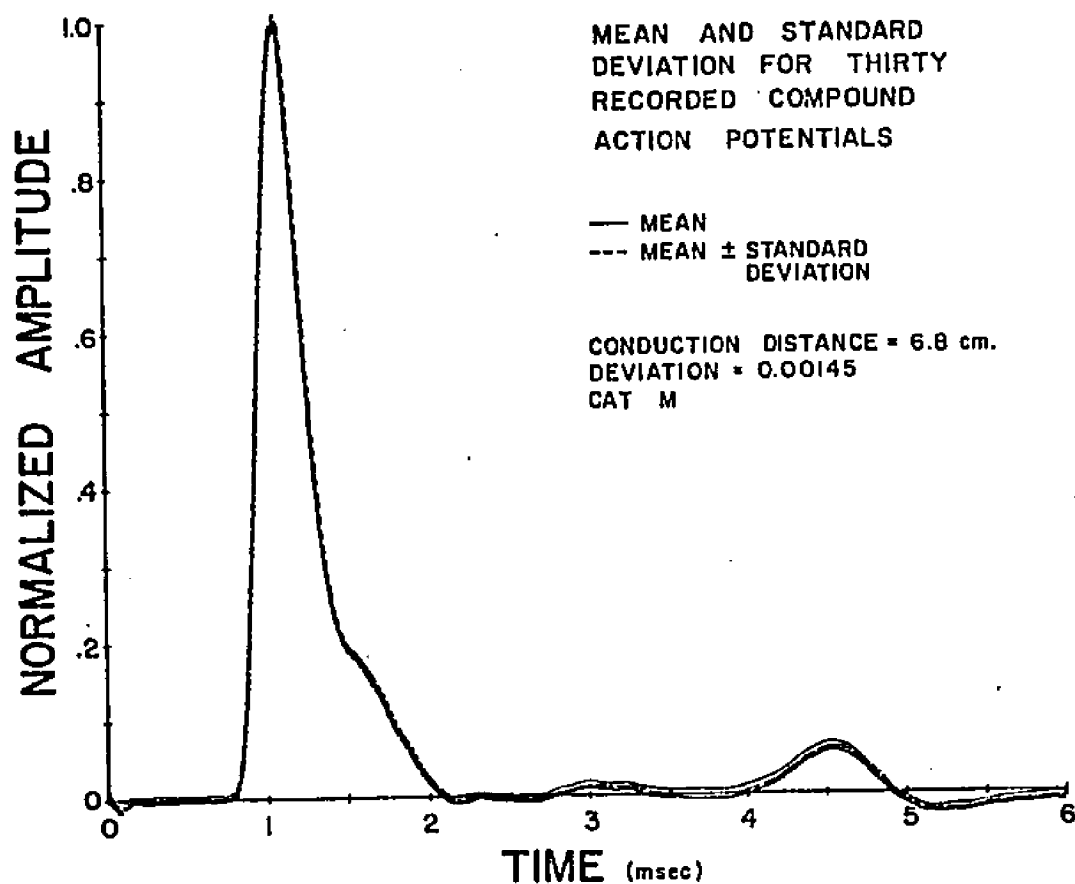


Figure 5-5. Variability of recorded CAP waveforms for cat M. The mean and standard deviation for thirty CAPs recorded one per second are plotted for the long conduction distance (6.8 cm.).

The deviation is redefined here by equation 5-1 for reconstructed or recorded CAPs with respect to the mean recorded CAP:

$$\text{deviation} \equiv \frac{\sum_{i=0}^L (V_{\max}(i\Delta t) - V_{\max \text{ recorded}}(i\Delta t))^2}{\sum_{i=0}^L (V_{\max \text{ recorded}}(i\Delta t))^2} \quad (5-1)$$

The deviations, defined by equation 5-1, for the standard deviation from the mean in figures 5-4 and 5-5 are 0.00114 and 0.00145 respectively. These small deviations show quantitatively that the variations in recorded CAP waveforms is quite small.

RECONSTRUCTED CAPS

The deviations (as defined by equation 5-1) of reconstructed CAPs from the mean CAP recorded for cat N are given in table 5-1. One CAP is reconstructed for each fiber diameter histogram compiled for cat N in Chapter IV. The name and figure number for each histogram are given. The default biophysical and axon potential wave parameter values given in table 3-1 are used for the reconstructions except for the measured 6.7 cm. conduction distance. The reconstructed and recorded CAP waveforms are plotted for fiber diameter histograms based on area and adjusted area ($X_1/X_2 \leq 0.50$) in figures 5-6 and 5-7 respectively. These figures show in detail the waveform

TABLE 5-1

Deviations for CAP reconstructions based on fiber diameter histograms for the same nerve (cat N).

<u>FIBER DIAMETER HISTOGRAM</u>	<u>FIGURE</u>	<u>DEVIATION</u>
Perimeter	4-4A	0.360
Area (Bin width=0.25 microns)	4-4B	0.174
Minor Chord	4-4C	0.488
Major Chord	4-4D	0.742
Arithmetic Mean, M_a	4-7A	0.257
Geometric Mean, M_g	4-7B	0.228
Harmonic Mean, M_h	4-7C	0.194
Adjusted Area, $X_1/X_2 \leq 0.50$	4-9A	0.136
Adjusted Area, $X_1/X_2 \leq 0.67$	4-9B	0.353
Adjusted Area, $X_1/X_2 \leq 0.83$	4-9C	0.662
Adjusted Area, $X_1/X_2 \leq 1.00$	4-9D	0.682
Neighbor, Area $\Delta\theta \leq 5^\circ$	4-11A	0.178
Neighbor, Area $\Delta\theta \leq 10^\circ$	4-11B	0.224
Neighbor, Area $\Delta\theta \leq 20^\circ$	4-11C	0.326
Neighbor, Area $\Delta\theta \leq 40^\circ$	4-11D	0.507
Neighbor, Area $X_1/X_2 \leq 0.05$	4-12A	0.210
Neighbor, Area $X_1/X_2 \leq 0.10$	4-12B	0.326
Neighbor, Area $X_1/X_2 \leq 0.20$	4-12C	0.532
Neighbor, Area $X_1/X_2 \leq 0.40$	4-12D	0.682
Neighbor, Area $\Delta\theta \leq 10^\circ$; $X_1/X_2 \leq 0.10$	4-13A	0.260
Area Bin Width=1.0 microns	4-14A	0.175
Area Bin Width=0.5 microns	4-14B	0.179
Area Bin Width=0.125 microns	4-14D	0.172

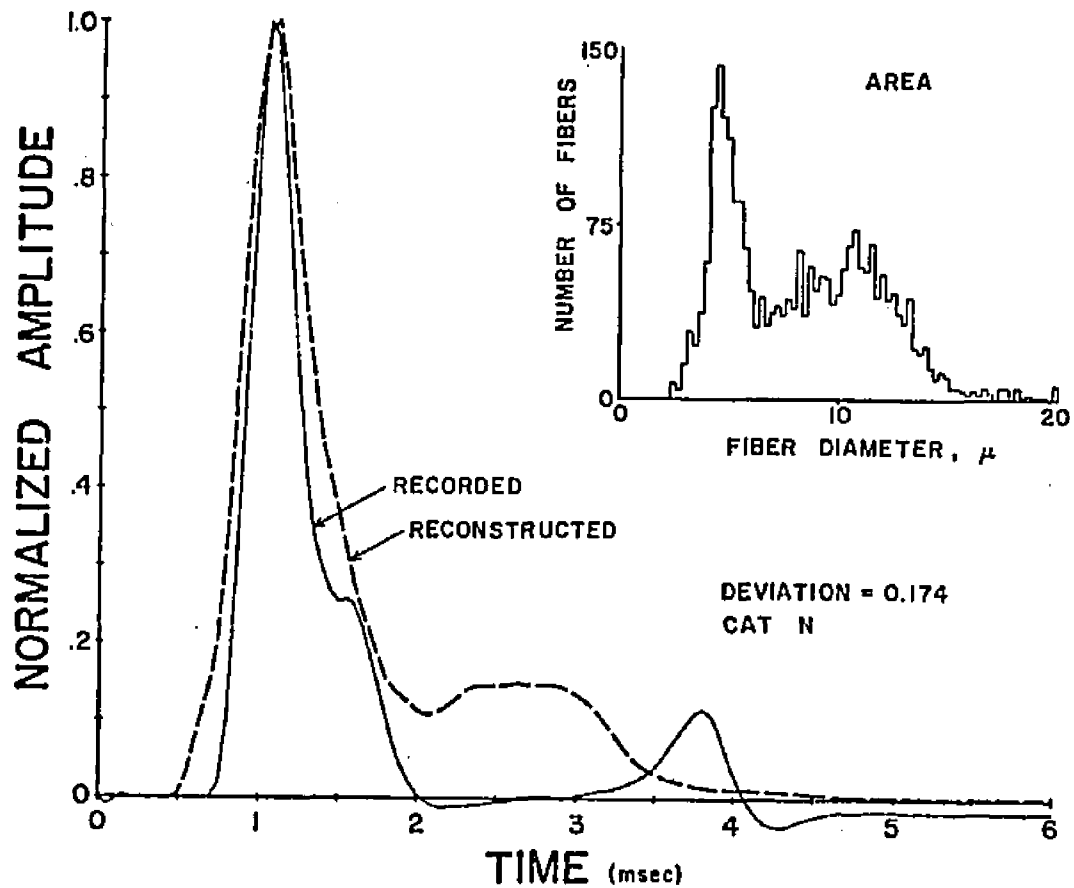


Figure 5-6. Recorded and reconstructed CAPs for cat N. The fiber diameter histogram was based on simple area (figure 4-4B) and the default biophysical and axon potential wave parameters in table 3-1 were used. The deviation was 0.174.

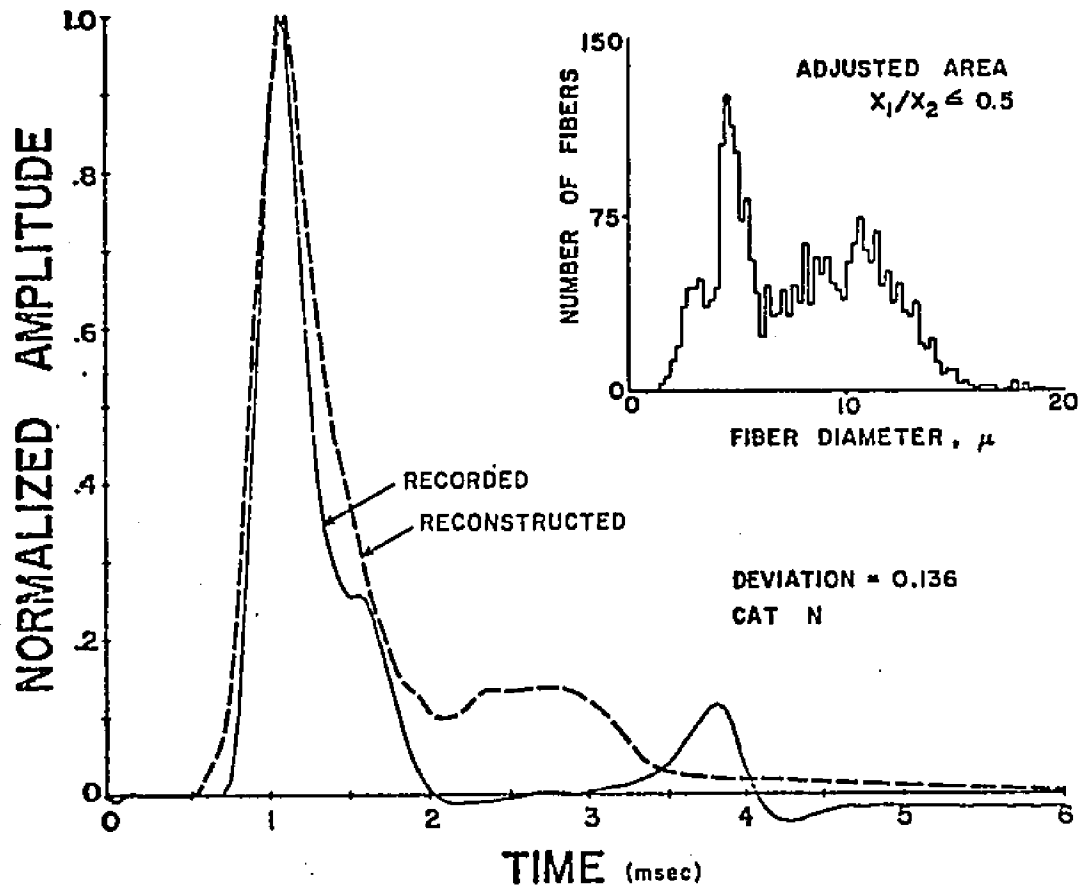


Figure 5-7. Recorded and reconstructed CAPs for cat N. The fiber diameter histogram was based on adjusted area with $X_1/X_2 \leq 0.5$ (figure 4-9C) and the default biophysical and axon potential wave parameters in table 3-1 were used. The deviation was 0.136.

differences that are responsible for two of the smaller deviations in table 5-1. The relatively small deviations in figures 5-6 and 5-7 apparently result from the close fit for the tallest peaks for large fibers.

The larger deviations in table 5-1 for histograms not based on area result primarily from poorer fits for the large dominant peak in the CAP. Note that progressively smaller means for the major and minor chords give decreasing deviations. Table 5-1 also shows that the deviation increases as all the types of area adjustment are applied to more of the noncircular fibers. As the diameter histograms shift toward smaller diameters for these adjustments the reconstructed CAPs have larger latencies and the deviation increases.

The second and last three entries in table 5-1 show that CAPs reconstructed from histograms based on area with different bin widths all have approximately the same deviation. This indicates that histogram bin width (see figure 4-14) is not a critical factor for CAP reconstructions. Recall that to obtain smooth reconstructed CAPs, the histogram is always interpolated linearly until the bin width is 0.0625 microns.

Table 5-1 should not be used to select a best estimate of fiber diameter for histogram compilation. The default values for the biophysical and axon potential wave

parameters, although based on the best information available, may be inaccurate. The deviations in table 5-1 would certainly be affected by the reconstruction parameter values. The results for Chapter III give some indication of how the deviation measure changes as the value of each parameter is varied. It is important to realize that the deviations in Chapter III are computed for a gaussian fiber diameter histogram. Other types of histograms could yield quite different deviation values.

CAP RECONSTRUCTION PARAMETER OPTIMIZATIONS

The values of the CAP reconstruction parameters are examined in this section. An anatomical fiber diameter histogram and the recorded CAP from the same nerve are both used to find reconstruction parameter values that minimize the deviation defined by equation 5-1.

One or more CAP model parameters are optimized based on calculations of the unconstrained minimum for the sum of the squared differences between the reconstructed CAP and the recorded CAP. A rapid descent method based on conjugate gradients for function minimization is used (Fletcher and Powell, 1963). This algorithm finds a local minimum for a function of "several" variables. If the function has more than one minimum, the minimum found is unpredictable and may depend on the initial parameter values. Only obviously inappropriate additional minimums are encountered for

the parameters studied below.

The optimization program is terminated when the deviation is less than or equal to the deviation computed for the recorded CAP standard deviation shown in figures 5-4 and 5-5. The optimization is also terminated if the deviation fails to decrease significantly for several iterations.

The choice of a fiber diameter estimate for the histogram used in the parameter optimizations is important because the results will vary somewhat for different histograms. The diameter histogram based on area adjusted by neighbor analysis for $\Delta\theta \leq 20^\circ$ (figure 4-11C) is used as the best available estimate of nerve morphology. This choice is based primarily on the qualitative evidence discussed in Chapter VII. The results in this section and Chapter VI could be repeated easily for any diameter histogram given in Chapter IV or for fiber diameter histograms obtained by other methods developed in the future.

Figure 5-8 shows the results for simultaneous optimization of the conduction velocity constant (CVC), the conduction velocity exponent (CVE), and the axon potential height exponent (APE). The conduction distance (DIST) is not optimized because it is measured directly during the experiment. The solid curve is the averaged recorded CAP. The reconstructed CAP for the initial default parameter values is plotted with short dashes (entry 14 in table

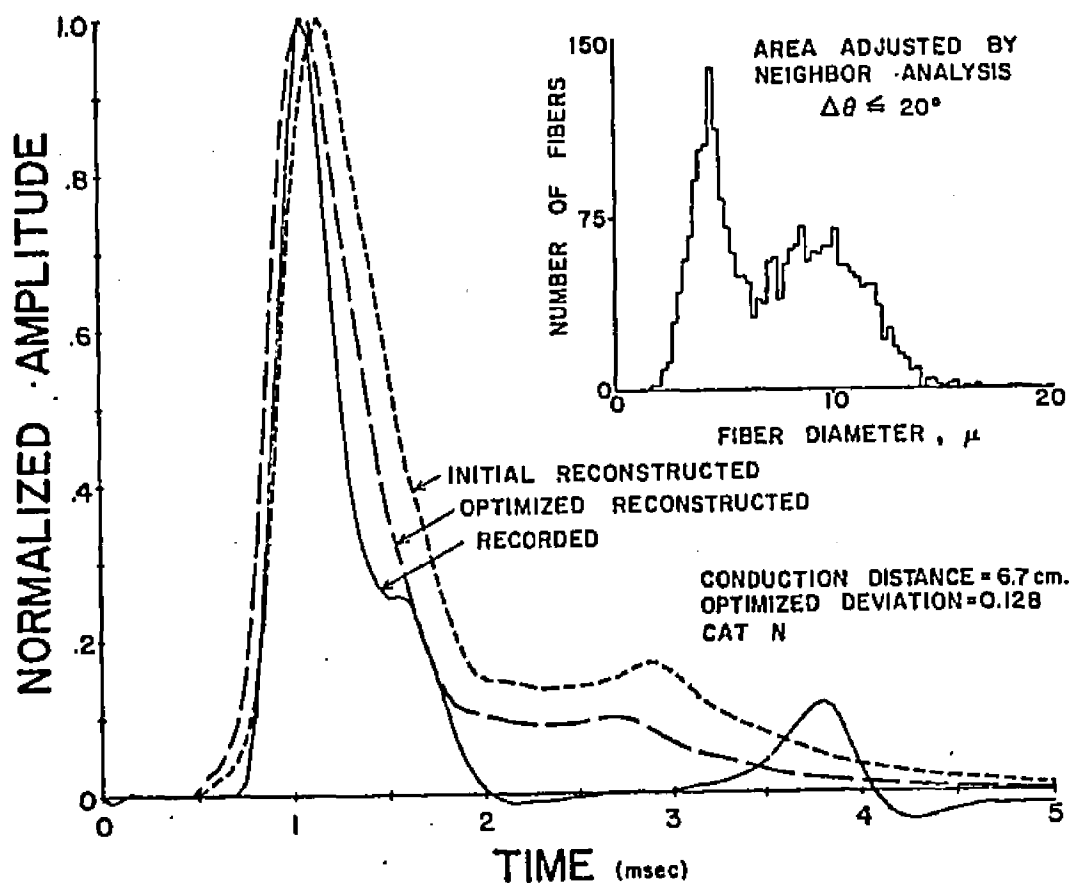


Figure 5-8. Recorded and reconstructed CAPs with parameter optimization of CVC, CVE and APE. The other parameters were fixed at the default values. The fiber diameter histogram was based on area adjusted by neighbor analysis for $\Delta\theta \leq 20^\circ$ (figure 4-11C). CVC=9.2; CVE=1.0; APE=1.6. The deviation was 0.128.

5-1). Finally, the curve for the reconstructed CAP for optimized parameter values of CVC, CVE and APE is plotted with long dashes. CVC and APE increase from 8.8 to 9.2 and from 1.0 to 1.6 respectively. All attempts to optimize CVE fail to show a significant departure from unity. The deviation is less than the minimum deviation (0.136) in table 5-1 for CAP reconstructions with default parameter values for any of the fiber diameter histograms in Chapter IV.

The default relationship for $g(d_1)$ (ratio of axon diameter to fiber diameter) that is used in all the above reconstructions decreases for small fibers. Recent data, cited in the introduction, show that "g" measured with the electron microscope remains nearly constant for all fiber diameters. If "g" is constant, then CVC and "g" in equation 3-4 can be combined:

$$t_1 = f(d_1) = \frac{\text{DIST}}{\text{CVS} \cdot d_1^{\text{CVE}} + \text{CVI}} \quad (5-2)$$

where conduction velocity slope (CVS) is the product of CVC and "g" ; and conduction velocity intercept (CVI) is added to simulate a non-zero intercept for the relationship between conduction velocity and fiber diameter. Extrapolation to zero fiber diameter for the line drawn by Hursh (1939) on his figure 2 (conduction velocity versus fiber diameter) results in a small negative CVI.

Figure 5-9 shows the recorded CAP (solid line) and the optimized reconstructed CAP (dashed line) for optimization of parameters CVS, CVI, and APE. Both CVS and APE increase from 6.0 to 6.7 and from 1.0 to 2.4 respectively. The intercept CVI remains at the initial zero value. The deviation decreases to 0.056 apparently because the fit for the tallest peak is now quite good. Note, however that the later smaller peaks in the recorded CAP are not reconstructed.

The reader has probably noted that none of the above reconstructions had a peak for the small fiber response that even approached the last small peak in the recorded waveform. Also, none of the reconstructions fell to zero after 2 msec. Furthermore, none of the fiber diameter histograms compiled for this nerve had a significant gap between the large and small fibers that could account for the gap between the peaks in the CAP. It became apparent that some nonlinear relationship between conduction velocity and fiber diameter was required to account for the much slower conduction velocities for small fibers. Several piecewise linear and exponential relationships between conduction velocity and fiber diameter were examined with little success. Finally, equation 5-2 was replaced by two linear equations that had different CVS's and CVI's for different ranges of fiber diameter.

The reconstructed CAP shown in figure 5-10 is for

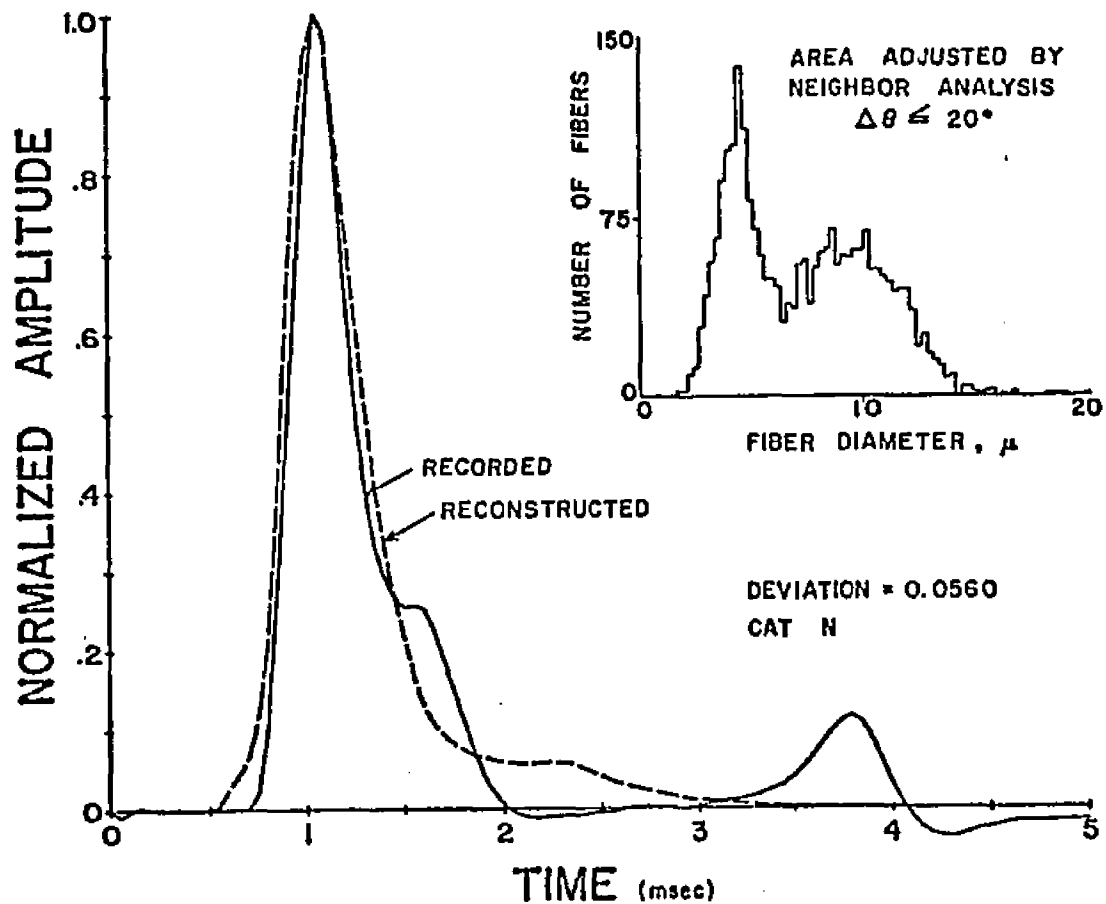


Figure 5-9. Recorded and reconstructed CAPs with parameter optimization for CVS, CVI and APE. The other parameters remained at the default values and the fiber diameter histogram based on area adjusted by neighbor analysis for $\Delta\theta \leq 20^\circ$ (figure 4-11C) was used. CVS= 6.7; CVI=0.0 APE=2.4. The deviation was 0.0560.

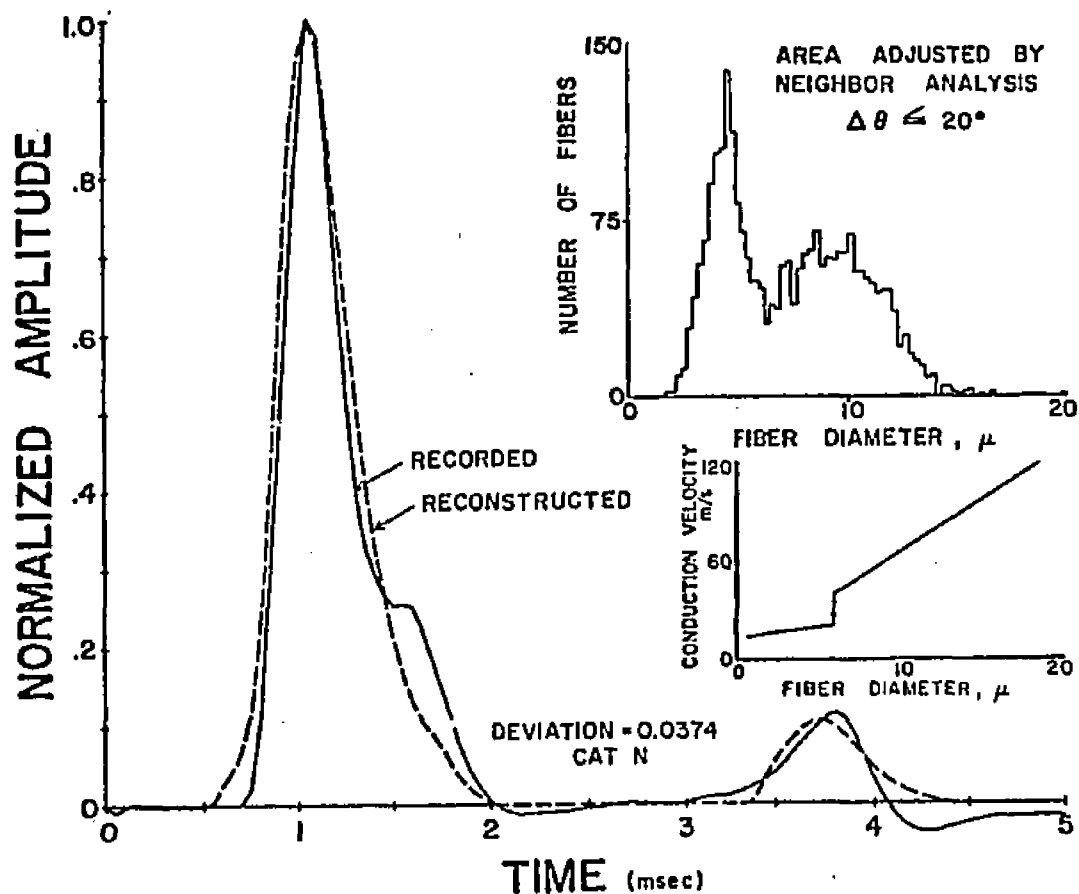


Figure 5-10. Recorded and reconstructed CAPs with parameter optimization for CVS and CVI for fibers less than 6 microns in diameter. All other parameter values were the same as for figure 5-9. The relationship between conduction velocity and fiber diameter specified in equation 5-3 was plotted as an inset. For less than 6 micron fibers: CVS=1.2 and CVI=13.0. The deviation was only 0.0374.

the piecewise linear relationship between latency and fiber diameter given by equation 5-3,

$$t_1 = f(d_1) \left\{ \begin{array}{ll} = \frac{\text{DIST}}{6.7 \cdot d_1 + 0.0} & d_1 \geq 6\mu \\ = \frac{\text{DIST}}{1.2 \cdot d_1 + 13.0} & d_1 < 6\mu \end{array} \right. \quad (5-3).$$

The denominators in equation 5-3 are conduction velocity as a function of fiber diameter, plotted as an inset in figure 5-10. The discontinuity for conduction velocity at 6 microns does separate the two components of the CAP reconstruction such that the recorded and reconstructed CAPs in figure 5-10 are quite similar. However, only a small inflection occurs on the decline of the first major peak in the reconstruction whereas a much larger inflection is present in the recorded CAP. The deviation for the CAP reconstruction in figure 5-10 is only 0.0374. This is the minimum deviation achieved for a reconstructed CAP.

Undoubtedly better fits could be obtained with more complicated nonlinear relationships between conduction velocity and fiber diameter. However, this approach to better reconstructions does not seem to be warranted until such time as more accurate fiber diameter histograms are available. Also, additional biophysical data are needed for the relationship between conduction velocity and fiber diameter, particularly for small fibers.

CHAPTER VI

PREDICTION OF FIBER DIAMETER HISTOGRAMS FROM CAPS

A procedure for predicting the shape of a fiber diameter histogram from a recorded CAP is presented in this chapter. The CAP recorded from cat N and the optimized biophysical and axon potential wave parameters found in Chapter V are used to predict a fiber diameter histogram. The predicted and the anatomical fiber diameter histograms are compared. Then, the CAP recorded from cat M and the biophysical and axon potential wave parameters found from the data for cat N are used to predict the fiber diameter histogram for cat M. The predicted and the anatomical diameter histograms are compared once again.

FIBER DIAMETER HISTOGRAM PREDICTION TECHNIQUES

The new technique presented here is used to predict the shape of a fiber diameter histogram from a recorded monophasic compound action potential. The measured conduction distance and some limits on the range of fiber diameter must also be specified.

Equation 3-8 is repeated here for reference:

$$V_{\max}(k\Delta t) = \sum_{i=1}^M n_i d_i^{\text{APE}} \cdot v(k\Delta t - f(d_i)), k=0,1,\dots,L \quad (6-1).$$

The n_i in each term of equation 6-1 appear only once as a multiplicative factor. This property of the $L+1$ linear equations makes it possible to express equation 6-1 in

matrix notation:

$$\begin{bmatrix} v_{\max_0} \\ v_{\max_1} \\ \vdots \\ \vdots \\ v_{\max_L} \end{bmatrix} = \begin{bmatrix} \alpha_{01} & \alpha_{02} & \dots & \alpha_{0M} \\ \alpha_{11} & \alpha_{12} & \dots & \alpha_{1M} \\ \vdots & \vdots & & \vdots \\ \vdots & \vdots & & \vdots \\ \alpha_{L1} & \alpha_{L2} & \dots & \alpha_{LM} \end{bmatrix} \cdot \begin{bmatrix} n_1 \\ n_2 \\ \vdots \\ \vdots \\ n_M \end{bmatrix} \quad (6-2)$$

where $\alpha_{ki} = d_i^{APE} \cdot v(k\Delta t - f(d_i))$. The vector $V_{\max}$ represents the discrete CAP and the vector N represents the ordinate values in the fiber diameter histogram. The $L+1$ by M matrix A with elements α includes all the biophysical and axon potential wave information. The CAP reconstructions simply require that the right side of either equation 6-1 or 6-2 be evaluated for the assumed biophysical and axon potential wave parameters and an anatomically derived fiber diameter histogram.

The fiber diameter histogram (vector N) can be found if the recorded CAP (vector $V_{\max}$) and a reliable set of biophysical and axon potential wave parameters (matrix A) are available. One requirement for a unique solution is that: $L+1 \leq M$. This simply means that there must be at least one linear equation for each unknown bin (n_i) in the fiber diameter histogram. Also the smallest fiber diameter and the biophysical parameters must be such that the maximum axon potential latency, t_1 , is less than $L \cdot \Delta t$.

An optimization approach that uses a conjugate gradient technique is utilized to evaluate the n_1 in equations 6-1 and 6-2. Initially the n_1 are all made equal and then each n_1 is adjusted to minimize the sum of the squared differences between the reconstructed and the recorded CAPs. This method is reasonable because matrix A must be large (about 120×80)¹ for precise reconstructions, yet matrix A is quite sparse (less than 5% non-zero elements). The optimization program written for this study saves a great deal of computer time by performing the multiplications in equation 6-2 only for non-zero α 's.

The inverse relationship between axon potential latency, t_1 , and fiber diameter, d_1 , (see equations 3-4, 3-5, 5-2, 5-3) makes the reconstructed axon potential waves overlap more extensively for small t_1 than for large t_1 . The n_1 for large fibers converge much faster than the n_1 for small fibers. More rapid overall convergence is achieved if diameter histogram bin width is made to decrease as fiber diameter decreases. This trick distributes the axon potential waves more evenly over time and reduces the computer time required for fiber diameter histogram predictions.

The optimization procedure for equal bin width histograms is illustrated in figure 6-1. Note that this figure

¹This estimate is based on 120 samples along the CAP abscissa (6 msec. at 0.05 msec/sample) and 80 bins in the fiber diameter histogram (bin width of 0.25 microns).

Figure 6-1.

Test of the histogram prediction technique. The "recorded" CAP in B was reconstructed from the "anatomical" histogram in A. The "initial" CAP was reconstructed from the uniform "initial" histogram. The "recorded" and the "initial" CAPs were compared and the "initial" histogram was adjusted so as to reduce the difference between the "recorded" and the new reconstructed CAP. This process was repeated until the reconstructed CAP ("predicted") nearly matched the "recorded" CAP. The resultant "predicted" histogram was essentially the same as the anatomical histogram originally used to reconstruct the "recorded" CAP.

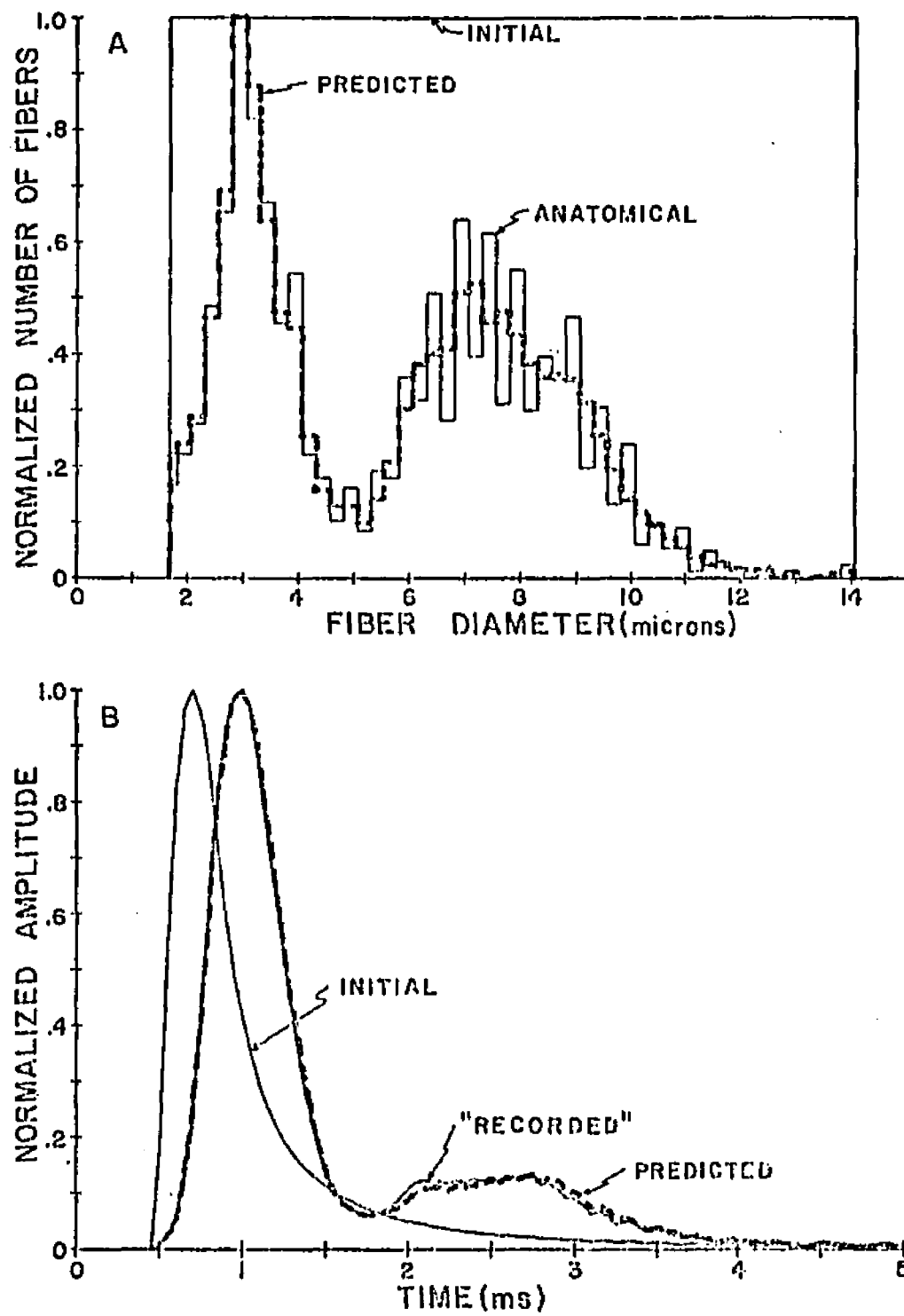


Figure 6-1.

contains three fiber diameter histograms (A) and three CAP waveforms (B). The following logical steps are important to understand this test of the procedure:

1. The reconstruction model described above is used to generate a "recorded" CAP waveform, based on the "anatomical" histogram shown (Gasser and Grundfest, 1939, figure 5). Realistic experimental conditions and biophysical parameter values are assumed. This preliminary step, not actually a part of the histogram prediction program, is necessary to establish a CAP waveform and its underlying fiber diameter histogram for the following test of the histogram prediction program.
2. The "recorded" CAP waveform is used as the input for the histogram prediction program. Starting with a uniform fiber diameter histogram (unbiased) labelled "initial", the program uses the reconstruction model to compute a first resultant CAP, also labelled "initial". The "recorded" and "initial" CAPs are then compared according to a cost function based on the mean square ordinate difference between the two waveforms.
3. The optimization program then alters the "initial" histogram systematically using conjugate gradients, and compares the resultant reconstructed CAP with

the "recorded" waveform. The histogram is changed by a succession of steps that work toward minimizing the difference between the resultant CAP waveform and the "recorded" waveform (the cost function is minimized).

4. The optimization procedure is terminated when the deviation for the reconstructed CAP with respect to the "recorded" waveform becomes less than the deviation for the variability in actual recorded CAPs (figures 5-4 and 5-5). The procedure is also terminated if the deviation fails to decrease significantly after several consecutive iterations. The final "predicted" CAP and the "predicted" histogram are shown in figure 6-1.

The agreement between the "anatomical" histogram used for step 1 and the "predicted" histogram is very good. Note that the "predicted" histogram has fewer spurious oscillations than the "anatomical" histogram. If the optimization is allowed to continue indefinitely, the two histograms should eventually match exactly because the "anatomical" histogram is used to get the "recorded" CAP in the first place. The fact that the "predicted" and the "anatomical" histograms are almost identical is strong evidence that this procedure can be used to predict fiber diameter histograms from recorded CAPs. It is important to realize that

this procedure predicts only the relative number of fibers of any particular diameter and not the actual number of fibers. Many factors affect the amplitude of recorded CAPs, so that even empirical estimates of an ordinate scale factor for the histogram are not likely to be reliable.

VARIABLE BIN WIDTH HISTOGRAMS

The histogram prediction program converges much faster for variable bin width diameter histograms, as mentioned above. Bin widths that decrease linearly with fiber diameter make the t_1 increase linearly as the d_1 decrease. Figure 6-2A shows a variable bin width histogram compiled directly from the raw data. The fiber diameter is based on area adjusted by neighbor analysis for $\Delta\theta \leq 20^\circ$. The histogram based on the same diameter estimate, compiled directly from the same raw data, for equal bin widths, is plotted in figure 6-2B (solid line). The variable bin width histogram in figure 6-2A is converted to the equal bin width histogram in figure 6-2B (dashed line) by an algorithm developed for this study. The two histograms in figure 6-2B are nearly identical. All three histograms in figure 6-2 have the same total number of fibers (2496). This and other tests of the conversion algorithm indicate that predicted variable bin width histograms can be faithfully converted to equal bin width histograms.

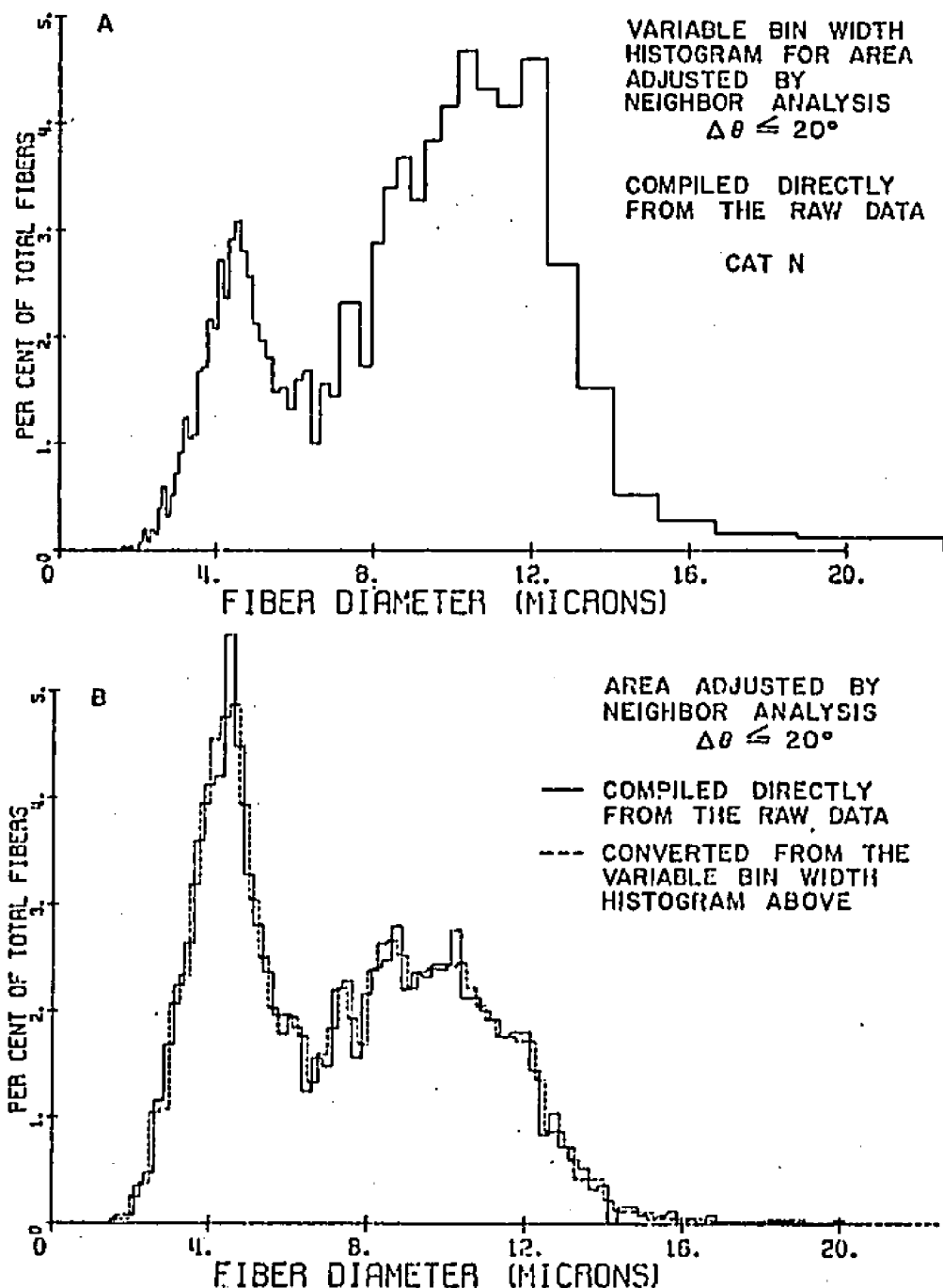


Figure 6-2. Variable and equal bin width diameter histograms. A. The variable bin width histogram was compiled directly from the data for each fiber. B. The equal bin width histogram (solid line) was compiled directly from the data for each fiber. The variable bin width histogram in A was converted to an equal bin width histogram in B (dotted line). The two histograms in B are very similar.

PREDICTED HISTOGRAMS FOR CAT N

Figure 6-3 is similar to figure 6-1, except the initial fiber diameter histogram and the initial CAP are omitted. Also, the diameter histograms have variable bin widths; and the "recorded" CAP is the actual averaged recorded CAP (solid curve) from cat N for a conduction distance of 6.7 cm.

The default biophysical and axon potential wave parameters given in table 3-1 are used to obtain the predicted variable bin width histogram in figure 6-3. The anatomical histogram in figure 6-3A is based on area without adjustments. The reconstructed and recorded CAPs for these conditions are plotted in figure 5-6. The recorded and predicted reconstructed CAPs in figure 6-3 match closely. The predicted histogram in figure 6-3A is negative for some small fiber diameters. This is a consequence of recorded CAP deflections below the baseline and entirely positive axon potential waves. This would not occur if the recorded CAP were truly monophasic. The predicted histogram peak for small fibers in figure 6-3A is very tall and narrow. The corresponding equal bin width histograms are plotted in figure 6-4. The predicted peak for small fibers is even taller relative to the anatomical histogram in this figure. Also note that the predicted histogram peaks for large and intermediate size fibers are much

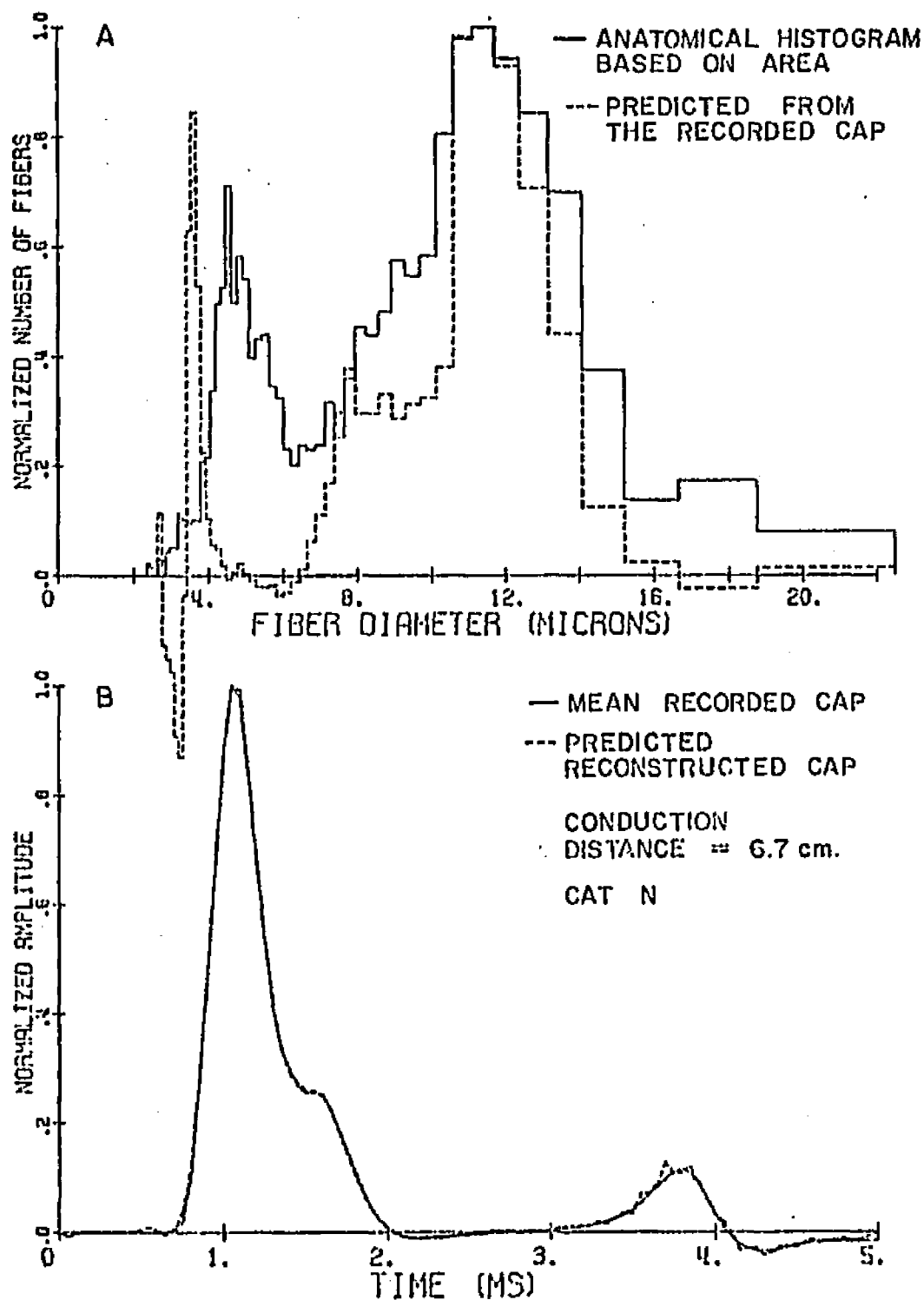


Figure 6-3. Diameter histogram prediction for default biophysical and axon potential wave parameters. Conduction distance = 6.7 cm. Cat N.

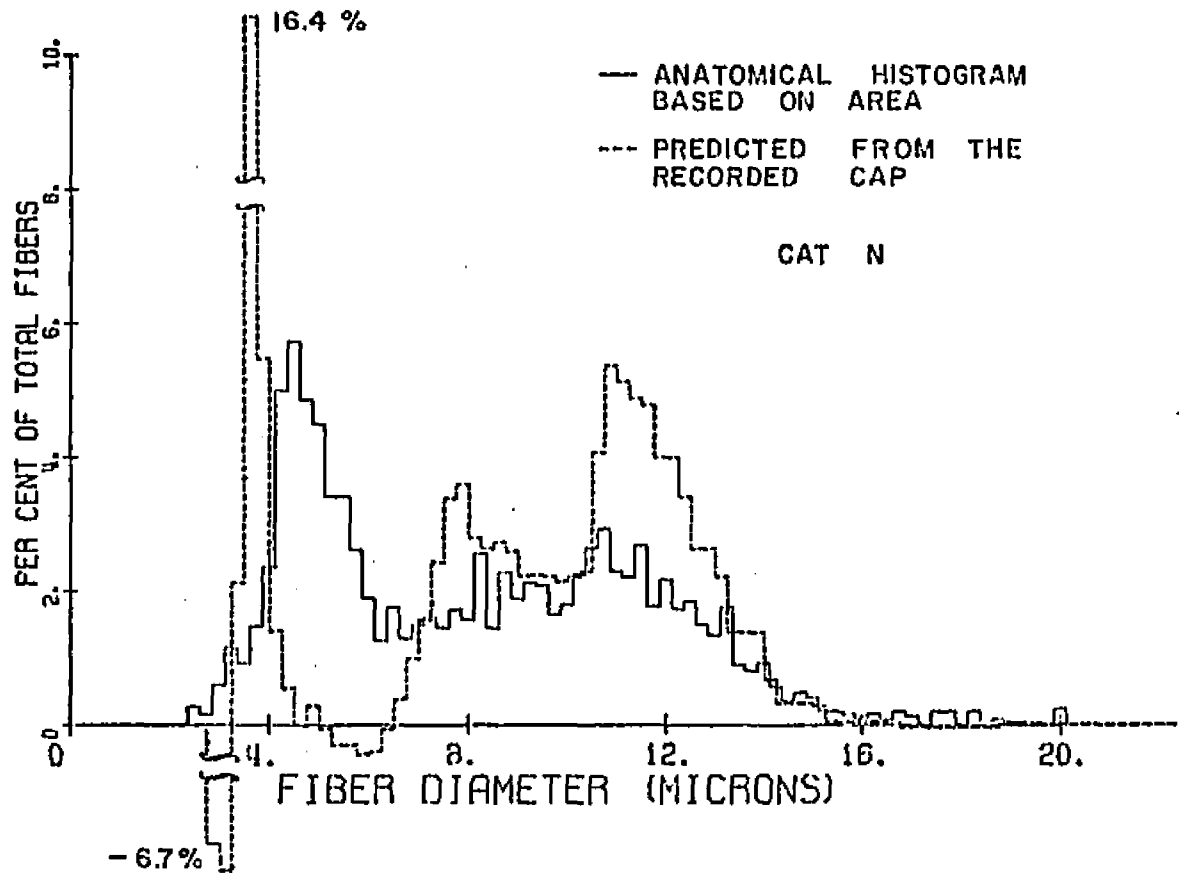


Figure 6-4. Equal bin width anatomical and predicted diameter histograms for default biophysical and axon potential wave parameters. Cat N.

larger than the corresponding anatomical histogram peaks in figure 6-4. It is apparent that histograms predicted for the default biophysical and axon potential wave parameters are not promising.

The biophysical and axon potential wave parameters used for the results in figure 6-5 are the optimized values found for the reconstruction shown in figure 5-10. The deviation for the predicted CAP in figure 6-5B is only 0.00338. Note that for the largest peak in the CAP, the recorded and predicted CAPs in figure 6-5B agree much better than the recorded and reconstructed CAPs in figure 5-10. The fit is comparable for the small fiber peaks in the same two figures.

The predicted fiber diameter histogram in figure 6-5A (dotted line) can be compared with the anatomically derived fiber diameter histogram from the same nerve (solid line). The fiber diameter for the anatomical histogram is based on area adjusted by neighbor analysis for $\Delta\theta \leq 20^\circ$ (figure 4-11C). The reasons for this choice are discussed in Chapter VII. The corresponding predicted and anatomical equal bin width histograms are shown in figure 6-6. The ordinate variable in figure 6-6 is percent of total fibers. Although the total number of fibers in the anatomical histogram is known (2496), the total number of fibers in the predicted histogram is not available. Therefore, the

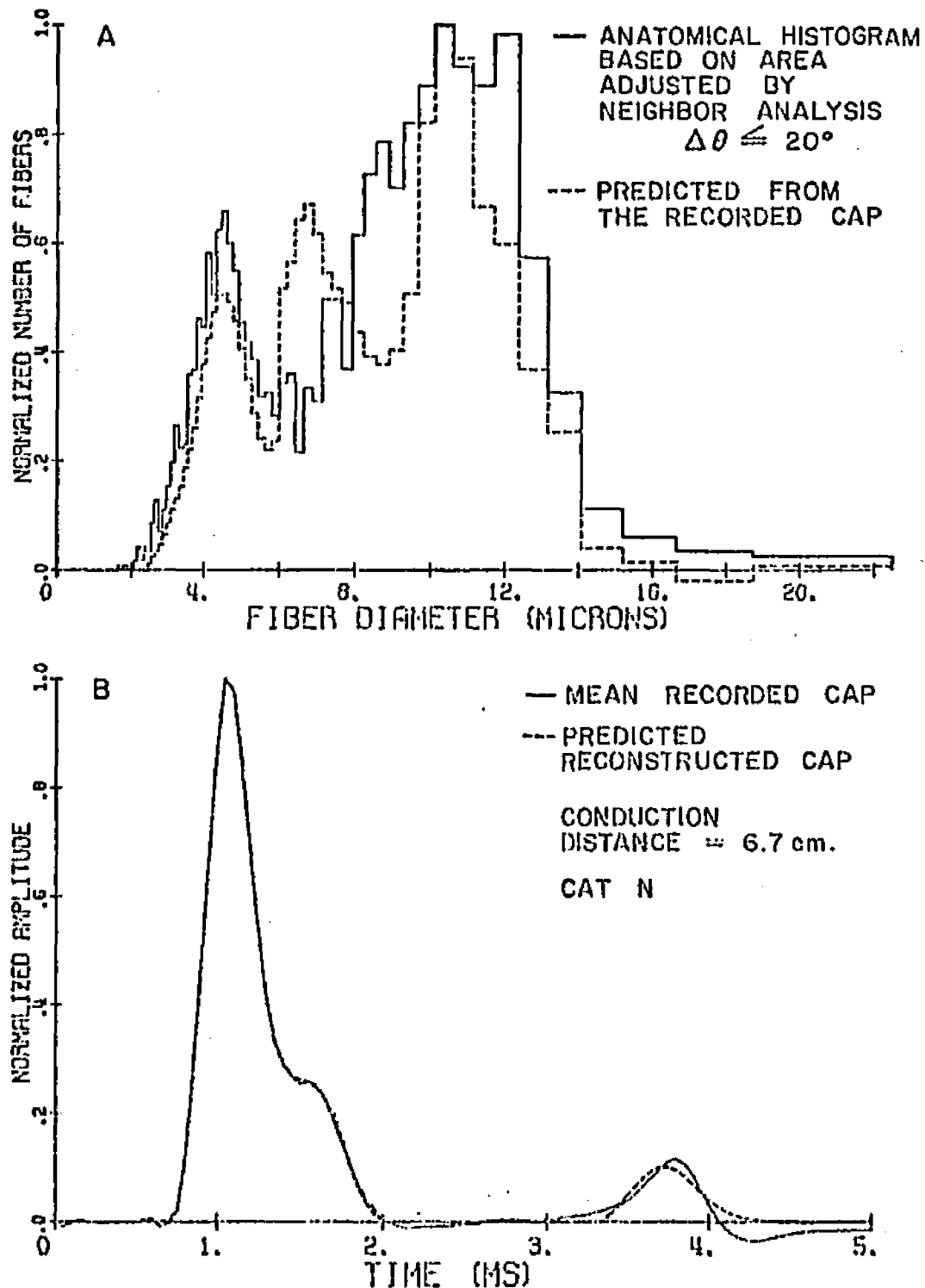


Figure 6-5. Diameter histogram for optimized biophysical and axon potential wave parameters. Non-default parameter values are: for $d_1 \geq 6\mu$: CVS=6.7 and CVI=0.0; for $d_1 < 6\mu$: CVS=1.2 and CVI=13.0; APE=2.4.

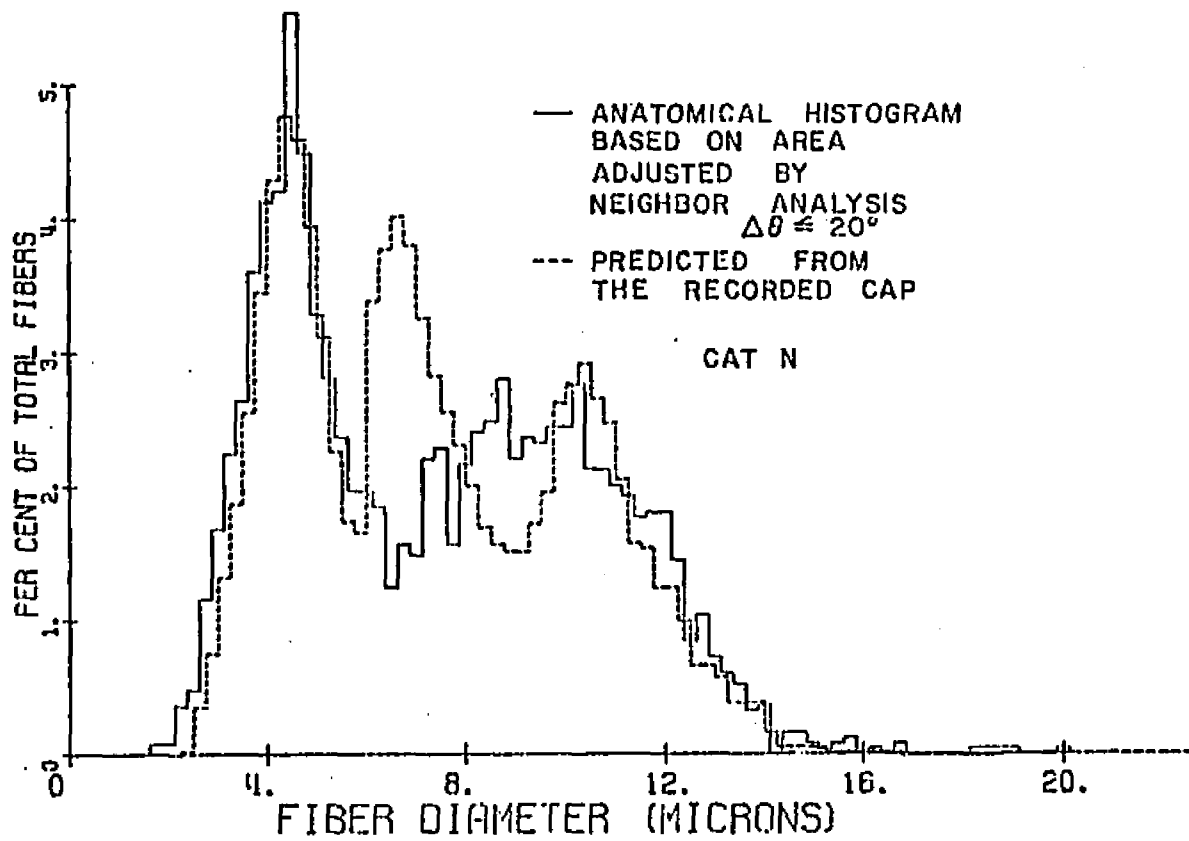


Figure 6-6. Equal bin width anatomical and predicted diameter histograms for optimized biophysical and axon potential wave parameters. Conduction distance= 6.7 cm. Cat N.

actual number of fibers in any bin of the predicted histogram is also unknown. Thus the predicted histogram gives only the relative number of fibers for each fiber diameter.

The two histograms in figure 6-6 are quite similar except for the region between 6 and 10 microns. The appearance of a third prominent intermediate peak in the predicted histogram is not surprising because figure 5-10 shows that the reconstructed CAP for this anatomical histogram does not have the same large inflection on the decline of the first major peak that is present in the recorded and predicted CAPs in figure 6-5B. The large change in the histogram at 6 microns occurs because in the model the functional relationship between conduction velocity and fiber diameter changes abruptly at 6 microns (figure 5-10, inset).

The central peak in the predicted histogram decreases in amplitude and moves slightly to the right if the discontinuous piece-wise linear relationship between conduction velocity and fiber diameter given by equation 5-3 is smoothed (simulation not shown). Such refinements of the piece-wise linear relationship between conduction velocity and fiber diameter do not seem appropriate until better empirical data for this functional relationship is forthcoming. This topic will be pursued further in Chapter VII.

The CAP recorded from cat N for the shorter conduction

distance (4.2 cm) is also used to predict a fiber diameter histogram, as shown in figure 6-7. The major difference between the results for figures 6-7 and 6-5 is the conduction distance. The predicted histogram should be approximately the same for both conduction distances. The recorded and predicted CAPs in figure 6-7 have very similar first peaks, while differences between the second peaks appear to be largely responsible for the 0.0050 deviation.

The predicted histogram in figure 6-7A is replotted for equal bin widths in figure 6-8. The predicted histogram for the shorter conduction distance is shifted somewhat to the right compared to the predicted histogram for the longer conduction distance in figure 6-6. The appearance of an intermediate predicted peak in figure 6-6 at about 7 microns is significant because no inflection is visible on the decline of the predicted CAP at the 4.2 cm. conduction distance (figure 6-5B). At the longer conduction distance for the same nerve a sharp inflection does appear (figure 6-3B) and a corresponding peak in the predicted histogram (figure 6-4) is also present. The appearance of the intermediate peak in figure 6-6 suggests that the predicted histogram is very sensitive to the shape of the CAP.

PREDICTED HISTOGRAMS FOR CAT M

The CAP recorded from cat M for a conduction distance of 6.8 cm. is used to predict a histogram for cat M. The

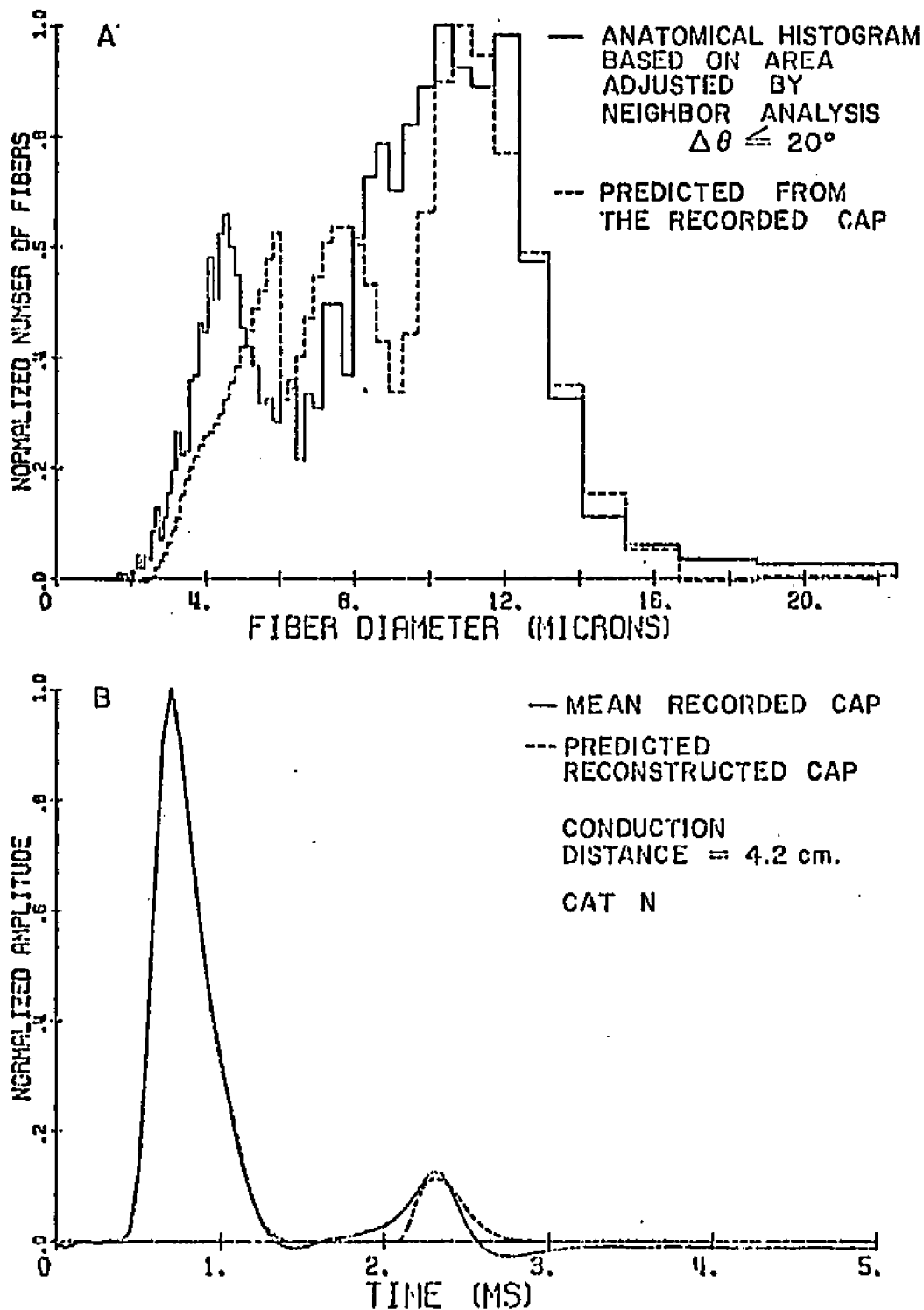


Figure 6-7. Diameter histogram prediction for optimized biophysical and axon potential wave parameters. Conduction distance = 4.2 cm. Cat N.

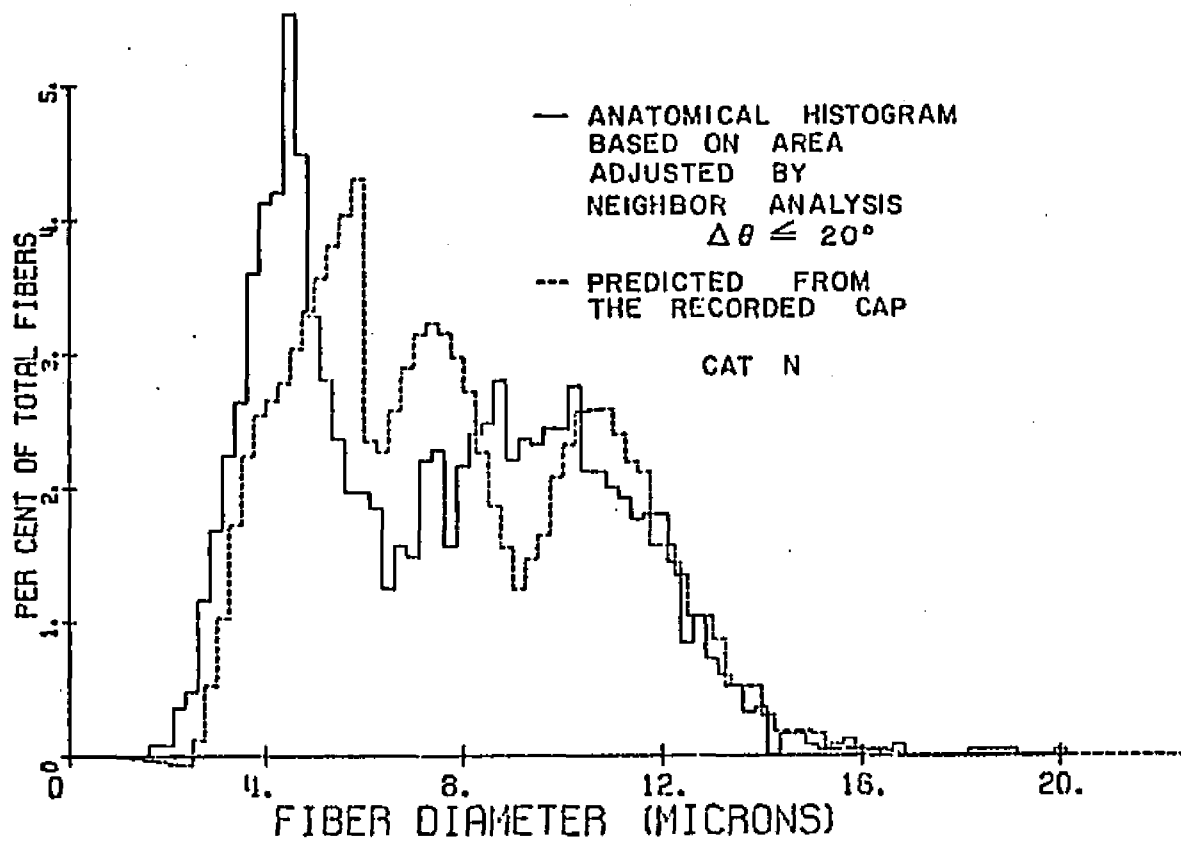


Figure 6-8. Equal bin width anatomical and predicted diameter histograms for optimized biophysical and axon potential wave parameters. Conduction distance = 4.2 cm. Cat N.

optimized biophysical and axon potential wave parameters for cat N are used for this prediction. This procedure tests the validity of these optimized parameter values with data from a different cat. The results are shown in figure 6-9. Note that the inflection on the decline of the first major peak in the recorded CAP is less than the corresponding inflection in the CAP for cat N for a comparable conduction distance. The intermediate peak in the predicted histogram for cat M is also smaller. Generally, the predicted histogram for cat M in figures 5-9A and 5-10 (equal bin widths) is shifted to the left as compared to the anatomical histogram for cat M based on area adjusted by neighbor analysis $\Delta\theta \leq 20^\circ$. Observe that the ordinate sensitivity is lower in figure 6-10 than for similar figures above. Note that the predicted histogram has a gap between 4 and 6 microns despite the discontinuity in the relationship between conduction velocity and fiber diameter at 6 microns. The peak predicted for small fibers is taller and broader than the anatomical peak for small fibers. Also, the peak for small fibers is shifted to the left by about 2 microns. The predicted and anatomical histograms in figure 6-10 are similar for large fiber diameters.

The predicted and anatomical histograms generally show reasonable agreement for fiber diameters greater than

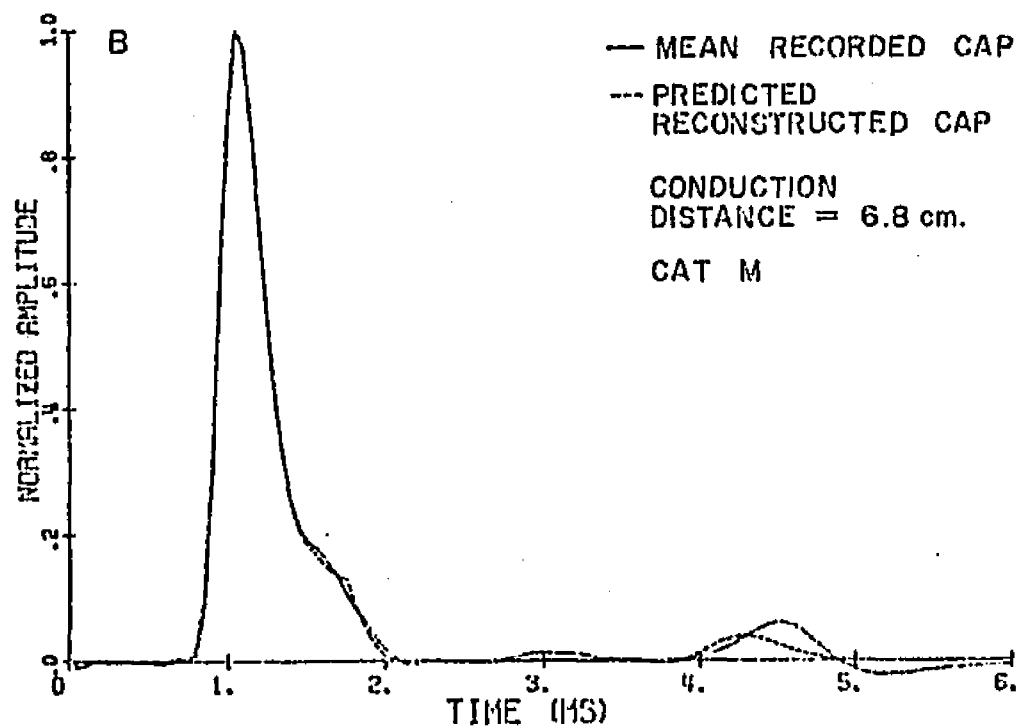
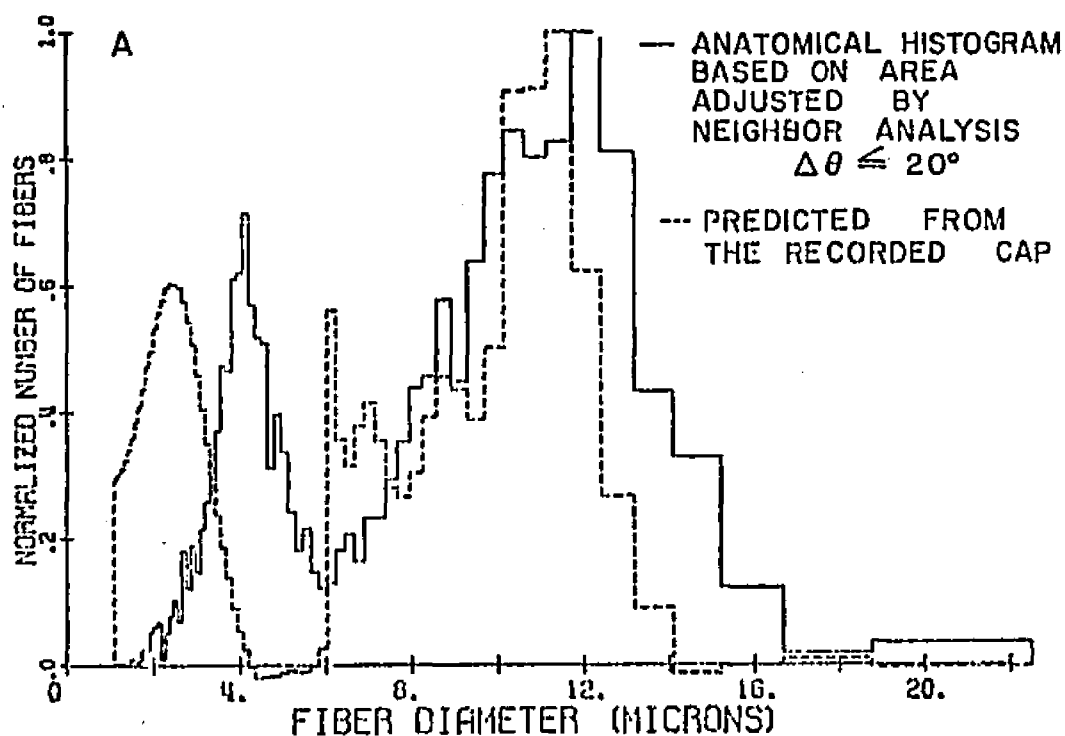


Figure 6-9. Diameter histogram prediction for optimized biophysical and axon potential wave parameters for Cat M. Conduction distance = 6.8 cm.

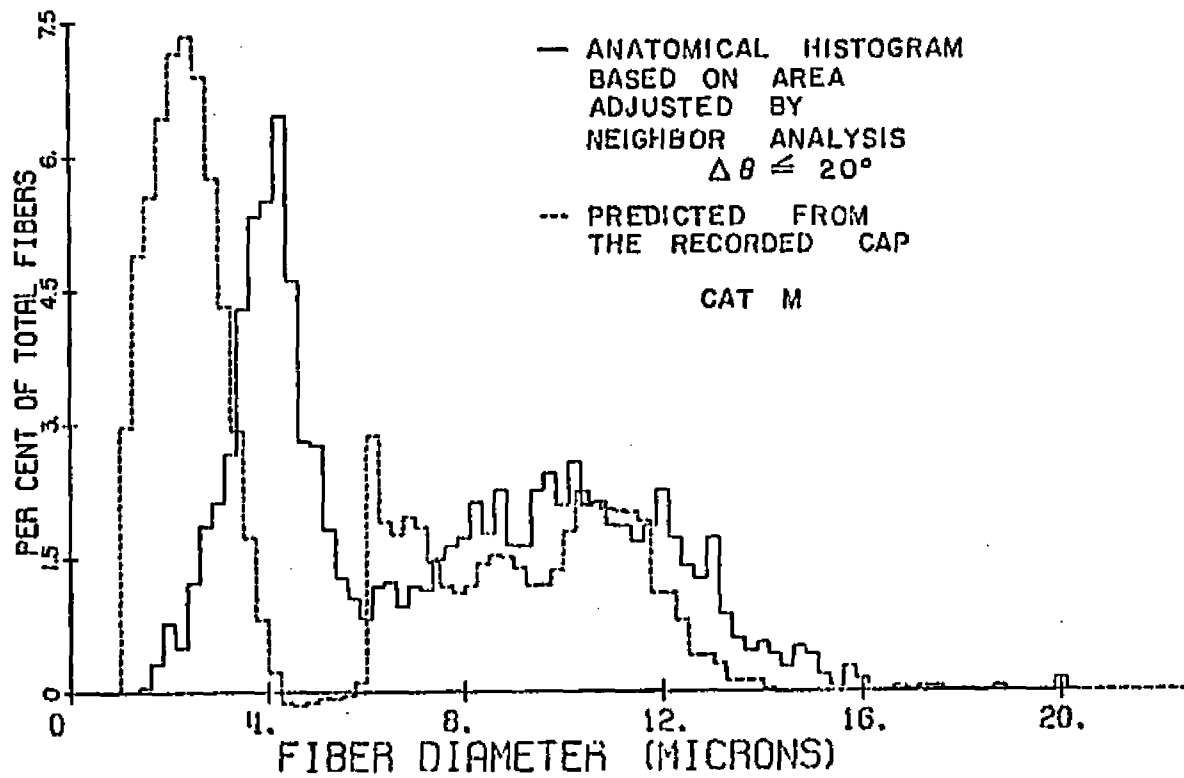


Figure 6-10. Equal bin width anatomical and predicted diameter histograms for optimized biophysical and axon potential wave parameters for Cat M. Conduction distance = 6.8 cm.

9 microns. However, a good prediction for fiber diameters less than 6 microns is obtained only when the biophysical and axon potential wave parameters are optimized for the same recorded CAP that is used to predict the diameter histogram.

CHAPTER VII

DISCUSSION

CAP RECONSTRUCTION MODEL ASSUMPTIONS

Early studies summarized by Erlanger and Gasser (1937) leave little doubt that the compound action potential (CAP) is indeed a summation of individual fiber responses. There is, however no direct experimental evidence to show that superposition holds for the many individual fiber responses recorded extracellularly. Thus, it is possible that minor interactions between individual fiber responses could occur within the nerve trunk adjacent to the recording electrode. Such interactions in this study are assumed to be negligible based on the indirect evidence given by Erlanger and Gasser (1937).

Single fiber conduction velocity could be affected by activity in neighboring fibers. Esplin (1962) gives convincing experimental evidence that conduction velocity in single myelinated fibers is unchanged when the fibers are activated alone or together with neighboring fibers. The myelin sheath may be a critical factor since there is electrophysiological evidence for this kind of interaction in unmyelinated fibers (Katz and Schmitt, 1940, 1942).

The position of a myelinated fiber within a nerve trunk might have an effect on the amplitude of the extracellular response recorded from the nerve trunk.

Schmitt (1971) states that Hambrecht (unpublished data) "has modeled a nerve trunk from published physiological data and has shown that when the trunk is raised in mineral oil for extracellular recording, a myelinated fiber anywhere in the trunk, produces approximately the same extracellular potential. The differences in axon potential amplitudes, recorded extracellularly, are due mainly to the different diameters of the active nerve fibers."

Buchthal and Rosenfalck (1966) disregarded differences in the distance from fibers to the recording electrode because "the electrode was at a point in the electrical field around the nerve where the action potential amplitude increased about proportionally with the number of fibers activated."

Figure 4-16B shows that for cat N the mean and standard deviation of the distances from each fiber to the nerve trunk perimeter are relatively constant as a function of fiber diameter. This means that fiber diameters are essentially distributed randomly throughout the cross-section so that diameter distribution bias (BIAS) is negligible.

The extracellular impedance that affects the recruitment of single fibers to electrical stimulation is the same impedance that attenuates the single fiber response recorded on the surface of a nerve trunk. It is well known that increasing the electrical stimulus amplitude recruits

progressively slower (smaller) fibers rather than giving a simple proportional increase to all portions of the CAP waveform. Thus, it seems reasonable to assume that fiber size rather than fiber position is the principal determinant of the size of the single fiber response recorded at the nerve trunk perimeter.

All CAP reconstructions included in the present study have either arbitrary or normalized ordinate scale factors. Thus, even if the fibers in the center of a nerve trunk give smaller responses recorded at the periphery, the effect would only be important in CAP reconstructions if fiber diameters are not randomly distributed in the cross-section. Figure 4-16B shows that fiber diameters are essentially randomly distributed. The fiber diameter histograms predicted in Chapter VI from normalized recorded CAPs also have unknown ordinate scale factors so these same arguments hold for the predicted histograms.

CAP RECONSTRUCTION MODEL PARAMETERS

The considerable empirical evidence cited in the introduction supports the assumption that myelinated fibers of the same diameter have very similar conduction velocities and very similar single fiber responses. Most authors agree that conduction velocity is approximately a linear function of fiber diameter (Hursh, 1939; Berry, Grundfest and Hinsey, 1944; Cragg and Thomas, 1964; Boyd, 1965; McLeod and Wray, 1967; Schnepf and Schnepf, 1971).

Most of these data, however, are for large fibers and the measurement errors tend to increase for small fiber data.

Two basic methods have been used to determine the conduction velocity constant (CVC). The first method (Hursh, 1939) requires that we assume the largest fiber conducts with the highest velocity. Nerves with different maximal velocities, are selected and the largest fiber diameters measured from histological sections. The slope of a line through the data points on a plot of conduction velocity versus fiber diameter is CVC. The results may be a low estimate for CVC if the voltage gain of the recording system is not sufficient to detect single fiber responses and if the largest fiber is significantly larger than the fibers that produce a measurable response. On the other hand, histological shrinkage can cause estimates of CVC to be too high.

A second method requires that we assume CVC is the ratio of the mean conduction velocity to the mean diameter for the myelinated fibers. The mean conduction velocity is usually measured to the peak in the CAP. Figure 3-3 shows, however that the position of the peak in the CAP depends on the standard deviation of the gaussian histogram, even though the mean diameter is constant. This second method is obviously not acceptable.

Experimental evidence indicates that the axon potential height exponent (APE) is unity (Blair and Erlanger,

1933; Zotterman, 1937; Gasser and Grundfest, 1939). These data show that single fiber axon potential height is approximately proportional to conduction velocity. Most of these data, however, are for large fibers and many sources of error are associated with measurements of single fiber axon potential amplitude. CAP reconstructions by others (Gasser and Erlanger, 1927; Buchthal and Rosenfalck, 1966; Landau, Clare and Bishop, 1968) along with the results in Chapter V indicate that a value of 2 for APE may be more appropriate.

Recent electron microscopic evidence cited in the introduction shows that "g" (ratio of axon to outside fiber diameter) remains approximately constant for all fiber diameters (Schwarzacher, 1954; Birren and Wall, 1956; Buchthal and Rosenfalck, 1966; Dyck, 1966; Friede and Samorjski, 1967; Bishop, Clare and Landau, 1971; Schnepf and Schnepf, 1971; Waxman and Bennett, 1972). Earlier studies with light microscopy find that "g" decreases for small diameter fibers. The recent evidence for a constant value for "g" is strong enough to suggest that "g" should not be considered in future reconstructions. The variation in "g" with diameter is not incorporated in the final reconstructions in Chapter V (equations 5-2 and 5-3).

RECONSTRUCTION PARAMETER SENSITIVITY ANALYSIS

Several conclusions can be drawn from the parameter

sensitivity analysis presented in Chapter III and from other reconstructions based on published fiber diameter histograms. The fiber diameter histogram is the most important determinant of CAP shape. The biophysical parameters examined have an important effect on CAP shape via the time delays, t_1 's, for the axon potential waves as a function of fiber diameter. With the exception of the axon potential height exponent (APE), the axon potential wave parameters and the shape of the axon potential wave are the least influential CAP parameters studied. For self-normalized CAPs the APE parameter determines the relative amplitude of the response for different diameter fibers. As APE increases, the contribution for the small fibers decreases relative to the responses for larger fibers. The importance of the APE parameter increases as the relative numbers of very large and very small fibers in the diameter histogram increase.

Table 7-1 is a summary of deviations selected from figures 3-4, 3-6 and 3-9. The deviations for each parameter will now be discussed.

The deviations for the fiber diameter histogram parameters are easy to understand if we keep in mind that large fibers have more influence on the waveform than small fibers, as shown in figure 3-12. Increases in MFD result

TABLE 7-1

Deviations calculated from scaled CAPs for percent variations of essential CAP parameters.

Parameter		% Variation					
		-30	-20	-10	+10	+20	+30
I	MFD	60.	35.	11.	14.	60.	140.
	STD ¹	7.0	2.8	0.63	0.51	1.9	3.8
II	DIST	93.	41.	9.8	7.7	26.	49.
	CVC	77.	37.	9.3	8.0	28.	55.
	CVE	160.	130.	46.	43.	120.	190.
III	APE	26.	14.	4.4	7.1	37.	110.
	APD	10.	4.6	1.2	1.2	4.6	10.
	APR	.25	.10	.05	.05	.10	.25

¹ Total number of fibers constant

in larger deviations than decreases in MFD simply because larger fibers contribute larger axon potential waves. The time delays, t_1 , for the axon potential waves vary inversely with fiber diameter, as shown by equation 3-4. The deviations for increases in MFD would be even larger relative to deviations for smaller MFD were it not for this inverse variation of the t_1 with fiber diameter. The deviation is larger for decreasing STD (total number of fibers remains constant) because the amplitude of the scaled CAP increases rapidly as the number of fibers close to the mean fiber diameter increases. Apparently the changes in CAP duration for STD variations are of relatively minor importance (figure 3-3).

The shape of the gaussian fiber diameter histogram is simple and there are relatively few very large and very small fibers. Other CAP reconstructions (not shown) for published diameter histograms that are more complex show that the overall shape and number of peaks in the CAP are primarily dependent on the shape of the fiber diameter histogram.

All the biophysical parameters (DIST, CVC and CVE) studied are influential parameters. The magnitudes of the deviations for all three biophysical parameters are comparable to deviations for equal percentage variations in MFD. As pointed out in Chapter III the DIST and CVC parameters have an inverse effect on the t_1 and hence on

the deviations. The CVE parameter gives the largest deviations in table 7-1. The great dependence of CAP reconstructions on the relationship between fiber diameter and conduction velocity indicates that good information about this relationship is important. The use of the CVE parameter simulates only one of many possible nonlinear relationships between fiber diameter and conduction velocity. In Chapter V a piecewise linear relationship is utilized effectively.

The axon potential wave parameters (APE, APD and APR) affect only the size and shape of the component axon potential waves. The APE parameter is an exponent that determines the relative amplitudes of the axon potential waves. The default value for APE is unity so it is not surprising that table 7-1 shows larger deviations for APE greater than one than for APE less than one. All the parameters studied that affect only the shape of the axon potential waves have relatively minor deviations.

Several differences are observed when deviation is calculated from self-normalized CAPs as shown in table 7-2. Note that for self-normalized CAPs, larger deviations result for decreases in MFD than for increases in MFD. The relative magnitudes of the deviation for changes in MFD are reversed for scaled CAPs, because the large CAP amplitude changes are lost when all the CAPs are self-normalized. The inverse relationship between the t_1

TABLE 7-2

Deviations calculated from self-normalized CAPs for percent variations of essential CAP parameters.

Parameter		% Variation					
		-30	-20	-10	+10	+20	+30
I	MFD	98.	44.	11.	8.9	31.	58.
II	DIST	72.	35.	9.0	8.3	31.	61.
	CVC	105.	45.	10.	7.4	25.	45.
	CVE	230.	160.	52.	38.	98.	136.
III	APE	.14	.062	.015	.015	.059	.13

and fiber diameter results in making decreases in MFD more influential for self-normalized CAPs. The relative effect of DIST and CVC are also reversed for the same reasons. The larger deviations in table 7-2 for decreases in CVE are also caused by the increased dependence on dispersive effects for self-normalized CAPs. The much smaller deviations for APE for self-normalized CAPs are discussed in Chapter III.

OPTIONAL MODEL FEATURES

The optional CAP reconstruction features listed in table 3-2 are used to simulate several conditions that could effect correlations between experimentally recorded CAPs and the anatomical fiber diameter histogram from the same nerve. Selected changes in fiber diameter histograms are studied and simulations for nonhomogeneous nerve bundles are explored.

CAPs reconstructed for diameter histograms with selected bins deleted show clearly that large diameter fibers contribute more to the CAP than small diameter fibers. The differences between the deviation curves in figure 3-13 for the gaussian test histogram will become greater as the number of very large and very small fibers in the histogram increases.

Figure 3-15 shows that for the gaussian histogram, simulated tissue shrinkage results in much larger

deviations than any of the deviations for comparable simulations of nerve bundle nonhomogeneities. In fact, the deviation for shrinkage is even greater than the deviation for proportionally identical reductions of MFD. A reasonable estimate of shrinkage for my tissue is 5% which corresponds to only a 0.036 deviation. The fiber diameter histograms in the present study are not adjusted to compensate for shrinkage (opposite histogram shift from shrinkage simulation) primarily because the degree of shrinkage is unknown. Also, other authors have refrained from shrinkage corrections, presumably for the same reason.

The results in figure 3-15 show that for the three different types of nerve bundle nonhomogeneities studied, tapering of all fibers by any given percentage results in more deviation than a comparable percentage of single fiber branching. Cross-section diameter distribution bias for the gaussian test histogram results in the smallest deviations. These simulations show that significant changes in the CAP can occur as a result of single fiber tapering and branching. Quantitative anatomical studies of nerve bundles at different levels are required before the possible effect of these nonhomogeneities on CAP reconstructions and predictions of fiber diameter histograms can be assessed.

ANATOMICAL FIBER DIAMETER HISTOGRAMS

The results given in Chapter IV indicate an attempt to obtain unbiased fiber diameter histograms from single cross-sections of histologically prepared peripheral nerve. Very few published photomicrographs of peripheral nerve cross-sections have a significant number of fiber shapes that are really circular. Some tissue fixed only in unbuffered osmic acid exhibits many round fibers, however, it is likely that excessive swelling occurs for this method of fixation (Ochs, 1965; Kasvand, Isnor and Romero-Sierra, 1970). A brief examination of my tissue with electron microscopy shows that the mitochondria and the myelin are generally intact. However, in the large fibers only, the axoplasm and axon membrane are often separated from the myelin to form large gaps. These gaps are not seen in the extracellular space between fibers and the myelin does not appear to be distorted. Most small diameter fibers do not have gaps. The apparent shrinkage of the axoplasm could occur during dehydration because the water content of the axoplasm is higher than that of the myelin sheath (Stoehr, 1929). Taylor (1942) and Sunderland and Roche (1958) both state that the axon shrinks more than the myelin. Nearly all published photomicrographs of myelinated fibers show only quite small fibers. Several published photomicrographs that

show at least part of some large fibers contain the gaps described above (Babel, Bischoff and Speedlin, 1970; Bourne, 1968).

Of course any histological procedure distorts the structure of living tissue. Very few quantitative estimates of shrinkage for peripheral nerve appear in the literature. The overall shrinkage for myelinated fibers found from data by Hursh (1939) is 7.9%. Sanders (1947) estimates that shrinkage during fixation and embedding is about 6 to 8%. A new accurate technique to measure tissue shrinkage is needed.

The methods used by other authors to measure fiber diameter are described in the introduction. Nearly all of these methods require subjective measurements for non-circular fiber cross-sections. Only the flying spot scanner used by Kasvand, Isnor and Romero-Sierra (1970) does not require direct human involvement. However, their procedure failed to identify about 30% of the small myelinated fibers and the procedure they used to find fiber diameter from fiber shape is applicable only to relatively round fibers.

The new procedure used in my study takes advantage of human's ability to identify the individual fibers and to follow the perimeter of each fiber. Then the computer

is programmed to compute many estimates of fiber diameter for the noncircular fiber shapes. The human's involvement in this method is well defined. Figure 4-19 shows that good repeatability of fiber perimeter measurements is obtained. Thus the method utilized in this study combines some of the best features of methods used by others. The results in Chapter IV show how versatile the perimeter data are for examining various estimates of fiber diameter for noncircular fibers.

The results in Chapter IV are the first known attempt to compare fiber diameter histograms based on different estimates of fiber diameter. The fiber diameter histograms based on area and minor chord in figure 4-4 are probably more accurate than histograms based on perimeter and major chord. The diameter estimates based on perimeter and major chord are much too large for obliquely cut and convoluted shaped fibers. The minor chord estimate of fiber diameter is influenced the least by obliquely cut fibers, however, the minor chord underestimates the diameter of fibers that are actually noncylindrical and this estimate is the least repeatable of the four primary estimates (table 4-1). Although the diameter estimate based on fiber area is too large for obliquely cut fibers, this estimate has the greatest repeatability (table 4-1) of the four primary estimates and is not particularly

affected by convoluted perimeters. For these reasons area is believed to be the best of the four primary estimates of fiber diameter considered.

The histograms based on the arithmetic, geometric and harmonic means of the major and minor chords (figures 4-7 and 4-8) seem to be reasonable intermediates between the simple major and minor chord histograms. However, a rational basis for accepting any such intermediate estimate is not apparent. The adjusted area histograms in figures 4-9 and 4-10 are an attempt to correct for obliqueness by adjusting the diameters of all fibers with a small enough ratio of minor to major chord. Once again there is no rational basis for expecting this method to correctly adjust the diameters of only the obliquely cut fibers.

The neighbor analysis procedure described in Chapter IV does seem to be capable of adjusting only the diameters of obliquely cut fibers. The adjustments based on the major chord angle are probably more accurate than adjustments based on differences in the ratio X_1/X_2 . A difficulty with the latter is that the ratios X_1/X_2 for a group of nearly round nonoblique fibers may be very similar. Also, the ratio X_1/X_2 may change rapidly for the neighbors of obliquely cut fibers when the obliqueness angle is changing for fibers within a fascicle.

The distance between fiber centroids that determines

which fibers are analyzed as neighbors is set at 20 microns. This choice is based on a qualitative examination of typical fibers on the photomicrographs. This fixed neighbor distance for all fiber diameters does mean that small fibers generally have more neighbors analyzed than large fibers. Certainly more complicated methods for choosing the neighbors could be investigated in future studies.

The parameter $\Delta\theta$ in the neighbor analysis is varied for the histograms based on area in figure 4-11. A $\Delta\theta$ of 20° (figure 4-11C) in the neighbor analysis results in a qualitatively reasonable shift in the histograms. Fiber diameter based on area is fully adjusted when multiplied by the square root of the ratio X_1/X_2 for the particular fiber. Figure 4-15B shows the mean ratio X_1/X_2 varies from about 0.6 to 0.7. Diameter adjustments for these ratios would be a factor of 0.78 to 0.84. Clearly the decreased diameters in figure 4-11C are on the average less than this. Also qualitative inspection of regions on the photomicrographs where fibers appear to be cut obliquely suggests that $\Delta\theta$ less than or equal to 20° is reasonable.

The reasons given above support the use of diameter based on area adjusted by neighbor analysis ($\Delta\theta \leq 20^\circ$) for the compilation of fiber diameter histograms. These fiber diameter histograms are used in Chapters V and VI where

relationships between fiber diameter histograms and compound action potentials are studied. Future studies will seek to improve the quantitative basis for the methods and parameter values used to derive fiber diameter histograms.

In regions of the fiber diameter histograms where the number of fibers is changing slowly with diameter, the diameter measurement errors will tend to cancel out. However, in regions of the histograms where the number of fibers change rapidly with diameter, the diameter measurement errors and the random variations of diameter along nerve fibers will result in histogram peaks that are too wide (Duncan, 1934; Gasser and Grundfest, 1939). The greater repeatability of the diameter estimate based on area (table 4-1) may be related to the taller, narrower small fiber peaks seen in figure 4-6B for diameter based on area.

The statistics for histograms compiled from the nine sequential histograms in figures 4-17 and 4-18 are not particularly helpful for comparing the variability of histograms based on the four primary estimates of fiber diameter. Apparently the number of fibers analyzed in each section is too small to compile reliable histograms. The difference between the histogram compiled for the small fascicle (165 fibers) and the histogram for the entire

section (2496 fibers) including the same small fascicle, is comparable to the difference between histograms for the small fascicle in the nine sequential sections. Also a published histogram (Gasser, 1935) for cat saphenous nerve with only 337 fibers does not have the same typical bimodal shape as all the complete histograms for cat saphenous nerve compiled by Gasser and Grundfest (1939), Boyd and Davey (1968) and myself (figure 7-1).

Figure 7-1 shows four published histograms for cat saphenous nerve (solid lines) and the histograms compiled from my data (dashed lines) for cat N based on the estimate of fiber diameter that is the closest approximation to the method described by each author. The bin widths are the same for each plot in figure 7-1.

The solid line histograms in figures 7-1A and 7-1D are published by Gasser and Grundfest (1939) and they remark that the two histograms "are similar". Although their two histograms both have two groups of fibers, their histogram in figure 7-1A is shifted to the left approximately 3 microns relative to their histogram in figure 7-1D. The two histograms in figure 7-1A have similar shapes but the bins with comparable numbers of fibers in Gasser and Grundfest's (1939) histogram are about 3 microns less than mine. Also my histogram in figure 7-1A has broader peaks and a larger overall range of fiber di-

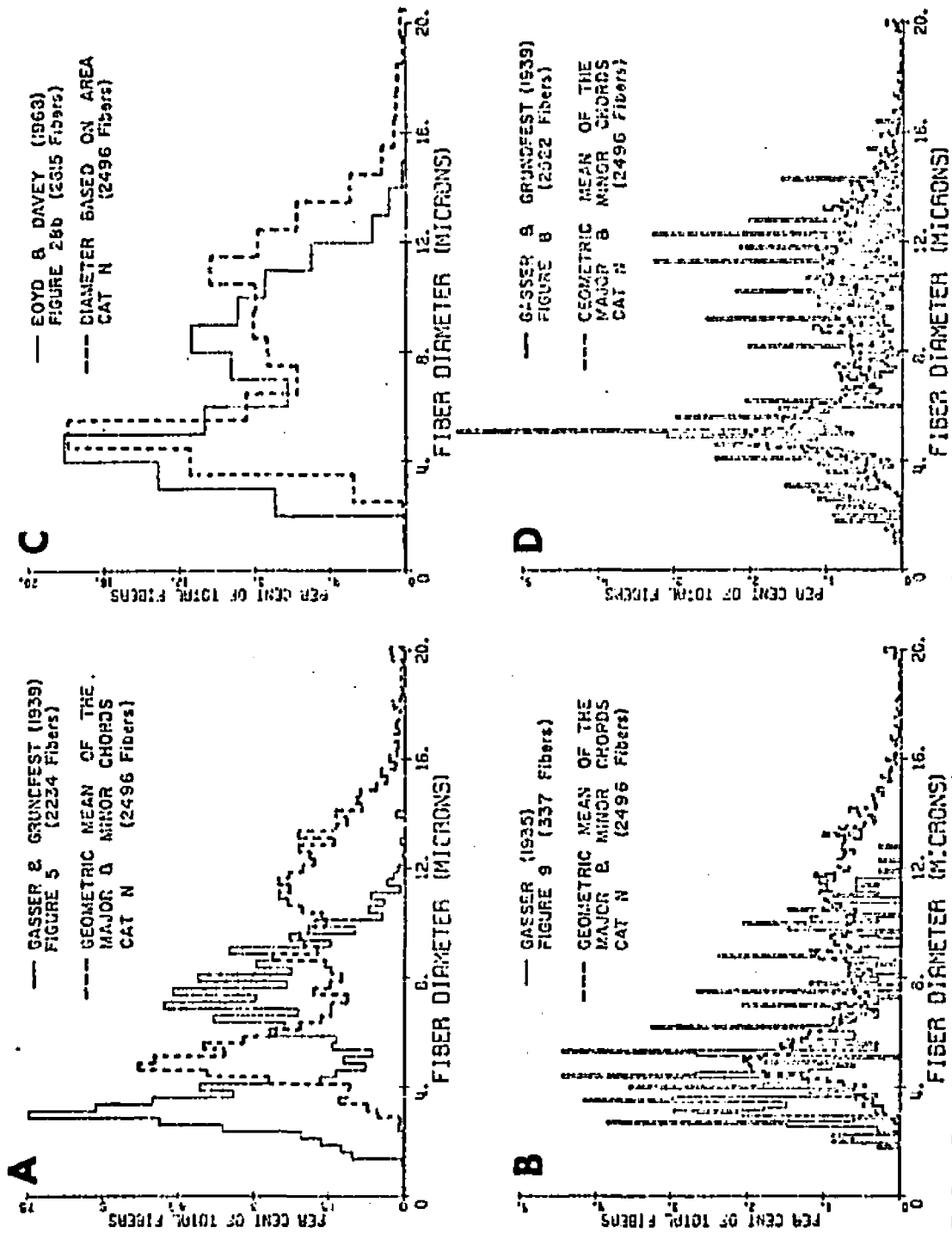


Figure 7-1. Published histograms (solid lines) and my corresponding histograms (dotted lines) for cat saphenous nerve.

ameters. The two histograms in figure 7-1D are remarkably similar except for the few very tall bins and the greater number of fibers less than 3 microns in diameter in the Gasser and Grundfest (1939) histogram. The large fluctuations in the number of fibers for adjacent bins in the histograms compiled by Gasser (1935) (figure 7-1B) and Gasser and Grundfest (1939) (figure 7-1D) probably result from the use of bin widths (0.1 microns) that are less than the resolution of the light microscope.

The diameter histogram for only 337 fibers published by Gasser (1935) is plotted in figure 7-1B. This histogram does not have the characteristic bimodal shape seen in all other cat saphenous nerve histograms, probably because so few fibers were measured. Average data from Boyd and Davey (1968) for five cat saphenous nerve transverse sections are plotted in figure 7-1C (solid line). Since they matched circles to the fiber shapes, the histogram based on area for cat N is also plotted in figure 7-1C (dotted line). The shape of the histogram is very similar to mine, although my area based histogram without any adjustments is shifted about one micron to the right relative to theirs.

Actually the histograms in figures 7-1C and 7-1D are remarkably similar when you consider the different methods used. Simple shifts in histograms could easily result

from slightly non-transverse sectioning angles. Also the tension on a peripheral nerve trunk during fixation may have quite an effect on the mean ratio of X_1/X_2 plotted versus fiber diameter. The tension on nerve trunks during fixation would probably affect the degree of obliqueness for many of the fibers seen in cross-sections and may even affect other aspects of the single fiber shapes.

COMPOUND ACTION POTENTIALS

The principal features of all the cat saphenous nerve CAPs recorded in this study are similar and in general agreement with CAPs recorded by Gasser and Grundfest (1939). The CAP waveforms recorded for this study are shown in figures 5-1, 5-2 and 5-3. Figure 7-2 is a reproduction from Gasser and Grundfest's (1939) figure 9. Their legend is quoted in figure 7-2. CAPs have an initial large peak such as the one labeled α in figure 7-2. An inflection or sometimes a small peak (labeled β) is always seen on the decline of the α wave for conduction distances greater than 6 cm. The potential falls to a value near zero for at least one millisecond following the β wave. Sometimes a small γ wave is present toward the end of this period as seen in figures 7-2 (at 2.3 ms.) and 5-3 (at 3 ms.). Finally, a small δ wave always appears for supra-maximal stimuli. Although the major features of these CAP waveforms are the same in all nerves studied, there are

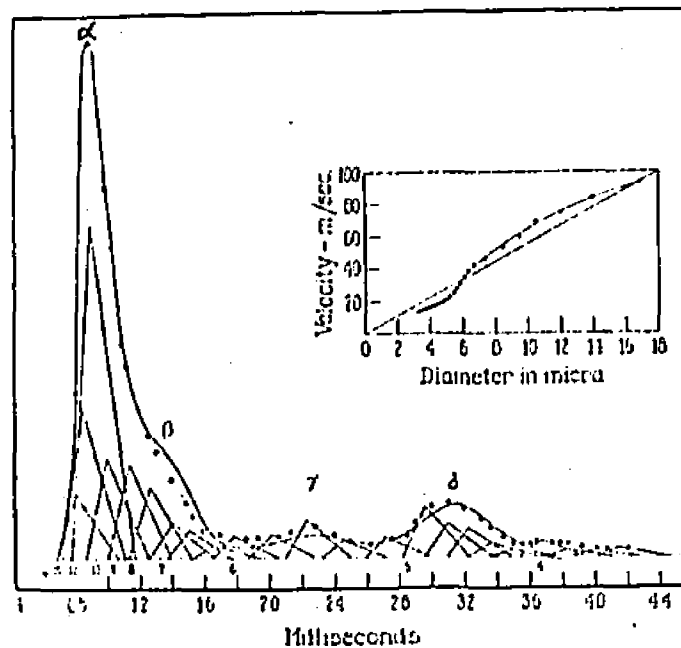


Figure 7-2. Figure 9 by Gasser and Grundfest (1939).

"Reconstruction of the action potential of the saphenous nerve of the cat from the data comprised in figure 8.² Conduction distance 6 cm. The solid line curve is the recorded potential. Heavy dots, summation of triangles. Note that the recorded action potential is diphasic. It undercuts the reconstruction following the first elevation, and the second elevation is so diphasic that the potential passes below the base line at the position of the 15 m.p.s. fibers. The latter, therefore, are visible in the reconstruction, but not in the potential. The fibers larger than 16 μ are grouped with 16 μ fibers."

²

Their figure 8 is plotted in figure 7-1D (solid line)..

Some significant differences in the relative amplitudes and durations of the various components of the CAP recorded from different cats (compare figures 5-2 and 5-3).

The Greek letters α , β , γ and δ were first used by Erlanger and Gasser (1924) to label the first four waves in order of appearance for the bull frog sciatic nerve. As other nerves were studied and functional consideration included, Erlanger and Gasser (1937) divided peripheral nerve fibers into three groups. Group A (subdivided into groups α , β , γ , δ) included the myelinated somatic afferent and efferent fibers. Group B comprised the preganglionic autonomic fibers and group C contained unmyelinated somatic afferent and postganglionic autonomic fibers. Later, the group A fibers were divided into groups α , β , γ for myelinated efferent fibers and groups I, II, III for myelinated afferent fibers in either cutaneous or muscle nerves.³ Sometimes group I is further subdivided into groups I_a and I_b .

The terminology described above is widely used in the literature. Unfortunately, problems arise when the groups overlap or have different ranges of diameter in different nerves. Therefore, the accepted terminology is

³ By an agreement at the Nobel Symposium I on "Muscle Afferents and Motor Control" held in Stockholm in 1965.

avoided when possible in this study because the generalized functional representation for the CAP given by equation 3-7 is preferred. A mathematical description of the fiber diameter histogram is likewise preferred.

The variability of the CAPs recorded for this study is small as shown by the small standard deviations in figures 5-4 and 5-5. Apparently the noise introduced by the recording instrumentation, tape recorder and the analog to digital converter are all negligible. The deviation defined by equation 5-1 is less than 0.0015 for both recorded CAPs. Generally it seems reasonable to state that two CAPs are practically equal if their deviation is less than 0.0015. It should be pointed out that we assume the waveform differences are not all concentrated in a small region of the curves. In this regard it is helpful to compute the single maximum difference between the waveforms as well as the deviation.

Figures 5-1, 5-2 and 5-3 show that despite the procedures used to obtain monophasic recordings, the CAP does fall below the baseline following the β and the δ elevations. Similar deflections below the baseline are present in Gasser and Grundfest's (1939) CAP shown in figure 7-2. It is still possible that all the fibers are not depolarized between the two recording electrodes. Also the after-

potentials in the individual fibers may contribute some downward deflection.

COMPOUND ACTION POTENTIAL RECONSTRUCTIONS

The deviations (defined by equation 5-1) in table 5-1 are quite large for the CAPs reconstructed from the fiber diameter histograms in Chapter IV, with the default biophysical and axon potential wave parameters. Figures 5-6 and 5-7 show that the reconstructions from histograms based on area come close to matching the α wave of the recorded CAP, but considerable differences between the curves exist for the β and δ waves.

The reconstructed α wave is wider than the recorded α wave, probably because measurement errors and obliquely cut fibers tend to make the peak in the diameter histogram for α fibers too broad. The reconstructed δ wave is also broader than the recorded δ wave, for presumably the same reason, and the reconstructed δ wave has a much shorter latency than the recorded δ wave.

Gasser and Grundfest (1939) used two factors to reduce similar discrepancies between their recorded and reconstructed CAPs. First their measurements showed the "g" (ratio of axon to fiber diameter) decreased for small fibers. When conduction velocities were made proportional to axon diameters, the reconstructed δ wave latency increased relative to the reconstructed α wave latency.

Second, because of the "virtual" cathode effect that results from current spread at the stimulating electrodes, they recorded the α and δ waves separately and then combined them before comparison with the reconstructed CAP. Recent anatomical studies with the electron microscope have shown that "g" does not decrease for small fibers. The α wave latency did not decrease with increased stimulus strength in my studies so the "virtual" cathode effect was negligible.

Some of these discrepancies undoubtedly result from errors associated with the measurements made for the compilation of fiber diameter histograms. The approach taken at this point is to choose the best available diameter histogram and then find biophysical and axon potential wave parameters that minimize the difference between the recorded and reconstructed CAPs. The resulting optimal parameter values are unique for sets of parameters that do not have redundant parameters. The diameter histogram based on area adjusted by neighbor analysis for $\Delta\theta \leq 20^\circ$ is used as the best available diameter histogram for the reasons given on pages 183 and 184.

Figures 5-8 and 5-9 show that only the reconstructed α wave is improved by parameter optimizations so long as the linear relationship between conduction velocity and fiber diameter is retained. Figure 5-10 shows the results

for an optimum piecewise linear relationship (equation 5-3) between conduction velocity and fiber diameter. The result in figure 5-10 has the smallest deviation (.0374) for this study and is the only reconstruction that gives a reasonable fit for the δ wave.

The nonlinear curve in the inset of figure 7-2 from Gasser and Grundfest (1939) is the conduction velocity-fiber diameter relationship found empirically for a good fit between their reconstructed and recorded CAPs. Notice that their relationship between conduction velocity and fiber diameter is similar to the corresponding functional relationship shown in the inset of figure 5-10. The available biophysical data for this relationship are not accurate enough to rule out these types of mildly nonlinear relationships between conduction velocity and fiber diameter.

The insets in figures 5-10 and 7-2 show the types of functional relationship between conduction velocity and fiber diameter that is needed for reasonable concurrence for the recorded and reconstructed CAPs. These relationships lead me to speculate that perhaps the biophysical properties are different for the myelinated fibers in the two major piles of fibers in cat saphenous nerve diameter histograms. Since anatomically derived diameter histograms are used to obtain the relationships in these two

insets, it is quite possible that the nonlinear relationships result from increasing measurement error for progressively smaller diameter fibers.

Apparently the only reasonable way to obtain a better fit between the reconstructed and the recorded CAPs in figure 5-10 is to find iteratively a detailed nonlinear relationship between conduction velocity and fiber diameter. Such a solution would undoubtedly require considerable computer time with my current simulation techniques.

PREDICTIONS OF FIBER DIAMETER HISTOGRAMS FROM CAPS

The results in Chapter VI are the first known attempt to predict the shape of a fiber diameter histogram from a recorded CAP. Figure 6-1 demonstrates that the procedure used can accurately predict the shape of the histogram if reliable reconstruction parameters are available. The conditions required for unique histogram predictions are reasonable (page 146).

Figures 6-3 and 6-4 indicate that a single linear relationship between conduction velocity and fiber diameter for all fiber diameters results in an unreasonably tall narrow peak for small fibers in the predicted histogram. The negative part of the predicted histogram in figure 6-4 is simply an artifact that results from the slightly biphasic character of the recorded CAP and the monophasic

axon potential wave in the model.

The results shown in figure 6-6 are encouraging because the predicted histogram is close to the anatomical histogram except for fibers between 6 and 10 microns. In figure 5-10 this corresponds to the β wave in the recorded CAP and the fit there is poor. Apparently smaller reconstruction deviations are the key to reducing the differences between the predicted and the anatomical histograms in figure 6-6.

The predicted histogram for a shorter conduction distance (figure 6-8) should be the same as the predicted histogram for a longer distance (figure 6-6). Although the results for large fibers are good, the predicted peak for small fibers is shifted to the right. It is possible that nerve bundle nonhomogeneities could contribute to this discrepancy. The prominent intermediate peak in the predicted histogram in figure 6-8 is particularly interesting because no β wave inflection is visible on the decline of α peak in the recorded CAP for this short conduction distance. The recorded CAP for the same nerve at a longer conduction distance does exhibit a significant β wave (figure 5-2). This indicates that the shape of the predicted diameter histogram is extremely sensitive to the CAP shape and therefore these studies may have important clinical and research implications.

The diameter histogram predicted for another nerve (cat M) shown in figure 6-10 does differ significantly from the anatomical result for the same nerve. Only the prediction for large fibers is reasonable, while the result for small fibers is shifted considerably toward smaller diameters from the anatomical result.

The δ wave latency for cat M is about 0.75 msec. greater than the δ wave latency for cat N, yet the two conduction distances are nearly equal (figures 5-2 and 5-3). The anatomical fiber diameter histograms for the nerves from the two cats are nearly identical (figure 4-6). Thus the differences between the histograms in figure 6-10 seem to be related to the different δ wave latencies in the recorded CAPs. Possibly the δ wave latency for cat M is increased by irreversible depolarization that might have spread from the adjacent crushed nerve end. The recording electrode separation was the same for recordings from the two nerves.

Generally the predicted histograms are closer to the anatomical histograms for the larger fibers. This is not particularly surprising because we know that large fibers influence the CAP much more than small fibers.

An alternative method for predicting fiber diameter histograms requires that the "pseudo inverse" of matrix A in equation 6-2 be computed.⁴ Then, the fiber diameter

⁴Suggested by an anonymous proposal reviewer.

histogram (vector N) can be found by multiplying the vector $V_{\max}$ times the inverse of the matrix A:

$$N = V_{\max} \cdot A^{-1} \quad (7-1).$$

The size of matrix A (120x80)⁵ required for precise predictions might make the matrix inversion prohibitively expensive. This second method might be preferred when appropriate biophysical and axon potential wave parameters are better established because the inverse matrix would only have to be computed once. However, such a "universal" inverse matrix would have to be recomputed for each different conduction distance.

FUTURE STUDIES

Several additional studies are required before the goal of developing a clinically useful nondestructive estimate of peripheral nerve structure based on electrical stimulation and recording techniques can be realized. First, additional refinements of the methods used in this study are needed to make the histogram predictions more accurate. Second, the methods must be extended to accommodate multiphasic CAPs because monophasic recording procedures damage the nerve. Finally, these procedures must be extended to CAPs obtained with clinically acceptable surface or needle electrode recording techniques. Of

course clinical testing and acceptance of this approach to

⁵This estimate is based on 120 samples along the CAP abscissa (6 msec. at 0.05 msec/sample) and 80 bins in the fiber diameter histogram (bin width of 0.25 microns).

improved diagnosis of peripheral neuropathies presents additional problems. I believe that acceptable solutions for all of these problems can be achieved.

The CAP reconstructions in Chapter V could probably be improved if the function identification procedure used in Chapter VI is applied to finding an exact nonlinear relationship between conduction velocity and fiber diameter. Of course different relationships would exist for fiber diameter histograms based on different estimates of fiber diameter. Certainly additional biophysical data are needed to improve our understanding of the relationship between conduction velocity and fiber diameter.

Additional basic studies are required to generate more accurate anatomical fiber diameter histograms. Perhaps the high ratio of fiber perimeter to fiber area for convoluted fibers could be used to identify these fibers so they could be analyzed differently. Anatomical studies at different levels along peripheral nerves are needed to establish whether significant single fiber tapering and branching occurs.

Finally it might be useful to extend the study presented here to group B and group C mammalian fibers. Also studies on other mammalian peripheral nerves are needed to find out if the results of this study are generally applicable to all mammalian nerves.

CHAPTER VIII

SUMMARY

1. The studies reported here are combined physiological and anatomical experiments on cat saphenous nerve. Extensive computer data processing and modelling were an integral part of these studies. A better understanding of the relationships between myelinated fiber diameter histograms and monophasic compound action potentials was the overall goal. Of particular interest throughout this study was the prediction of relative fiber diameter histograms from monophasic CAPs.
2. The properties of a model for reconstructing compound action potentials from fiber diameter histograms were investigated. The shape of the fiber diameter histogram was found to be the major determinant of the CAP shape. The biophysical parameters that relate single fiber conduction velocity to fiber diameter were also influential determinants of the CAP shape. The parameters of the axon potential wave that specify the shape of the single fiber responses in the model were the least influential parameters studied. The effects of nonhomogeneous nerve trunks were also simulated and studied with the model.
3. Fiber diameter histograms were compiled based on the following primary estimates of fiber diameter: $\text{perimeter}/\pi$,

diameter of a circle with equivalent area, major chord and the minor chord. The diameters based on area were the most accurate except for obliquely cut fibers. A method was developed to adjust the fiber diameters of obliquely cut fibers based on the shape and orientation of neighboring fibers. The mean smallest distance from each fiber centroid to the nerve bundle perimeter was nearly constant for all fiber diameters.

4. The recorded CAPs had small standard deviations and were significantly different for the two cats. Recorded and reconstructed CAPs were compared for the different underlying fiber diameter histograms. Selected model parameter values were optimized to minimize the sum of the squared differences between the recorded and the reconstructed CAPs. A nonlinear relationship between conduction velocity and fiber diameter was necessary to account for the gap between peaks in the recorded CAP.

5. The model was used to predict relative fiber diameter histograms from monophasic recorded CAPs. The histogram predictions also require that biophysical constants, the measured conduction distance and limits on the range of fiber diameter be given. The predicted and the anatomically derived histograms agreed better for large diameter fibers than for small diameter fibers.

APPENDIX A

FIBER DIAMETER HISTOGRAMS AVAILABLE IN THE LITERATURE FOR NERVES AND TRACTS

 ***** NERVES BY REFERENCE NUMBER *****

A=NUMBER, PER CENT, OR DENSITY ON HISTOGRAM ORDINATE

B=MINIMUM FIBER DIAMETER (MICRONS)

C=MAXIMUM FIBER DIAMETER (MICRONS)

D=BIN WIDTH (MICRONS)

E=HISTOGRAM MODALITY

F=REFERENCE NUMBER

NERVE	ANIMAL	A	B	C	D	E	F
SAPHENOUS	CAT	# 1.	14.	.25	2	1	1
SAPHENOUS	RABBIT	# 1.4	11.	.1	1	1	1
PHRENIC	CAT	# 1.6	14.	.2	2	1	1
SAPHENOUS	CAT	# 1.	19.	.1	2	1	1
OPTIC	CAT	# .5	13.5	.25	1	2	2
MEDIAL	CAT	# 1.5	17.5	1.	1	3	3
MEDIAL (13)	CAT	# 1.5	17.5	1.	2	3	3
POSTERIOR	CAT	# 1.5	17.5	1.	1	3	3
POSTERIOR (14)	CAT	# 1.5	17.5	1.	1	3	3
PERONEAL	CAT	# 2.7	19.1	.1	3	4	4
9TH MOTOR ROOT	BULLFROG	# 6.0	20.3	.1	1	4	4
9TH SENSORY ROOT	BULLFROG	# 2.7	20.1	.1	1	4	4
8TH MOTOR ROOT	BULLFROG	# 5.5	19.	.1	1	4	4
8TH SENSORY ROOT	BULLFROG	# 3.5	23.	.1	1	4	4
7TH MOTOR ROOT	BULLFROG	# 7.5	20.5	.1	1	4	4
7TH SENSORY ROOT	BULLFROG	# 5.	18.	.1	1	4	4
SCIATIC	BULLFROG	# 5.	21.	.1	3	4	4
PERONEAL	GREEN FRNG	# 3.4	14.	.1	1	4	4
PHRENIC	DNG	# 4.5	13.	.1	1	4	4

PHRENIC	DOG	#	4.8	12.2	.19	1	4
SAPHENOUS	DOG	#	1.	17.2	.19	1	4
CHORDA-LINGUAL	DOG	#	2.5	13.5	.1	1	4
CHORDA-TYMPANI	DOG	#	1.5	8.3	.1	1	4
OPTIC (3)	CAT	2	1.0	10.5	.5	1	5
OPTIC	CAT	#	.5	10.5	.5	1	6
OPTIC TRACT	CAT	#	.5	10.5	.5	1	6
OLFATORY (UM)	GARFISH	#	.11	.47	.02	1	7
SURAL (18 N ALCOHOLIC THALIDAMIDE)	HUMAN	2	2.	16.	1.	2	8
RADIAL (17)	HUMAN	2	2.	16.	1.	1	8
FACIAL (12 INTRAMEDULLARY GENU)	RAT	2	0.	4.2	.7	1	9
CORTICOSPINAL TRACT (4 M UM)	RAT	2	.2	3.	.1	1	10
FASCICULUS GRACILIS (4 M UM)	RAT	2	.2	5.	.1	1	10
FASCICULUS CUNEATUS (4 M UM)	RAT	2	2.	8.	.1	2	10
MEDIAN (4)	HUMAN	2	1.	21.	1.	2	11
DORSAL ROOT (UM)	RAT	2	1.	1.2	.1	1	12
DORSAL ROOT (UM)	CAT	2	.2	1.3	.1	1	12
DORSAL ROOT (UM)	COW	2	.2	2.1	.1	1	12
SURAL (9)	HUMAN	2	0.	15.	1.	+	13
SURAL (23 AN)	HUMAN	2	0.	15.	1.	1	13
SURAL (2 N AN RILEY-DAY SYNDROME)	HUMAN	#	.1	15.	.1	+	14
SURAL (2 UM N AN)	HUMAN	#	.1	5.	.1	1	14
VENTRAL AND DORSAL ROOTS (6 L4 L5 L6 S1)	RAT	2	1.	20.	1.	+	15
GASTRO (LATERAL) AND SOLEUS (9)	RAT	2	1.	16.	1.	1	15
GASTROCNEMIUS MEDIAL (11)	RAT	2	1.	17.	1.	1	15
GIJANEUS (7)	RAT	2	1.	12.	1.	1	15
SURAL (IN 5AN 2ANUM FRIEREICH ATAXIA)	HUMAN	#	1.	16.	1.	1	16
MEDIAN (IN 1AN)	BABOON	2	3.	19.	1.	2	17
MEDIAL POPLITEAL (6N 6AN)	RABBIT	#	2.	15.	1.	1	18
GASTROCNEMIUS MEDIAN (EF)	CAT	2	2.	18.	1.	2	19
EXTENSOR LONGUS DIGITOR (EF)	CAT	2	2.	16.	1.	2	19
RICEPS FEMORIS (EF)	CAT	2	2.5	17.5	1.3	2	19
SEMTENDINOSUS (EF)	CAT	2	2.5	18.8	1.3	2	19

SOLEUS (EF)	CAT	#	1.25	13.8	1.3	2	19
GASTROCNEMIUS LATERAL (EF)	CAT	#	1.25	17.5	1.3	2	19
GASTROCNEMIUS LATERAL W/O SOLEUS (EF)	CAT	#	1.25	17.5	1.3	2	19
7TH VENTRAL SPINAL ROOT	CAT	#	1.25	18.8	1.3	2	19
8TH VENTRAL SPINAL ROOT	CAT	#	2.	20.	2.	2	19
GASTROCNEMIUS MEDIAN (EF 2)	CAT	#	1.25	18.8	1.3	2	19
GASTROCNEMIUS MEDIAN (AF)	CAT	#	1.	18.	1.	2	19
DIGITAL DORSAL	CAT	#	1.25	12.5	1.3	1	19
TIBIALIS POSTERIOR (IN 1EF)	CAT	#	2.	17.	1.	2	20
POPLITEUS (IN 1EF)	CAT	#	2.	18.	1.	2	20
EXTENSOR DIGITORUM LONGUS (IN 1EF)	CAT	#	2.	17.	1.	2	20
PLANTARIS (IN 1EF)	CAT	#	2.	19.	1.	2	20
POPLITEUS (IN 1EF)	CAT	#	1.	21.	1.	2	20
GASTROCNEMIUS MEDIAN (IN 1EF)	CAT	#	1.	18.	1.	2	20
FLEXOR DIGITORUM LONGUS LATERAL (IN 1EF)	CAT	#	2.	16.	1.	2	20
TIBIALIS ANTERIOR (IN 1EF)	CAT	#	2.	18.	1.	2	20
PERONEUS LONGUS (IN 1EF)	CAT	#	2.	18.	1.	2	20
SOLEUS (IN 1EF)	CAT	#	2.	16.	1.	2	20
FLEXOR DIGITORUM BREVIS (IN 1EF)	CAT	#	2.	15.	1.	2	20
SURAL (IN 1EF)	CAT	#	1.	13.	1.	2	20
POPLITEUS (IN 1EF)	CAT	#	1.	22.	1.	2	20
GASTROCNEMIUS MEDIAN (IN 1AF)	CAT	#	1.	20.	1.	2	20
FLEXOR DIGITORUM LONGUS LATERAL (IN 1AF)	CAT	#	1.	19.	1.	2	20
TIBIALIS ANTERIOR (IN 1AF)	CAT	#	1.	18.	1.	2	20
TIBIALIS POSTERIOR (IN 1AF)	CAT	#	1.	18.	1.	2	20
EXTENSOR DIGITORUM LONGUS (IN 1AF)	CAT	#	1.	18.	1.	2	20
SOLEUS (IN 1AF)	CAT	#	1.	17.	1.	2	20
SAPHENOUS (IN 1AF)	CAT	#	1.	16.	1.	2	20
SOLEUS (AVERAGE OF 3)	CAT	#	1.	15.	1.	2	20
INTEROSSEOUS MEMBRANE (AVERAGE OF 2)	CAT	#	1.	17.	1.	2	20
SAPHENOUS (AVERAGE OF 5)	CAT	#	1.	15.	1.	2	20
SURAL (AVERAGE OF 5)	CAT	#	1.	15.	1.	2	20
KNEE JOINT RECEPTOR (BY RECEPTOR TYPE)	CAT	#	4.	15.	1.	1	21

POSTERIOR ARTICULAR (AVERAGE OF 6)	CAT	# 0.	19.	1.	1	22
SOLEUS MUSCLE RECEPTOR (BY RECEPTOR TYPE)	CAT	# 3.	17.	1.	1	23
PYRAMIDAL TRACT (2)	OPPOSSUM	# 0.	4.	1.	1	24
SURAL (17 DEVELOPMENTAL AGES)	HUMAN	% 0.	14.	1.	+	25
GASTROCNEMIUS LATERAL (9 DEVELOPING)	CAT	% 1.2	19.	.55	+	26
TIBIAL ANTERIOR (9 DEVELOPING)	CAT	% 1.2	15.5	.55	+	26
GASTROCNEMIUS MEDIAL (4N 2 IMOBILIZED)	RAT	% 1.	15.	1.	1	27
OPTIC NERVE (2)	MALLARD DUCK	# .3	6.	.4	1	28
SURAL (IN 3 ACRYLAMIDE)	RABBIT	D 2.	16.	1.	2	29
3RD DIGITAL NERVE OF THE HAND (2N 3AN)	RABBIT	D 2.	14.	1.	2	29
EXTENSOR DIGITORUM BREVIS (2N 3AN)	RABBIT	# 2.	16.	1.	1	29
OCULOMOTORIUS (2)	CAT	# 1.	12.	.32	1	30
ACUSTICUS (2)	CAT	# .6	5.	.32	1	30
CALLOSAL FIBERS (1)	CAT	# .2	8.4	.2	1	31
ULNAR (1 AXON DIA AND OTHER MEASURES)	OPPOSSUM	# 1.	12.5	.5	2	32
MEDIAN (1 AXON DIA AND OTHER MEASURES)	OPPOSSUM	# 1.	14.5	.5	1	32
SURAL (2 KRABBE'S DISEASE 1N)	HUMAN	% 1.	10.	1.	1	35
TRIGEMINAL (MINOR PORTION N=814)	MONKEY	% 1.	19.	1.	2	36
ABDUCENS (N=1613)	CAT	% 2.	21.	1.	2	36
ABDUCENS (N=4376)	HUMAN	% 1.	16.	1.	2	36
ABDUCENS (N=1510)	MONKEY	% 4.	17.	1.	1	36
ABDUCENS (PARTLY SUMMED UP N=689)	MONKEY	% 4.	17.	1.	2	36
HYPOGLOSSUS (N=1325)	MONKEY	% 3.	14.	1.	1	36
HYPOGLOSSUS (N=374)	CAT	% 3.	12.	1.	1	36
HYPOGLOSSUS (N=1065)	HUMAN	% 1.	15.	1.	2	36
FACIALIS (BEFORE CHORDA TYMPANI N=5498)	CAT	% 1.	16.	1.	1	36
FACIALIS (SMALL FIBER GROUP N=522)	CAT	% 0.	13.	1.	1	36
FACIALIS (N=3172 LARGE FIBER GROUP)	CAT	% 1.	15.	1.	1	36
RADIX ANTERIOR OF 4TH CERVICAL (N=784)	MONKEY	% 1.	19.	1.	2	36
RADIX ANTERIOR OF 5TH LUMBAR (N=884)	MONKEY	% 1.	18.	1.	2	36
RADIX ANTERIOR OF 5TH SACRAL (N=951)	MONKEY	% 1.	17.	1.	2	36
RADIX ANTERIOR OF 6TH CERVICAL (N=1566)	CAT	% 1.	24.	1.	1	36
RADIX ANTERIOR OF 5TH LUMBAR (N=2167)	CAT	% 1.	27.	1.	2	36

RADIX ANTERIOR OF 2TH SPINAL (N=868)	TOAD	%	2.	20.	1.	2	36
RADIX ANTERIOR OF 10TH SPINAL (N=1268)	TOAD	%	1.	17.	1.	2	36
RADIX ANTERIOR OF 4TH CERVICAL (N=513)	RABBIT	%	2.	18.	1.	3	36
RAMUS PECTRALIS THORACALIS LONGUS (N=1371)	PIGEON	%	2.	15.	1.	1	36
RAMUS PECTRALIS THORACALIS LONGUS (N=381)	DOM. FOWL	%	2.	13.	1.	1	36
RAMUS PECTRALIS THORACALIS LONGUS (N=501)	MONKEY	%	1.	16.	1.	2	36
RAMUS PECTRALIS THORACALIS LONGUS (N=635)	CAT	%	1.	24.	1.	2	36
RAMUS MUSCULARIS TIBIALIS (N=612)	RABBIT	%	1.	22.	1.	2	36
RAMUS MUSCULARIS TIBIALIS (N=381)	CAT	%	1.	23.	1.	2	36
RAMUS MUSCULARIS TIBIALIS (N=445)	MONKEY	%	2.	17.	1.	2	36
RAMUS MUSCULARIS TIBIALIS (N=1384)	MAN	%	1.	14.	1.	2	36
RAMUS MUSCULARIS MUSCULOTANEI (N=476)	CAT	%	1.	22.	1.	2	36
RAMUS MUSCULARIS MUSCULOTANEI (N=1043)	MAN	%	1.	15.	1.	2	36
RAMUS MUSCULARIS MEDIANI (N=549)	CAT	%	1.	20.	1.	2	36
RAMUS MUSCULARIS MEDIANI (N=1332)	MAN	%	1.	17.	1.	2	36
RAMUS MUSCULARIS RADIALIS (N=468)	MONKEY	%	1.	21.	1.	3	36
RAMUS MUSCULARIS ULNARIS (N=1211)	MAN	%	1.	17.	1.	2	36
PHRENICUS (N=1899)	MAN	%	0.	17.	1.	2	36
PHRENICUS (N=696)	CAT	%	2.	20.	1.	2	36
RADIX ANTERIOR 2ND THORACAL (N=2337)	CAT	%	1.	19.	1.	2	36
RADIX ANTERIOR 2ND THORACAL (N=669)	MONKEY	%	0.	15.	1.	2	36
RADIX ANTERIOR 5TH SPINAL (N=331)	TOAD	%	1.	19.	1.	2	36
RADIX ANTERIOR 2ND THORACAL (N=2746)	MAN	%	1.	18.	1.	2	36
RADIX ANTERIOR 2ND THORACAL (N=1039)	MAN	%	0.	7.	1.	1	36
RADIX ANTERIOR 2ND THORACAL (N=1113)	MAN	%	2.	18.	1.	2	36
RADIX ANTERIOR 2ND SACRAL (N=927)	CAT	%	0.	22.	1.	2	36
RADIX ANTERIOR 11TH SPINAL (N=473)	TOAD	%	1.	18.	1.	2	36
ACCESSORIUS (N=1789)	MONKEY	%	1.	15.	1.	2	36
ACCESSORIUS (N=1225 MOTOR GROUP)	MONKEY	%	1.	15.	1.	2	36
ACCESSORIUS (N=564 PARASYMPATHICUS)	MONKEY	%	1.	13.	1.	1	36
RADIX SPINALIS ACCESSORII (N=2448)	MAN	%	1.	16.	1.	2	36
RADIX MYELENCEPHALICUS ACCESSORII	MAN	%	0.	14.	1.	1	36
TROCHLEARIS (N=1724)	CAT	%	2.	19.	1.	2	36

Species	Condition	N	Mean	SD	SE	DF
MONKEY		23	1.0	1.0	2	36
DOM. FOWL		7	1.0	1.0	1	37
DOM. FOWL		6	1.0	1.0	1	37
DOM. FOWL		5	1.0	1.0	1	37
DOM. FOWL		5	1.0	1.0	1	37
RABBIT		1	1.8	1.0	1	38
DNG		1	17.2	1.0	1	39
DNG		1	13.8	1.0	2	39
BULLFROG		1	14.4	1.0	2	39
MAN		?	?	?	1	40
MONKEY		?	?	?	1	40
BABOON		2	18	1.0	2	41
CAT		1	13	.5	2	42
CAT		2	19	.5	2	42
HUMAN FETUS		1	8	.5	1	43
HUMAN FETUS		1	8	.5	1	43
HUMAN FETUS		1	8	.5	1	43
HUMAN FETUS		1	8	.5	1	43
HUMAN FETUS		1	7	.5	1	43
HUMAN FETUS		1	6	.5	1	43

***** NERVES ALPHABETICAL *****						
CAT		2	21	1.0	2	36
HUMAN		1	16	1.0	2	36
MONKEY		4	17	1.0	1	36
MONKEY		4	17	1.0	2	36
MONKEY		1	13	1.0	1	36
MONKEY		1	15	1.0	2	36
MONKEY		1	15	1.0	2	36
CAT		6	5	.32	1	30
CAT		2	17.5	1.3	2	19
CAT		2	8.4	.2	1	31

TROCHLEARIS (N=1379)
 VAGUS (N=9762)
 RECURRENT (N=3454)
 NESOPHAGEAL (N=1826)
 PULMONO-DESOPHAGEAL (N=899) TRANSECTION
 MESENTERIC (UM, 2 CONTROL, 1 TRANSITION)
 FEMORAL, MOTOR BRANCH (N=372)
 SAPHENOUS (BRANCH OF FEMORAL) (N=390)
 SKIN, DORSAL (M, UM)
 OPTIC (2, AREA NOT D)
 OPTIC (3, AREA NOT D)
 GASTROCNEMIUS (MED. HEAD, DEAFERENT)
 SURAL (4 METHODS)
 GASTROCNEMIUS, LATERAL (4 METHODS)
 VENTRAL ROOTS (L2, L3, L4, L5, S1-5 EACH)
 DORSAL ROOTS (L2, L3, L4, L5, S1-5 EACH)
 OBTURATOR (5)
 GASTROCNEMIUS, MEDIAL (5)
 GASTROCNEMIUS, LATERAL (5)
 SURAL (5)

CHORDA-LINGUAL	DOG	#	2.5	13.5	.1	1	4
CHORDA-TYMPANI	DOG	#	1.5	8.3	.1	1	4
CORTICOSPINAL TRACT (4 M UM)	RAT	%	.2	3.	.1	1	10
CUTANEOUS (7)	RAT	%	1.	12.	1.	1	15
DIGITAL DORSAL	CAT	%	1.25	12.5	1.3	1	19
3RD DIGITAL NERVE OF THE HAND (2N 3AN)	BABOON	D	2.	14.	1.	2	29
DORSAL ROOTS (L2, L3, L4, L5, S1-5 EACH)	HUMAN FETUS	%	1.	8.	.5	1	43
DORSAL ROOT (UM)	RAT	%	.1	1.2	.1	1	12
DORSAL ROOT (UM)	CAT	%	.2	1.3	.1	1	12
DORSAL ROOT (UM)	COW	%	.2	2.1	.1	1	12
EXTENSOR DIGITORUM BREVIS (2N 3AN)	BABOON	#	2.	16.	1.	1	29
EXTENSOR DIGITORUM LONGUS (1N 1AF)	CAT	#	1.	18.	1.	2	20
EXTENSOR DIGITORUM LONGUS (1N 1EF)	CAT	#	2.	17.	1.	2	20
EXTENSOR LONGUS DIGITOR (EF)	CAT	%	2.	16.	1.	2	19
FACIAL (12 INTRAMEDULLARY GENU)	RAT	%	0.	4.2	.7	1	9
FACIALIS (N=3172 LARGE FIBER GROUP)	CAT	%	1.	15.	1.	1	36
FACIALIS (BEFORE CHORDA TYMPANI N=5498)	CAT	%	1.	16.	1.	1	36
FACIALIS (SMALL FIBER GROUP N=522)	CAT	%	0.	13.	1.	1	36
FASCICULUS GRACILIS (4 M UM)	RAT	%	.2	5.	.1	1	10
FASCICULUS CUNEATUS (4 M UM)	RAT	%	2.	8.	.1	2	10
FEMORAL, MOTOR BRANCH (N=372)	DOG	#	1.5	17.2	.1	1	39
FLEXOR DIGITORUM BREVIS (1N 1EF)	CAT	#	2.	15.	1.	2	20
FLEXOR DIGITORUM LONGUS LATERAL (1N 1EF)	CAT	#	2.	16.	1.	2	20
FLEXOR DIGITORUM LONGUS LATERAL (1N 1AF)	CAT	#	1.	19.	1.	2	20
GASTRO (LATERAL) AND SOLEUS (9)	RAT	%	1.	16.	1.	1	15
GASTROCNEMIUS LATERAL (EF)	CAT	%	1.25	17.5	1.3	2	19
GASTROCNEMIUS, LATERAL (4 METHODS)	CAT	%	2.	19.	.5	2	42
GASTROCNEMIUS, LATERAL (5)	HUMAN FETUS	%	1.	7.	.5	1	43
GASTROCNEMIUS LATERAL (9 DEVELOPING)	CAT	%	1.2	19.	.55	+	26
GASTROCNEMIUS LATERAL W/O SOLEUS (EF)	CAT	%	1.25	17.5	1.3	2	19
GASTROCNEMIUS MEDIAL (11)	RAT	%	1.	17.	1.	1	15
GASTROCNEMIUS, MEDIAL (5)	HUMAN FETUS	%	1.	8.	.5	1	43
GASTROCNEMIUS (MED. HEAD, DEAFFERENT)	BABOON	#	2.	18.	1.	2	41

RAMUS MUSCULARIS TIBIALIS (N=381)	CAT	23.	1.	23.	1.	2	36
RAMUS MUSCULARIS TIBIALIS (N=1384)	MAN	14.	1.	14.	1.	2	36
RAMUS MUSCULARIS TIBIALIS (N=445)	MONKEY	17.	1.	17.	1.	2	36
RAMUS MUSCULARIS TIBIALIS (N=612)	RABBIT	22.	1.	22.	1.	2	36
RAMUS MUSCULARIS MEDIANI (N=549)	CAT	20.	1.	20.	1.	2	36
RAMUS MUSCULARIS MEDIANI (N=1332)	MAN	17.	1.	17.	1.	2	36
RAMUS MUSCULARIS MUSCULOTANEI (N=476)	CAT	22.	1.	22.	1.	2	36
RAMUS MUSCULARIS MUSCULOTANEI (N=1043)	MAN	15.	1.	15.	1.	2	36
RAMUS MUSCULARIS RADIALIS (N=468)	MONKEY	21.	1.	21.	1.	3	36
RADIX MYELENCEPHALICUS ACCESSORII	MAN	14.	1.	14.	1.	1	36
RAMUS PECTORALIS THORACALIS LONGUS (N=635)	CAT	24.	1.	24.	1.	2	36
RAMUS PECTORALIS THORACALIS LONGUS (N=381)	DOM. FOWL	13.	1.	13.	1.	1	36
RAMUS PECTORALIS THORACALIS LONGUS (N=501)	MONKEY	16.	1.	16.	1.	2	36
RAMUS PECTORALIS THORACALIS LONGUS (N=1371)	PIGEON	15.	1.	15.	1.	1	36
RAMUS MUSCULARIS ULNARIS (N=1211)	MAN	17.	1.	17.	1.	2	36
RADIX SPINALIS ACCESSORII (N=2448)	MAN	16.	1.	16.	1.	2	36
RECURRENT (N=3454)	DOM. FOWL	6.	1.	6.	1.	1	37
SAPHENOUS	CAT	14.	.25	14.	.25	2	1
SAPHENOUS	CAT	19.	.1	19.	.1	2	1
SAPHENOUS	RABBIT	11.	.1	11.	.1	1	1
SAPHENOUS	DOG	17.2	.19	17.2	.19	1	4
SAPHENOUS (IN IAF)	CAT	16.	1.	16.	1.	2	20
SAPHENOUS (AVERAGE OF 5)	CAT	15.	1.	15.	1.	2	20
SAPHENOUS (BRANCH OF FEMORAL) (N=390)	DOG	13.8	.1	13.8	.1	2	39
SCIATIC	BULLFROG	21.	.1	21.	.1	3	4
SEMTENDINOSUS (EF)	CAT	18.8	1.3	18.8	1.3	2	19
7TH SENSORY ROOT	BULLFROG	18.	.1	18.	.1	1	4
8TH SENSORY ROOT	BULLFROG	23.	.1	23.	.1	1	4
9TH SENSORY ROOT	BULLFROG	20.1	.1	20.1	.1	1	4
SKIN, DORSAL (M, UM)	BULLFROG	14.4	.1	14.4	.1	2	39
SOLEUS (EF)	CAT	13.8	1.3	13.8	1.3	2	19
SOLEUS (IN IEF)	CAT	16.	1.	16.	1.	2	20
SOLEUS (IN IAF)	CAT	17.	1.	17.	1.	2	20

SOLEUS (AVERAGE OF 3)	CAT	#	1.	15.	1.	2	20
SOLEUS MUSCLE RECEPTOR (BY RECEPTOR TYPE)	CAT	#	3.	17.	1.	1	23
SURAL (18 N ALCOHOLIC THALIDAMIDE)	HUMAN	%	2.	16.	1.	2	8
SURAL (9)	HUMAN	%	0.	15.	1.	+	13
SURAL (23 AN)	HUMAN	%	0.	15.	1.	1	13
SURAL (2 N AN RILEY-DAY SYNDROME)	HUMAN	#	.1	15.	.1	+	14
SURAL (2 IM N AN)	HUMAN	#	.1	5.	.1	1	14
SURAL (IN SAN ZANUM FRIEREICH ATAXIA)	HUMAN	#	1.	16.	1.	1	16
SURAL (IN IEF)	CAT	#	1.	13.	1.	2	20
SURAL (AVERAGE OF 5)	CAT	#	1.	15.	1.	2	20
SURAL (7 DEVELOPMENTAL AGES)	HUMAN	%	0.	14.	1.	+	25
SURAL (IN 3 ARRYLAMIDE)	BAROON	D	2.	16.	1.	2	29
SURAL (2 KRABBE'S DISEASE IN)	HUMAN	%	1.	10.	1.	1	35
SURAL (4 METHODS)	CAT	%	1.	13.	.5	2	42
SURAL (5)	HUMAN FETUS	%	1.	6.	.5	1	43
TIBIAL ANTERIOR (9 DEVELOPING)	CAT	%	1.2	15.5	.55	+	26
TIBIALIS ANTERIOR (IN IEF)	CAT	#	2.	18.	1.	2	20
TIBIALIS ANTERIOR (IN IAF)	CAT	#	1.	18.	1.	2	20
TIBIALIS POSTERIOR (IN IEF)	CAT	#	2.	17.	1.	2	20
TIBIALIS POSTERIOR (IN IAF)	CAT	#	1.	18.	1.	2	20
TRIGEMINAL (MINOR PORTION N=814)	MONKEY	%	1.	19.	1.	2	36
TROCHLEARIS (N=1724)	CAT	%	2.	19.	1.	2	36
TROCHLEARIS (N=1379)	MONKEY	%	1.	23.	1.	2	36
ULNAR (1 AXON DIA AND OTHER MEASURES)	OPUSSUM	#	1.	12.5	.5	2	32
VAGUS (N=9762)	DOW. FOWL	#	0.	7.	1.	1	37
VENTRAL AND DORSAL ROOTS (6 L4 L5 L6 S1)	RAT	%	1.	20.	1.	+	15
VENTRAL ROOTS (L2, L3, L4, L5, S1-5 EACH)	HUMAN FETUS	%	1.	8.	.5	1	43
7TH VENTRAL SPINAL ROOT	CAT	%	1.25	18.8	1.3	2	19
8TH VENTRAL SPINAL ROOT	CAT	%	2.	20.	2.	2	19

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APPENDIX B

FIRER DIAMETER HISTOGRAM DATA

THE FIRST DATA LINE FOR EACH HISTOGRAM GIVES RESPECTIVELY, THE NUMBER OF RINS, RIN WIDTH, MAXIMUM DIAMETER, AND MINIMUM DIAMETER. THE NUMBERS OF FIRERS IN EACH BIN ARE GIVEN IN 16 FIELDS OF 5 CHARACTERS EACH FOR EACH LINE OF DATA. THE RIN FOR THE LARGEST FIRER DIAMETER IS GIVEN FIRST.

PERIMETER CAT N FIGURE 4-4A

80	0.2500	20.0000	0.0000	6.0	2.0	7.0	4.0	10.0	5.0	6.0	5.0	14.0	12.0
41.0	2.0	5.0	4.0	2.0	6.0	2.0	6.0	2.0	6.0	2.0	6.0	2.0	6.0
19.0	14.0	14.0	13.0	20.0	23.0	24.0	34.0	39.0	44.0	44.0	45.0	47.0	51.0
51.0	74.0	56.0	53.0	42.0	34.0	38.0	47.0	44.0	45.0	57.0	41.0	57.0	44.0
36.0	49.0	27.0	40.0	33.0	37.0	50.0	63.0	83.0	91.0	92.0	112.0	97.0	60.0
19.0	22.0	21.0	8.0	3.0	3.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

AREA CAT N FIGURES 4-4A, 4-14C, 4-15A, 4-16A AND DOTTED CURVES IN FIGURES 4-9A-D, 4-11A-D, 4-12A-D, 4-13A, 4-13B.

80 0.2500 20.0000 0.0000

80	0.2500	20.0000	0.0000	1.0	2.0	1.0	5.0	0.0	5.0	0.0	3.0	5.0	2.0	4.0
6.0	0.0	1.0	1.0	1.0	2.0	1.0	5.0	0.0	5.0	0.0	3.0	5.0	2.0	4.0
2.0	4.0	5.0	10.0	12.0	9.0	14.0	23.0	20.0	22.0	44.0	33.0	37.0	46.0	43.0
54.0	44.0	67.0	55.0	57.0	73.0	66.0	56.0	45.0	41.0	52.0	47.0	57.0	36.0	64.0
39.0	43.0	36.0	40.0	37.0	32.0	44.0	31.0	47.0	65.0	85.0	85.0	121.0	143.0	125.0
59.0	37.0	23.0	29.0	15.0	4.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

MINOR CHORD CAT N FIGURE 4-4C

65 0.2500 16.2500 0.0000

65	0.2500	16.2500	0.0000	1.0	2.0	1.0	6.0	5.0	8.0	11.0	12.0	18.0	23.0	26.0
2.0	1.0	4.0	2.0	1.0	0.0	1.0	2.0	6.0	5.0	8.0	11.0	12.0	18.0	23.0
14.0	39.0	40.0	32.0	40.0	40.0	46.0	47.0	65.0	60.0	40.0	73.0	59.0	64.0	70.0
54.0	57.0	59.0	53.0	52.0	47.0	39.0	53.0	40.0	42.0	48.0	55.0	68.0	55.0	75.0
107.0	117.0	105.0	94.0	69.0	59.0	54.0	33.0	18.0	16.0	4.0	2.0	0.0	0.0	0.0
0.0														

MAJOR CHORD CAT N FIGURE 4-4D

[illegible][illegible][illegible][illegible][illegible]

AREA CAT M FIGURE 4-5B AND DOTTED CURVES FIGURE 4-10A-D. 4-13B

[illegible][illegible][illegible]

ARITHMETIC MEAN CAT M FIGURE 4-RA														
80		0.2500		20.0000		0.0000								
37.0	2.0	1.0	2.0	1.0	4.0	3.0	7.0	2.0	0.0	3.0	4.0	5.0	8.0	10.0
12.0	12.0	14.0	9.0	26.0	22.0	28.0	29.0	28.0	43.0	41.0	37.0	42.0	52.0	46.0
47.0	38.0	57.0	37.0	34.0	47.0	35.0	26.0	31.0	39.0	47.0	36.0	38.0	31.0	19.0
9.0	20.0	18.0	19.0	23.0	27.0	26.0	29.0	29.0	52.0	76.0	75.0	16.0	127.0	98.0
65.0	27.0	23.0	19.0	15.0	17.0	9.0	7.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0

GEOMETRIC MEAN CAT M FIGURE 4-8R													
80		0.2500		20.0000		0.0000							
15.0	1.0	2.0	2.0	2.0	3.0	4.0	6.0	3.0	3.0	4.0	6.0	5.0	8.0
9.0	13.0	11.0	17.0	14.0	22.0	22.0	34.0	29.0	32.0	40.0	38.0	54.0	53.0
43.0	41.0	49.0	44.0	43.0	35.0	42.0	31.0	36.0	34.0	44.0	41.0	38.0	26.0
20.0	18.0	16.0	18.0	18.0	21.0	27.0	37.0	25.0	33.0	57.0	88.0	124.0	113.0
80.0	46.0	32.0	22.0	17.0	15.0	18.0	13.0	2.0	1.0	0.0	0.0	0.0	0.0

HARMONIC MEAN CAT M FIGURE 4-8C

[illegible]

ADJUSTED AREA CAT N FIGURE 4-9A

	76	0.2500	19.0000	0.0000																
1.0	1.0	0.0	3.0	0.0	4.0	1.0	0.0	0.0	2.0	2.0	2.0	1.0	2.0	4.0	3.0					
9.0	9.0	7.0	12.0	22.0	18.0	20.0	38.0	32.0	34.0	46.0	38.0	51.0	45.0	68.0	55.0					
60.0	74.0	63.0	55.0	39.0	43.0	46.0	57.0	50.0	57.0	37.0	63.0	38.0	45.0	32.0	43.0					
33.0	32.0	45.0	23.0	42.0	56.0	82.0	73.0	102.0	113.0	126.0	105.0	44.0	39.0	36.0	48.0					
44.0	44.0	36.0	19.0	13.0	6.0	3.0	0.0	0.0	0.0	0.0	0.0									

ADJUSTED AREA CAT N FIGURE 4-9B

[illegible]

ADJUSTED ARFA CAT N FIGURE 4-9C

[illegible]

ADJUSTED AREA CAT N FIGURE 4-9D

[illegible]

ADJUSTED AREA CAT M FIGURE 4-10A

76	0.2500	19.0000	0.0000
1.0	1.0	0.0	0.0
13.0	9.0	12.0	24.0
50.0	36.0	45.0	33.0
21.0	25.0	26.0	19.0
40.0	54.0	54.0	38.0

ADJUSTED AREA CAT M FIGURE 4-10B

70	0.2500	17.5000	0.0000
1.0	2.0	1.0	0.0
16.0	36.0	26.0	29.0
49.0	38.0	53.0	40.0
32.0	32.0	51.0	51.0
18.0	2.0	0.0	0.0

ADJUSTED AREA CAT M FIGURE 4-10C

64	0.2500	16.0000	0.0000
1.0	1.0	5.0	5.0
28.0	33.0	32.0	42.0
38.0	35.0	41.0	31.0
93.0	114.0	127.0	116.0

ADJUSTED AREA CAT M FIGURE 4-10D

61	0.2500	15.2500	0.0000
2.0	2.0	1.0	4.0
38.0	42.0	46.0	39.0
32.0	31.0	48.0	31.0
115.0	112.0	73.0	67.0

AREA ADJUSTED BY NEIGHBOR ANALYSIS CAT M FIGURE 4-11A

80	0.2500	20.0000	0.0000
3.0	0.0	1.0	0.0
3.0	2.0	3.0	11.0
42.0	46.0	49.0	67.0
41.0	42.0	44.0	34.0
88.0	53.0	30.0	39.0

AREA ADJUSTED BY NEIGHBOR ANALYSIS CAT N FIGURE 4-11B

AREA ADJUSTED BY NEIGHBOR ANALYSIS CAT N													
80		0.2500		20.0000		0.0000							
1.0	0.0	1.0	0.0	2.0	2.0	1.0	2.0	1.0	1.0	2.0	2.0	1.0	1.0
1.0	4.0	1.0	1.0	3.0	8.0	6.0	2.0	8.0	16.0	24.0	21.0	29.0	35.0
47.0	38.0	45.0	66.0	50.0	53.0	76.0	72.0	50.0	46.0	67.0	53.0	61.0	49.0
49.0	43.0	52.0	38.0	32.0	29.0	52.0	46.0	39.0	48.0	65.0	83.0	87.0	135.0
107.0	63.0	45.0	39.0	29.0	12.0	7.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0

[illegible][illegible]

AREA ADJUSTED BY NEIGHBOR ANALYSIS CAT N FIGURE 4-12A												
80		0.2500		20.0000		0.0000						
5.0	0.0	0.0	0.0	1.0	2.0	0.0	2.0	1.0	1.0	2.0	2.0	4.0
4.0	4.0	1.0	6.0	2.0	7.0	11.0	6.0	16.0	12.0	15.0	23.0	44.0
41.0	52.0	46.0	60.0	63.0	57.0	67.0	71.0	53.0	59.0	61.0	51.0	45.0
56.0	38.0	44.0	43.0	42.0	37.0	32.0	41.0	46.0	56.0	68.0	97.0	138.0
110.0	43.0	38.0	30.0	22.0	8.0	5.0	3.0	0.0	0.0	0.0	0.0	0.0

[illegible]

AREA ADJUSTED BY NEIGHBOR ANALYSIS CAT N FIGURE 4-12C

[illegible][illegible][illegible][illegible]

20	1.0000	20.0000	0.0000
7.0	4.0	11.0	13.0
286.0	68.0	4.0	0.0
		15.0	32.0
		74.0	144.0
		196.0	259.0
		199.0	201.0
		183.0	142.0
		11.0	447.0

[illegible]

AREA CAT N BIN WIDTH= 0.125 MICRONS FIGURE 4-14D

[illegible]

MEAN RATIO OF MINOR TO MAJOR CHORD CAT N FIGURE 4-15B

80	0.2500	20.0000	0.0000MEAN
80	0.2500	20.0000	0.0000MEAN

0.3240.0 0.4180.3300.6760.5100.3970.4740.0 0.5340.4240.0 0.3960.5430.6950.5480.6320.5080.6220.6010.6450.6200.5530.6130.6520.6310.7070.6600.7050.7180.6930.7140.6920.7010.7220.6990.6890.6810.7010.6470.7070.6680.7020.7140.6880.6740.6960.6510.6710.6210.6280.6150.6590.6280.5650.6510.6210.6600.6580.6240.6620.6370.6010.5750.5570.5460.5930.5360.5450.5920.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0

MEAN RATIO OF MINOR TO MAJOR CHORD + STD CAT N FIGURE 4-15B

08	0.250	20.000	0.000+STD
80			

0.3240.0 0.4180.3300.6760.6910.3970.6240.0 0.7270.5150.0 0.4160.7640.8570.6740.9100.6440.7640.7470.7400.7720.7030.7520.7900.7760.8420.8180.8340.8420.8510.8690.8060.8250.8650.8140.8120.8090.8330.7770.8550.8280.8230.8190.8340.8020.8360.7780.7860.7890.7630.7740.7850.7920.7370.8280.7920.8030.8090.7740.7950.7810.7400.7510.6840.7170.7640.7090.7690.7390.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0

MEAN RATIO OF MINOR TO MAJOR CHORD -STD CAT N FIGURE 4-15R

80	0.2500	20.0000	0.0000-STD
0.3240.0	0.4180.3300.6760.3290.3970.3240.0	0.3420.3340.0	0.3760.3230.5340.423
0.3550.3720.4800.4550.5500.4680.4030.4750.5140.4860.5720.5020.5760.5650.5940.534			
0.5590.5770.5790.5840.5660.5520.5690.5170.5600.5080.5810.6090.5420.5450.555			
0.5250.5560.4540.4930.4570.5230.4640.3920.4730.4500.5160.5080.4740.5300.4940.462			
0.3990.4310.3740.4220.3620.3220.4450.0	0.0	0.0	0.0 0.0 0.0 0.0

MEAN DISTANCE TO THE PERIPHERY CAT N FIGURE 4-16R

80	0.2500	20.0000	0.0000MEAN
263.	0.275.269.	168.243.284.232.	0.296.251.0.219.242.311.291.
232.	245.289.303.	273.274.296.243.254.234.243.	258.245.240.243.232.
244.	242.243.240.	255.248.246.225.219.227.229.	267.232.235.233.231.
224.	213.261.248.	243.224.253.245.244.249.263.	238.227.251.240.229.
232.	230.250.209.	248.222.244.0.0.0.0.	0.0.0.0.0.0.

MEAN DISTANCE TO THE PERIPHERY +STD CAT N FIGURE 4-16R

80	0.2500	20.0000	0.0000+STD
263.	0.275.269.	168.306.284.306.	0.343.317.0.259.299.377.321.
245.	330.315.392.	319.357.377.305.308.303.323.	343.327.307.321.310.
322.	327.331.318.	338.327.337.301.308.317.309.	351.307.317.304.316.
310.	285.352.323.	312.295.331.310.321.325.354.	313.310.333.328.313.
318.	333.335.286.	331.357.342.0.0.0.0.	0.0.0.0.0.0.

MEAN DISTANCE TO THE PERIPHERY -STD CAT N FIGURE 4-16R

80	0.2500	20.0000	0.0000-STD
263.	0.275.269.	168.181.284.159.	0.248.185.0.178.184.244.262.
220.	160.264.213.	227.191.216.181.199.165.163.174.	164.172.165.155.
166.	157.156.162.	172.168.155.150.130.138.150.183.	157.153.161.145.
137.	141.170.173.	174.154.176.179.167.172.171.163.	144.170.151.144.
146.	127.164.133.	166.87.145.0.0.0.0.	0.0.0.0.0.0.

MEAN SEQUENTIAL HISTOGRAM FOR MAJOR CHORD CAT N FIGURE 4-17A, 4-17B

37	0.50	18.50	0.0
1.3	0.9	1.2	2.7
5.4	4.0	6.4	5.1
0.1	0.0	0.0	0.0

MEAN SEQUENTIAL HISTOGRAM FOR MAJOR CHORD +STD CAT N FIGURE 4-17A DOTTED												
37	0.50	18.50	0.0									
1.8	1.5	2.8	5.5	6.1	5.4	5.3	6.4	8.1	10.1	7.7	10.5	10.3 11.0 10.1
7.6	6.2	9.7	7.9	14.4	14.8	15.5	14.5	12.1	10.9	12.3	7.1	4.8 1.3 2.3 0.4
0.4	0.0	0.0	0.0	0.0								
MEAN SEQUENTIAL HISTOGRAM FOR MAJOR CHORD -STD CAT N FIGURE 4-17A DOTTED												
37	0.50	18.50	0.0									
0.8	0.3	-0.3	-0.2	1.3	1.7	0.9	1.1	3.2	5.4	3.7	5.3	4.2 5.9 5.2 3.4
3.3	1.8	3.2	2.4	2.9	5.8	5.6	3.0	2.2	1.3	-0.5	0.1	-1.7 -0.4 -1.0 -0.2
-0.2	0.0	0.0	0.0	0.0								
MEAN SEQUENTIAL HISTOGRAM FOR MAJOR CHORD +SEM CAT N FIGURE 4-17B DOTTED												
37	0.50	18.50	0.0									
1.4	0.9	1.5	3.6	4.3	3.9	3.7	4.5	6.3	8.4	6.1	8.7	8.2 8.7 9.0 8.0
6.0	4.6	7.6	6.0	12.3	12.6	13.3	12.5	9.8	8.7	10.5	4.9	2.8 0.5 1.0 0.1
0.1	0.0	0.0	0.0	0.0								
MEAN SEQUENTIAL HISTOGRAM FOR MAJOR CHORD -SEM CAT N FIGURE 4-17B DOTTED												
37	0.50	18.50	0.0									
1.3	0.8	1.0	1.8	3.0	3.2	2.6	3.0	5.0	7.2	5.2	7.1	6.2 7.6 7.2 5.5
4.9	3.4	5.3	4.3	5.0	8.1	7.9	5.1	4.4	3.5	1.3	7.2	0.4 0.4 0.4 0.1
0.1	0.0	0.0	0.0	0.0								
MEAN SEQUENTIAL HISTOGRAM FOR MINOR CHORD CAT N FIGURES 4-17C, 4-17D												
28	0.50	14.00	0.0									
1.4	1.6	2.0	2.8	3.3	5.6	6.1	6.6	7.3	8.6	8.2	7.2	10.2 6.2 7.2 7.9
8.6	8.7	12.6	10.4	9.4	8.0	5.3	4.1	1.9	0.9	0.2	0.0	
MEAN SEQUENTIAL HISTOGRAM FOR MINOR CHORD +STD CAT N FIGURE 4-17C DOTTED												
28	0.50	14.00	0.0									
2.2	3.9	3.9	5.1	5.5	9.1	8.4	8.8	10.2	11.2	10.3	9.9	13.8 9.9 10.2 11.2
12.2	13.9	19.7	14.3	14.3	15.2	10.8	9.4	4.6	2.6	0.9	0.0	
MEAN SEQUENTIAL HISTOGRAM FOR MINOR CHORD -STD CAT N FIGURE 4-17C DOTTED												
28	0.50	14.00	0.0									
0.7	-0.8	0.1	0.5	1.2	2.0	3.8	4.3	4.5	6.0	6.1	4.5	6.6 2.6 4.3 4.6
4.9	3.4	5.4	6.6	4.6	0.8	-0.1	-1.2	-0.8	-0.8	-0.4	0.0	

MEAN SEQUENTIAL HISTOGRAM FOR MINOR CHORD +SEM CAT N FIGURE 4-17D DOTTED
 28 0.50 14.00 0.0
 1.5 2.2 2.4 3.4 3.8 7.0 6.7 7.1 8.2 9.3 8.7 8.0 11.7 7.7 8.2 9.1
 10.0 11.7 18.2 12.1 12.0 13.7 8.6 7.2 2.7 1.2 0.3 0.0
 MEAN SEQUENTIAL HISTOGRAM FOR MINOR CHORD -SEM CAT N FIGURE 4-17D DOTTED
 28 0.50 14.00 0.0
 1.4 0.9 1.6 2.2 2.8 4.1 5.5 6.0 6.4 7.8 7.7 6.4 8.8 4.8 6.3 6.7
 7.1 5.6 6.9 8.8 6.9 2.3 2.0 1.0 1.1 0.6 0.2 0.0
 MEAN SEQUENTIAL HISTOGRAM FOR AREA CAT N FIGURE 4-18A, 4-18B
 29 0.50 14.50 0.0
 1.6 2.3 1.7 3.8 4.9 5.9 6.7 7.3 8.6 7.4 11.0 8.0 7.3 7.3 4.3 4.4
 6.1 11.8 13.2 13.3 9.7 5.3 4.9 3.7 1.0 0.4 0.3 0.0 0.0
 MEAN SEQUENTIAL HISTOGRAM FOR AREA +STD CAT N FIGURE 4-18A DOTTED
 29 0.50 14.50 0.0
 2.3 4.8 3.7 7.0 7.1 8.7 9.0 10.1 12.2 10.1 12.8 11.2 10.2 10.8 6.8 7.2
 9.9 19.7 19.8 21.2 18.3 9.3 11.7 10.7 3.6 1.5 1.3 0.0 0.0
 MEAN SEQUENTIAL HISTOGRAM FOR AREA -STD CAT N FIGURE 4-18A DOTTED
 29 0.50 14.50 0.0
 0.8 -0.2 -0.4 0.5 2.6 3.1 4.3 4.6 4.9 4.8 9.2 4.8 4.5 3.9 1.9 1.7
 2.3 3.8 6.6 5.5 1.0 1.3 -1.9 -3.4 -1.6 -0.6 -0.7 0.0 0.0
 MEAN SEQUENTIAL HISTOGRAM FOR AREA +SEM CAT N FIGURE 4-18B DOTTED
 29 0.50 14.50 0.0
 1.6 3.0 2.1 4.9 5.5 6.8 7.3 8.2 10.0 8.2 11.4 9.1 8.2 8.6 5.0 5.3
 7.7 18.8 18.1 20.2 18.0 7.1 10.0 9.2 1.8 0.6 0.4 0.0 0.0
 MEAN SEQUENTIAL HISTOGRAM FOR AREA -SEM CAT N FIGURE 4-18B DOTTED
 29 0.50 14.50 0.0
 1.5 1.6 1.2 2.6 4.3 5.0 6.1 6.5 7.1 6.7 10.6 6.9 6.4 6.0 3.7 3.6
 4.5 4.8 8.4 6.5 1.4 3.6 -0.2 -1.9 0.2 0.3 0.2 0.0 0.0
 MEAN SEQUENTIAL HISTOGRAM FOR AREA NEIGHBOR ANALYSIS CAT N FIGURE 4-18C, 4-18D
 27 0.50 13.50 0.0
 1.1 1.8 1.8 4.2 5.0 5.7 7.8 8.8 8.1 9.6 8.9 8.1 7.3 7.7 5.8 6.9
 10.0 14.8 14.2 10.9 7.7 4.1 1.4 0.7 0.1 0.0 0.0

MEAN SEQUENTIAL HISTOGRAM FOR AREA NEIGHBOR ANALYSIS +STD CAT N FIGURE 4-18C DOT
 27 0.50 13.50 0.0
 1.4 3.5 3.1 7.0 7.2 8.2 10.0 12.3 11.0 12.3 11.0 10.1 10.1 10.1 10.6 7.7 10.1
 15.5 22.9 19.9 18.6 14.9 8.4 3.3 2.0 0.4 0.0 0.0
 MEAN SEQUENTIAL HISTOGRAM FOR AREA NEIGHBOR ANALYSIS -STD CAT N FIGURE 4-18C DOT
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 MEAN SEQUENTIAL HISTOGRAM FOR AREA NEIGHBOR ANALYSIS +SEM CAT N FIGURE 4-18D DOT
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 MEAN SEQUENTIAL HISTOGRAM FOR AREA NEIGHBOR ANALYSIS -SEM CAT N FIGURE 4-18D DOT
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 6.6 7.4 10.6 4.3 1.9 2.1 1.1 0.5 0.1 0.0 0.0

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