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This study is aimed at elucidating the response of neural membranes to temporally specific acoustic impulses and utilizing this information to better understand the electrochemical microstructure of such membranes. We have found that single, short duration, low energy, pulses of ultrasound delivered at specific times within a window of about 50 msec preceding a standard electrical stimulus appreciably changes the compound action potential (CAP) evoked. These ultrasonic pulses were focused, 2 to 7 MHz, 0.5 msec durations and had peak intensities of 100-800 W/cm^2. The resulting local absorbed energies (less than 100 mJ/gm) would thus produce temperature rises less than 0.025°C which would preclude bulk heating as a basis of the effect. When used as the prestimulus, neither an electrical pulse nor an RF burst would produce similar effects on the CAP. Low frequency mechanical prestimuli, delivered by a vibrating stylus, does however mimic the effect of an ultrasound pulse. At this point in our investigation it appears that the low frequency radiation pressure transient produced by the ultrasound pulse is the proximal effector and, it is acting on relatively slow stretch-sensitive channels in the neural membrane.

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Progress Report on Contract N00014-87-K-0313

Principal Investigator: Dr. Howard Wachtel

Contractor: University of Colorado, Boulder

Contract Title: Micromechanical Properties of Neuronal Membranes

INTRODUCTION

Most studies of the excitability of neural membranes make primary use of electrical and chemical techniques. Recently, however, it has become evident that the "micromechanical" properties of neural membranes can play a major role in the excitation process. In particular the existence of "stretch sensitive" channels has been documented in a wide variety of neuronal membranes not previously thought of as "mechanoreceptors." We wished to further elucidate the relationship between mechanical (acoustic) perturbations of the neuronal membrane and consequent alterations in its electrical excitability. Our approach has, so far, involved the application of low energy ultrasonic pulses, as well as sonic vibrations, to the isolated frog sciatic nerve at specific times in the milliseconds preceding its electrical stimulation. Resultant changes in the compound action potential (CAP) are then interpreted in terms of the likely effects taking place at the neuronal membrane level.

Ultrasound effects on the structures of the central and peripheral nervous system have received previous attention, and the ability to induce irreversible histological change with sufficient intensity has been demonstrated (Fry et al., 1954b; Ballantine et al., 1956; Lele, 1967). At acoustic energy depositions below these levels, reversible functional effects in neural systems have been observed without apparent histological alteration, including direct stimulation of neurons *in vivo* (Gavrilov et al., 1973; Gavrilov et al., 1977a) and *in vitro* (Mori et al., 1987; Gavrilov et al., 1978), suppression or blockading of action potentials (Young and Henneman, 1961a; Young and Henneman, 1961b; Fry and Fry, 1958), and direct modification of receptor potentials in mechanoreceptors (Gavrilov et al., 1977b). Reversible effects have also been reported on the electrical characteristics of other tissue types, including electrical conduction in frog muscle (Welkowitz and Fry, 1956), mammalian myocardium (Mortimer et al., 1984) and frog skin (Coble and Dunn, 1976).

At the membrane level of the neuron, time constants for mechanisms associated with the generation and propagation of action potentials are typically on the order of fractions of milliseconds or milliseconds. Thus, discrimination of ultrasound effects on these mechanisms necessitates the use of appropriately brief acoustic pulses coupled with a recording system capable of examining electrical characteristics of the neural preparation at

different latencies, with temporal resolution on the order of these time constants. To explore the possibility of such time-specific effects, the relative excitability of a segment of frog sciatic nerve, following its irradiation by a single, focused ultrasound pulse, was studied over a range of latencies, from 0 to 100 msec post-pulse.

METHODS AND MATERIALS

Whole sciatic nerves from pithed, 3–4" (snout to vent) frogs of the species Rana pipiens were excised and placed in an ultrasound exposure chamber (Fig. 1) containing Ringers solution (mM: NaCl, 115.0; KCl, 2.1; $CaCl_2$, 1.8; MgCl, 2.0; TRIS, 1.0; Glucose, 3.3). The excised nerve trunks measure 3–4 cm in length from the lower tibio-fibula to the spinal ganglia, and have a nominal diameter of 2 mm. The upper dish of the chamber has a 5-mm hole at its center over which the nerve is placed. The focused ultrasound transducer (Panametrics, model V304, f=2") in the lower portion of the chamber focuses the field to a 1-3 mm spot (half-power width) on the section of nerve exposed by the dish opening above depending on frequency and location in the z-dimension. The carrier signal of a Yaesu RF transceiver (model FT-101) triggered by a Grass stimulator (model 548) and amplified (Ameritron, model AL-1200 RF amplifier) is used to drive the ultrasound transducer. Field intensities were measured using a point hydrophone (Specialty Engineering Associates, model PVDFZ44) calibrated using the calorimetric methods of Fry and Fry (1954a) and Parker (1983).

The trigger signal to the ultrasound driver is also channeled to the delayed trigger input of a second Grass Stimulator (model S44) which provides an electrical stimulus output of variable amplitude, duration and latency. On either side of the exposed nerve segment lie two Ag/AgCl electrodes separated by approximately 5 mm. Through these electrodes, a 15-μsec electrical stimulus from the second stimulator is delivered, the intensity of which is adjusted prior to each experiment to yield a compound action potential (CAP) of an amplitude of approximately half of its value at saturation. Thus a percentage of fibers contributing to the CAP can be presumed to be stimulated just over threshold, and another can be presumed to be stimulated just below threshold. Any modification of excitability by the ultrasound pulse therefore results in relatively greater or fewer numbers of fibers being stimulated over threshold, which is reflected by an increase or decrease, respectively, of the CAP amplitude relative to the no-ultrasound control. A second pair of Ag/AgCl electrodes records the CAP's approximately 1.5 cm distally, where the end of the nerve bundle exits the bath. Both the stimulus and recording electrodes were oriented away from the cut ends of the nerve trunk to minimize the contribution of any injury potentials.

Two important variations of the basic protocol were utilized in addition to that described above. In one parallel experiment, the acoustic pulse was applied to a section of the nerve bundle between the points of

stimulus and recording to determine its effect on a propagating CAP initiated outside the irradiated region. The second variation of the basic protocol utilized a transducer-driven glass stylus to apply a direct mechanical pre-stimulus to the nerve trunk in the region which was subsequently electrically stimulated at 0–100 ms latencies. The transducer consists of a high-frequency response (3–20 kHz) loudspeaker coil driven by a 500-μsec voltage pulse of square-wave form. The stylus was attached with epoxy to the transducer and positioned over the top of the nerve with a micropositioner such that light contact exists at all times. The 3-mm diameter of the stylus was chosen to involve a segment of the nerve bundle comparable to that of the ultrasound pre-stimulus. Sufficiently rapid mechanical response of this system was confirmed by optically tracking the actual displacement of the stylus tip with respect to the applied voltage pulse. The time course of the applied voltage can thus be presumed to accurately reflect the displacement of the stylus tip during these experiments.

RESULTS

Excitability Modification by Ultrasound

The relative excitability of a segment of sciatic nerve, as reflected by changes in CAP amplitude, was studied over a range of latencies from 0–100 msec post-pulse. Specific temporal windows were found in which the irradiated section showed enhancement or suppression of excitability before settling back to reference level. The sequence of the excitability changes following an acoustic pulse generally followed one of two temporal response patterns. Figure 2 shows recordings of CAP's at six different latencies which illustrate excitability changes at characteristic points in samples of data from each of the two response patterns. These two distinct patterns will henceforth be referred to as early-suppression (ES) - and early-enhancement (EE) -type responses.

Figure 3 presents the envelope function of the CAP amplitudes for each of the response types in the interval of 0–100 ms post-pulse, which more completely illustrates the continuum of the excitability modification following a single acoustic pre-stimulus. The relatively short lifetimes of the modification ($\tau < 50$ ms) in both cases is notable.

Generally the response pattern of a given nerve is clearly either an ES- or EE-type response. Occasionally, nerves exhibit temporal response patterns which appear to be combinations of the two response types at nominal pulse intensities (e.g., 250 W/cm$^{2)}$. In such cases, we have observed that a relative reduction of the ultrasound pre-stimulus intensity (e.g., to 150 W/cm$^{2)}$ results in a new response pattern which most closely resembles the ES-type, while a relative increase (e.g., 400 W/cm$^{2)}$ introduces what resembles a strong superimposed EE-type response (Fig. 4). Thus the form of the excitability modification, as well as its amplitude, at times exhibits pre-stimulus intensity

sensitivity. It is not known with certainty what determines the response type in the general case where one predominates at all pre-stimulus intensities.

There is some evidence that suggests a predisposition of A fibers (large-diameter, myelinated) towards the ES-type response and of B fibers (small-diameter, myelinated) towards the EE response, based on experiments in which the relatively greater conduction velocity of the A fibers was used to separate its component of the CAP from that of the B fibers (Fig. 5). In such cases, A and B fiber groups respond independently to the pre-stimulus, with A fibers generally exhibiting ES-type response and B fibers generally exhibiting EE-type response.

Effect of Frequency

The data presented thus far was acquired using pulses of 2 MHz ultrasound. The possibility of a frequency dependence of the effects was assessed by utilizing ultrasound pulses at 4 and 7 MHz, of comparable duration and range of intensities. The general form of the responses (i.e. ES- and EE-type) was found to be consistent whether elicited by 2, 4 or 7 MHz pulses. The relative efficiency of the different frequencies for inducing the effects was studied by comparing the acoustic pulse energies required to elicit a predetermined degree of enhancement or suppression at a given latency. If this comparison is made based on incident pulse energies, then high frequencies are observed to be generally more effective at eliciting a given excitability change. It is well known, however, that in this frequency region the absorption and attenuation coefficients of biological tissue increase linearly with frequency. If one therefore compares the relative effectiveness of these different frequencies based on interactive rather than incident intensities, no frequency dependence for either the form or degree of excitability modification is observed. Interactive intensity was defined as $I_{int}=I_o(1-e^{-2azf})$ for the purposes of this plot, where I_o is the incident pulse intensity (W/cm^2), a is the attenuation coefficient of the nerve trunk (Np/cmMHz), f is the ultrasound frequency (MHz), and z is the nominal nerve trunk diameter. Figure 6 gives acoustic pulse intensity-duration plots for 20% suppression at the 7 msec latency of a typical ES-type response at 2, 4 and 7 MHz. The generally superimposed plots illustrate the relative frequency independence of the effects over a broad range of pulse intensities and durations. It should be noted that the location of the nerve trunk in the focal region was varied at the different frequencies such that the half-power field width (i.e. spot size) was constant in each case.

Effect of Ultrsound Spot Size

The 3-mm spot size utilized in most of these experiments represents a defocused field condition, which was used to minimize the effect of any slight variations in the lateral position of the nerve trunk in the exposure chamber from experiment to experiment. Several additional experiments were conducted in which the acoustic spot size was reduced, while maintaining the

same pulse intensity. Thus although the spatial power density is in each case the same, the total energies vary with the area of the spots, and less total energy is deposited at the smaller spot sizes.

Figure 7 shows intensity-duration plots for 20% suppression at 7 msec latency of an ES-type response at 1.25, 1.63, and 2.88 mm half-power field widths. Note that over the entire range of pulse intensities studied, the smaller spot size is more effective at eliciting a given excitability modification even though the total energy content of the pulse is less. These results clearly illustrate the importance of the spatial distribution of the acoustic pulse as an experimental parameter in this study, in addition to its intensity and duration.

Effect on Propagating CAP

When the acoustic pulse was applied to a section of the nerve trunk between the points of stimulus and recording, a partial blockade of the propagating CAP was observed. This effect was maximized when the conditioning ultrasound pulse preceded the arrival of the CAP by 6–7 msec, corresponding closely to the peak of the first window of suppression in the ES-type response pattern. Conduction blockade at 14 msec latency was not regularly observed. No effect was observed when the arrival of the ultrasound pulse and CAP coincided, nor was any increase in CAP amplitude observed at any latency in this experiment. Intensities of the pre-stimuli required to elicit changes in a propagating CAP are generally four to five times greater than those required to modify excitability to the same degree at the same latency in the previous experiment.

Direct Mechanical Pre-stimulus

When the transducer-driven glass stylus was utilized to apply a direct mechanical pre-stimulus to the nerve trunk (500-μsec duration) analogous excitability modifications were observed compared to those elicited using the single ultrasound pulse as the pre-stimulus. The temporal response patterns compared closely to those characterized earlier as ES- and EE-type responses.

The implications of this experiment are significant in assessing the relative importance of several experimental parameters, most notably the frequency dependence of the general effect, and associated thermal and cavitation mechanisms. These will be addressed in the Discussion section of this paper.

DISCUSSION

As with all phenomenological observations, direct causality between the experimental parameters and system responses cannot be assumed, necessitating careful consideration of the range of factors which may underlie the observed effects. Several modes of possible interaction of the ultrasound field with the nerve trunk are considered below.

Thermal Effects

Ultrasonic fields often interact with living systems as a result of bulk heating produced by acoustic absorption. Many of the early studies reporting nerve conduction effects utilized cw- or long-pulse ultrasound, and correlated the observed changes to the accompanying temperature rise (Lehmann and Biegler, 1954). In this study, although peak pulse powers are high, total energy deposition is low, less than 100 mJ/gm, owing to the short duration of exposure. For a typical nerve trunk at 2 MHz, this corresponds to a general temperature rise of less than 0.025° C. Effects due to bulk heating are therefore doubtful.

The possibility of microthermal effects, due to differential heating of specific structures of the axons, was considered, as was the significance of the relatively high rate-of-change of temperature calculated during the pulse (0.025° C/500 μsec), for which a theoretical basis of effect exists (Barnes, 1984). Both of these mechanisms are, however, strongly precluded by the analagous effects observed following the 500-μsec direct mechanical stimulus, since acoustic absorption and, hence, heating of the frequency band of this pulse would be negligible.

Transient Cavitation

At the acoustic intensities utilized in this study, small gas bubbles in the nerve tissue or medium may exhibit complex dynamical behavior, including transient cavitation characterized by violent collapse (Flynn, 1964; Neppiras, 1980). Very high temperatures in the vicinity of the bubbles can dissociate water vapor into the free radicals H^+ and OH^-, which can interact with other components, resulting in further chemical changes.

The potential role of cavitation in eliciting the observed excitability effects was thus considered. Although some transient cavitation may occur with each pulse, the similar excitability modifications which occurred following the direct mechanical stimulus, with which cavitation would not be expected, argue against a significant contribution of this mechanism.

Stimulus Coupling Artifacts

Direct effects of the ultrasound field on the stimulus electrodes and on the coupling of the stimulus current with the nerve trunk were also considered. The location of the stimulus electrodes on either side of the nerve and separated by 5 mm places them outside the maximum field spot of 3 mm, thereby making direct effects of the ultrasound field on the electrodes unlikely. Likewise, the recording electrodes located outside of not only the field but also the bath itself are not subject to direct interaction with the ultrasound.

Coupling of the stimulus current with the nerve trunk could be affected by movement of the bathing medium, the nerve itself, or both. The sensitivity of the set-up to nerve movement was studied by varying the position of the nerve with respect to the electrodes using a micromanipulator. Movements of several millimeters in any given

direction were necessary to alter the CAP amplitude to the same degree observed following the ultrasound exposure. Microscopic examination during and following the ultrasound pulse revealed no gross movement of the fiber bundle with respect to the stimulus electrodes.

Stimulus current coupling could also be affected by displacement of the aqueous bath which serves as the coupling medium. We have observed that movement of the bath is reflected in the electrical stimulus artifact which precedes the CAP. The relative stability of the stimulus artifact at all latencies, while the CAP amplitude changed markedly, is therefore inconsistent with what would be expected if the coupling artifact were acting. The height of the bath also was not observed to influence the experimental results.

Basis of Effect

In approaching the question of identifying the basis of the observed effects, several points are notable. First is the observation of the analogous effects of the 500-μsec ultrasound pulse and that of the 500-μsec, direct mechanical stimulus. This finding suggests that the perturbation associated with the ultrasound pulse envelope, rather than the oscillatory nature of the mechanical perturbation within the pulse, is of greatest importance. The radiation force envelope may thus be implicated as the active parameter. This hypothesis is consistent with the observed frequency independence of the effect.

Radiation force is derived from the average momentum of an acoustic beam carried per unit time past a plane normal to the propagation axis, given by

$$\text{Momentum} = \frac{I_a A}{c},$$

where I_a is the time-average field intensity, A is its cross-sectional area, and c is the speed of sound in the medium. Interaction of the field with the nerve trunk causes a change in momentum, resulting in a normal force given by

$$\text{Fnorm} = \frac{\left(I_{abs} + 2I_{sc}\right) A\cos\theta}{c} \qquad [1]$$

where I_{abs} and I_{sc} are absorbed and scattered fractional intensities of the field incident on the nerve, A is the cross-sectional area of the irradiated region, c the speed of sound, and θ is the angle between the beam axis and the normal to nerve surface. This expression, although strictly true only for a continuous plane wave in a homogeneous, nonviscous fluid medium, provides a reasonable estimate for radiation force developed in this work.

The fraction of incident intensity absorbed and scattered by the nerve can be estimated by

$$I_{abs} = I_o\left(1 - e^{-2\alpha z f}\right) \qquad [2]$$

$$I_{sc} = I_o\left(1 - e^{-2\sigma z f}\right) \qquad [3]$$

where I_o is the time average incident intensity during the pulse, α and σ are the absorption and scattering coefficients, respectively (Np/cm MHz), z is the nerve thickness (cm) and f the acoustic frequency (MHz).

Assuming an acoustic spot size of 0.3 cm, a nerve trunk diameter of 0.2 cm, $c=1.5 \times 10^5$ cm/s, $I_o=500$ W/cm^2, $\alpha=0.039$ Np/cmMHz, and $\sigma=0.047$ Np/cmMHz, the radiation force developed during a single pulse of 2, 4 and 7 MHz ultrasound is calculated to be 2.1×10^{-5}, 4.1×10^{-5} and 7.2×10^{-5} dynes respectively. These forces correspond to radiation pressures across the nerve trunk of 3.5×10^{-4}, 6.8×10^{-4} and 1.2×10^{-3} dynes/cm^2.

A comparison of the mechanical forces developed by the tranducer-driven stylus and those developed by the radiation pressure of the ultrasound can be made based on calculations of the total pulse energy in each case resulting in an undirectional force on the nerve trunk. In the case of the ultrasound pulse, this would correspond to the fraction of incident pulse energy which is transduced to radiation pressure, or

$$E_{rad} = (I_{abs} + 2I_{sc})\, At_{pulse}$$

Assuming the same ultrasound parameters as above at 2 MHz, the component of pulse energy translated into radiation pressure is 1.6 mJ.

In the case of the direct mechanical stimulus, the energy applied to the nerve can be estimated based on the measured electrical energy going into the transducer and the estimated conversion efficiency to mechanical energy. Typical applied electrical pulse power in these experiments was 3.1 W. Assuming a conversion efficiency of 50%, the resulting mechanical energy applied through the stylus is

$$E_{stylus} = {}^1/_2\,(P_{ele} \cdot t_{pulse}) = 0.8 \text{ mJ}.$$

Thus the total pulse energies directed as a mechanical force normal to the nerve trunk in both cases is seen to be comparable, further supporting the view that the ultrasound energy interacting with the nerve as radiation pressure is the active component of the pulse.

It is interesting to note that auditory nerve responses in cats to pulses of 5 MHz, 30 W/cm^2 ultrasound from a transducer placed against the dura matter have been reported, and attributed to radiation pressure transients accompanying these pulses (Foster and Wiederhold, 1978). The authors assumed complete absorption of the ultrasound energy in the brain tissue, and thus calculated the peak radiation pressure per pulse to be 2.0×10^{-4} dyne/cm^2. Other investigators have reported human hearing sensation to 2.35 MHz pulsed ultrasound applied to the head with frequency equal to the

pulse repetition frequency (Gavrilov et al., 1980). This action of ultrasound was attributed in part to modulation of radiation pressure acting on the aural labyrinth, with an intensity threshold of 7-110 W/cm^2 (pulse duration of 1 msec) depending on the localization of the focal zone. Unpulsed ultrasound at the same intensity failed to elicit any auditory sensation. The expectation that radiation pressure transients accompanying pulsed ultrasound may have direct action on neural tissues is thus not without precedent in the literature, although the importance of this mode of interaction of ultrasonic field and tissue has perhaps received disproportionately little attention overall.

A second important observation is that even the earliest window of effect, the excitability enhancement peaking at 5 msec in the EE-type response, is relatively slow in the context of typical neuroelectric phenomena associated with action potential generation, such as channel switching times, or even refractory periods, which generally do not extend beyond 2–3 msec in the frog sciatic fibers. A body of evidence does exist, however, for the existence of "slow" channel types or conductance states of both potassium (Dubois, 1981; Ilyin, 1980) and sodium (Benoit et al., 1985) with time constants extending well into this range. Another important observation is that no emulation of these effects could be elicited using either a sub-threshold DC or a 2 MHz RF electrical pre-stimulus, suggesting that the mechanisms involved are uniquely receptive to mechanical stimulation, yet translate their action into electrical excitability changes.

Such translation of mechanical into electrical energy is, of course, the defining characteristic of a mechanoreceptor, in which mechanical perturbation of the nerve ending results in changes in membrane permeability to Na and K, and hence to membrane potentials. It is interesting to note in this context that these channels are thought to be different from those involved in the production of action potentials (Kuffler et al., 1984). The possibility that similar mechanisms are playing a role in the effects that we have observed with axonal segments will be considered in future studies.

Observations of membrane sensitivity to mechanical stimulus have not been limited to mechanoreceptors, however. Stretch-activated (SA) ion channels have been reported in a variety of animal cells (Guharay and Sachs, 1984; Ohmori, 1984; Sigurdson et al., 1987; Lansman et al., 1987; Christensen, 1987), including neural membrane, where coexisting stretch-inactivated (SI) channels have also most recently been described (Morris and Sigurdson, 1989). These channels are presumed to be transmembrane structures which are activated by a conformational change during membrane tension. In these investigations, single-channel response to stretch was measured by applying suction through a patch clamp electrode. Guharay and Sachs (1984) estimated a typical membrane tension T in their experiments to be 0.67 dyne/cm. Preliminary calculations which define tension T in terms of the membrane elasticity constant K_A and fractional increase in membrane area $\Delta A/A$ resulting from the radiation pressure of the ultrasound pulse predict tensions which are comparable to those calculated by Guharay and Sachs. It should be

noted that there is considerable uncertainty associated with attempting to quantify membrane tension in the both patch clamp context (see W.J. Sigurdson et al., 1987), and the myelinated nerve case, however it does provide a starting basis for comparison. In addition, it is interesting to note that analysis of the kinetics of SA channel activity suggests a gating mechanism which involves multiple open and closed states, with time constants falling in the range of latencies where we have observed effects.

A candidate for the mechanism underlying the excitability changes observed may thus be the transient gating of SA- and/or SI ion channels, whose resulting currents could serve to modulate excitability by altering threshold. Although we are unaware of any studies in which these channels have been documented at nodes of myelinated axon, we know of no evidence to preclude this, especially given the seemingly ubiquitous distribution of SA channels among the variety of cells studied.

Effect of Spot Size

The relatively greater effectiveness of the smaller spot sizes, even with their reduced energy content, can be perhaps explained in this context of stretch-activated neural events. The electrode geometry in this study is such that the region of the nerve trunk undergoing direct electrical stimulation is probably limited to a few millimeters, and thus any mechanism which elicits excitability changes must be active in this relatively limited region to be observed.

Radiation pressure acting on a region of membrane would be expected to result in a local displacement, which in turn would result in an increase in area or stretch of the membrane surface. SA channels are presumed to be sensitive to tension directed parallel to the membrane surface. Thus the membrane regions in which stretch-activation would be most likely to occur may be those across which the pressure gradient, and not simply the peak pressure, is maximized along its surface. The smaller ultrasound spot sizes may therefore be more effective at eliciting an observable excitability change because of the relatively larger gradient in the spatial distribution of radiation pressure in the region undergoing electrical stimulation. One implication of this finding is that a maximal effect might be observed when the degree stretch is maximized over a distance equivalent to the spacing between the nodes of Ranvier for a particular fiber.

In the experiment in which the effect of the ultrasound pulse on a propagating CAP was studied, several points merit discussion. The observation that maximal blockade was observed when the acoustic pulse precedes the arrival of the CAP by 6–7 msec is consistent with the major suppression of excitability in the ES-type response peaking at near the same latency. It is thus likely that the mechanisms underlying these two observed effects are the same.

The relatively greater ultrasound pulse intensity required to elicit comparable suppression of the propagating CAP might be explained by the inherent safety factor (typically ~5) of myelinated axon with regard to the

amplitude of an action potential arriving at a node versus that required for depolarization past threshold. The lack of a partial blockade at 14 msec latency, corresponding to the suppression of the EE-type response, may thus be the result of the inherently less sensitive nature of this particular experiment.

At this stage of the investigation it is rather difficult to approach the question regarding the precise origins of the ES- and EE-type responses. Our experiments have demonstrated both a sensitivity to the ultrasound intensity and the fiber class being observed with regard to which response type predominates. Most striking is perhaps the independent response of the A and B fiber groups when their separate components of the CAP can be resolved as distinct peaks. It is interesting to note in this context that at least two separate investigations have ranked the relative sensitivities of the different peripheral fiber groups to ultrasound as being non-uniform, with B fibers being the most sensitive, followed by C fibers, while A fibers are reported to be least sensitive (Anderson et al., 1951; Herrick, 1953). Thus if the temporal response patterns (i.e. ES- or EE-type) are determined by two or more underlying membrane events, each with different thresholds and associated time constants (e.g. gating of SA potassium, SA sodium and SI potassium channels), then an ultrasound pulse of a given intensity might evoke a different temporal response in an A or B fiber due to their differing ultrasound sensitivities. This general view is also consistent with the sensitivity of the response form to the pulse intensity which was sometimes observed. We hope to resolve this question and others regarding the exact bases of the general phenomenon in future studies using single fibers.

The implications of these observations in the clinical context are difficult to completely assess until the exact modality of interaction of the ultrasound and nerve is revealed. As a general comment, however, it can be said that any means by which neural function can be predictably and reversibly modified provides a basis for use as a system of inputting and sculpting information in the nervous system. Prosthetic, analgesic, and other therapeutic uses could thus be envisioned. Specific characterization of the ionic, metabolic and morphologic events underlying the observed excitability modifications may lead to the coalescence of these general concepts into specific clinical applications.

SUMMARY

These studies have shown that single, brief pulses of ultrasound have the capacity to elicit temporally specific modification of excitability in myelinated axons. The low total energy of the ultraound pulses and the comparable effects seen with direct mechanical prestimuli preclude the possibility of this phenomenon having a primarily thermal basis. These transient modifications of excitability, perhaps mediated by the activation of stretch-sensitive ionic channels, suggest a potential clinical application of

high peak-power acoustic pulses in the functional modulation of neuroelectric signals.

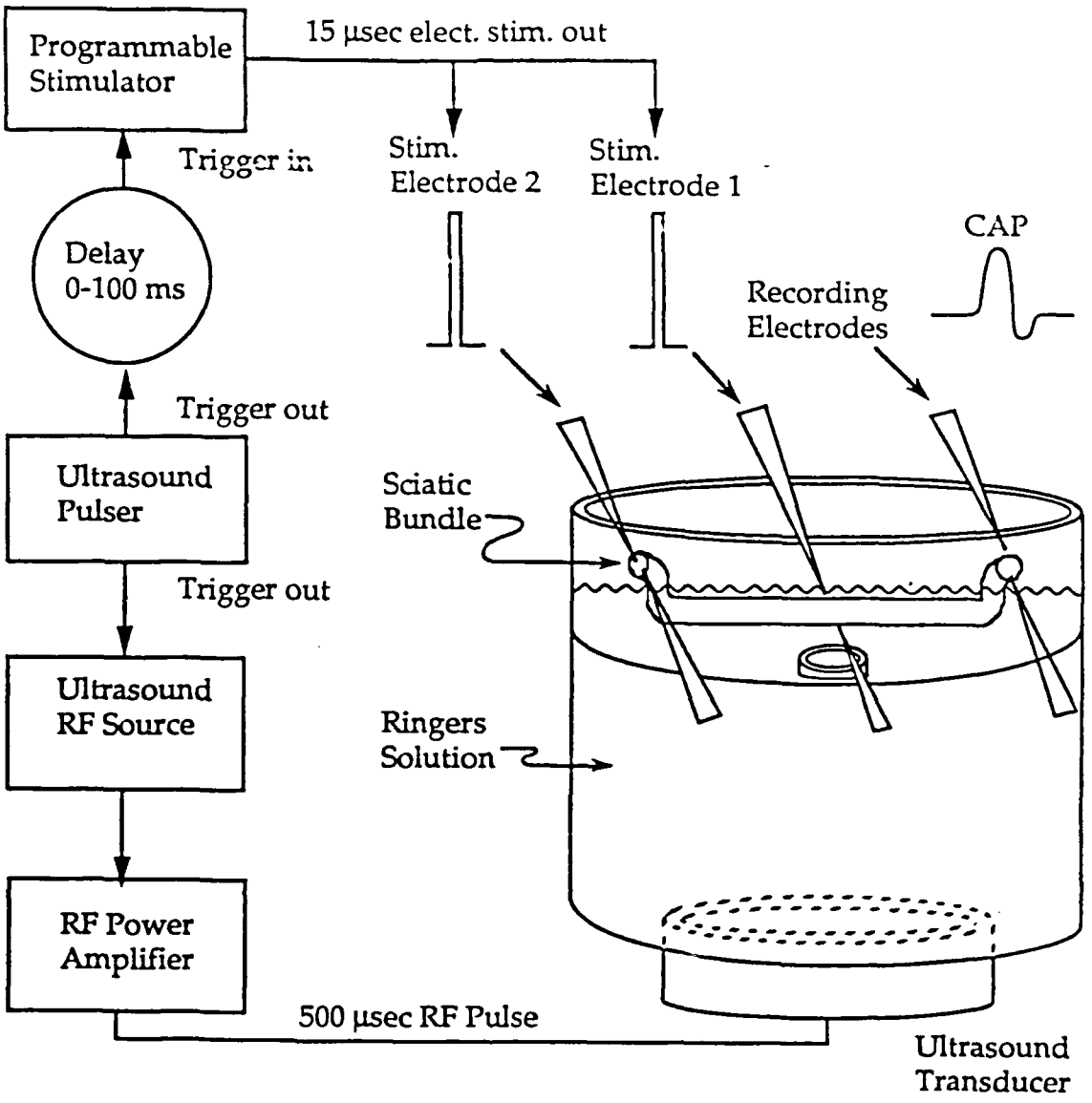

Figure 1. Schematic representation of the ultrasound exposure system. Stimulus electrode 1 delivers the 15-µsec electrical stimulus to the nerve segment exposed to the ultrasound pulse used in the excitability modification study. Stimulus electrode 2 delivers the stimulus to the end of the bundle to study the ultrasound effect on a propagating compound action potential. Recording electrodes measure the CAP at the end of the nerve trunk elevated above the bathing medium.

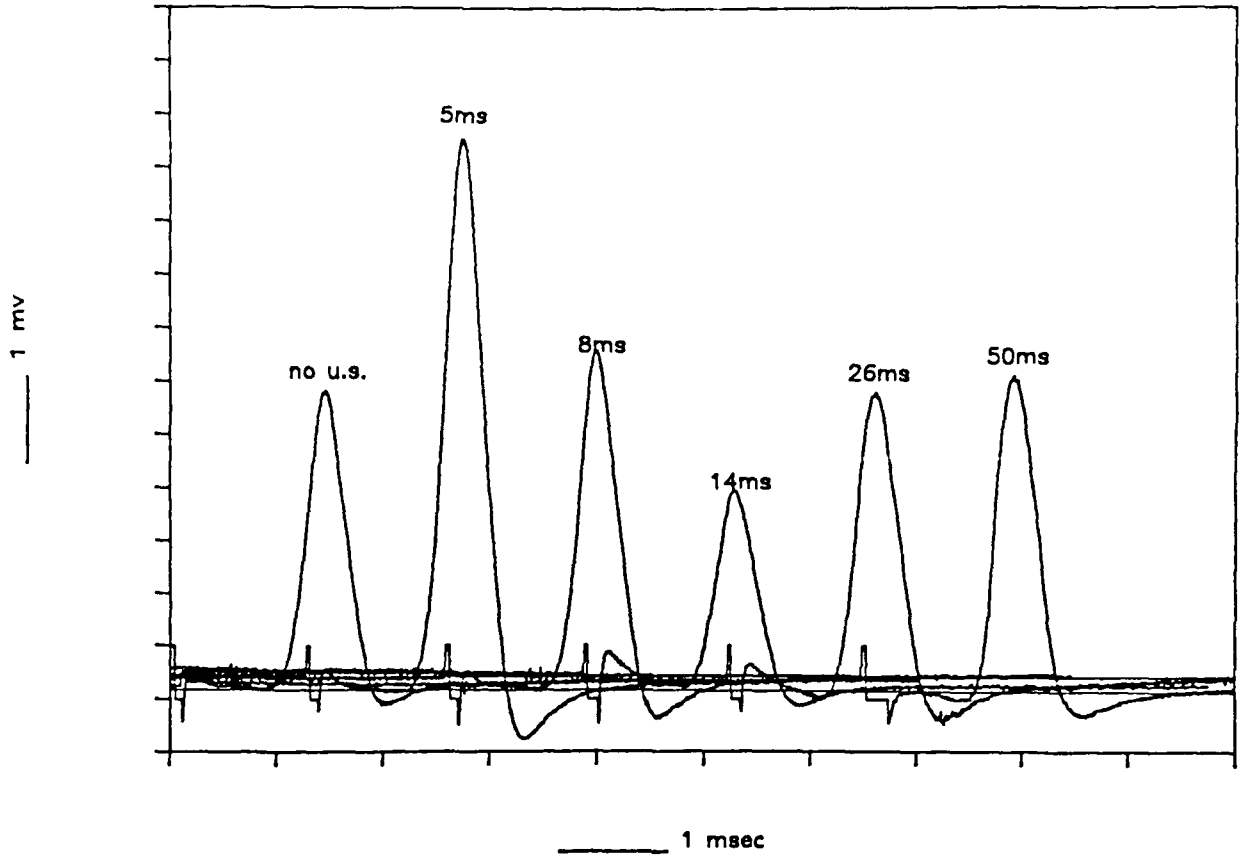

Figure 2. Compound action potentials (CAPs) recorded from the sciatic bundle at various latencies from 0-50 ms following application of a 500-μsec, 400-W/cm^2, 2-MHz ultrasound pulse to the stimulus region of the axonal bundle. A) Excitability modification of the ES-type response. B) Excitability modification of the EE-type response. In both response plots, the first CAP of each series is elicited by a 15-μsec electrical stimulus of sufficient intensity to generate a CAP amplitude of approximately half of its possible maximum in the no-ultrasound (no-u.s.) condition. The electrical stimulus was maintained at this setting for the rest of the recordings shown in the series. (Modification of excitability by the ultrasound pulse is reflected by an increase or decrease, respectively, of the CAP amplitude relative to the no-ultrasound control.) The spike preceding each CAP in this and subsequent figures is an artifact of the electrical stimulus used to generate the CAP. The temporal separation (approximately 1 ms) represents the relatively slower propagation speed of the ionic conduction underlying the CAP.

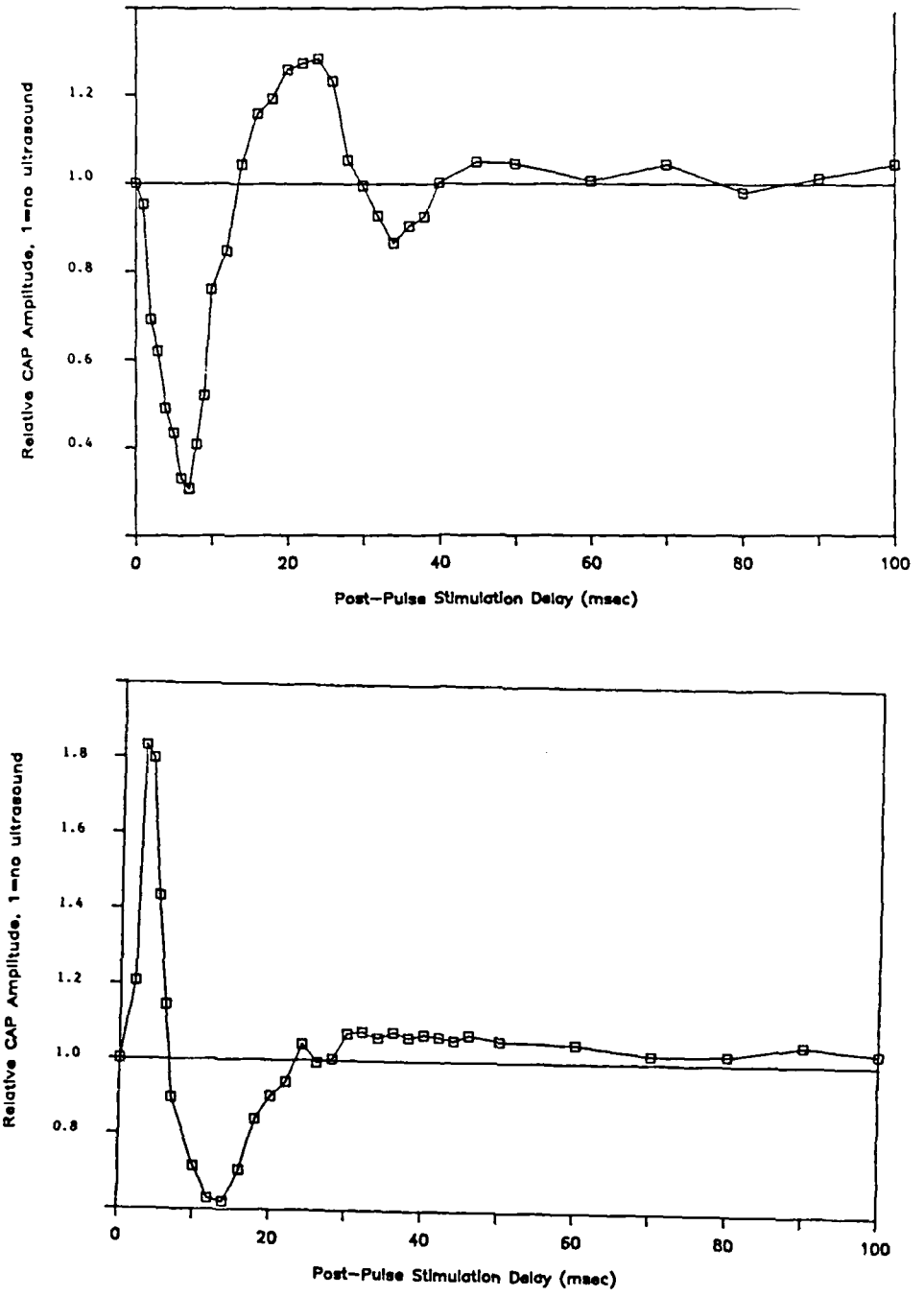

Figure 3. Plot of compound action potential (CAP) peak amplitudes of : (A) the ES-type response, and (B) the EE-type response, at delays ranging from 0-100 msec following a 500-µsec, 400-W/cm^2 acoustic pre-stimulus. Each plot is represented by peak values of approximately 50 CAPs. In each series the strength and duration of the electrical stimulus used to elicit the half-maximal CAPs are identical. Changes in CAP amplitudes at different latencies indicate the modification of excitability induced by the acoustic pre-stimulus pulse at t=0 msec. The horizontal line in each series represents the reference CAP amplitude of the no-ultrasound condition. The results of a typical experiment are shown.

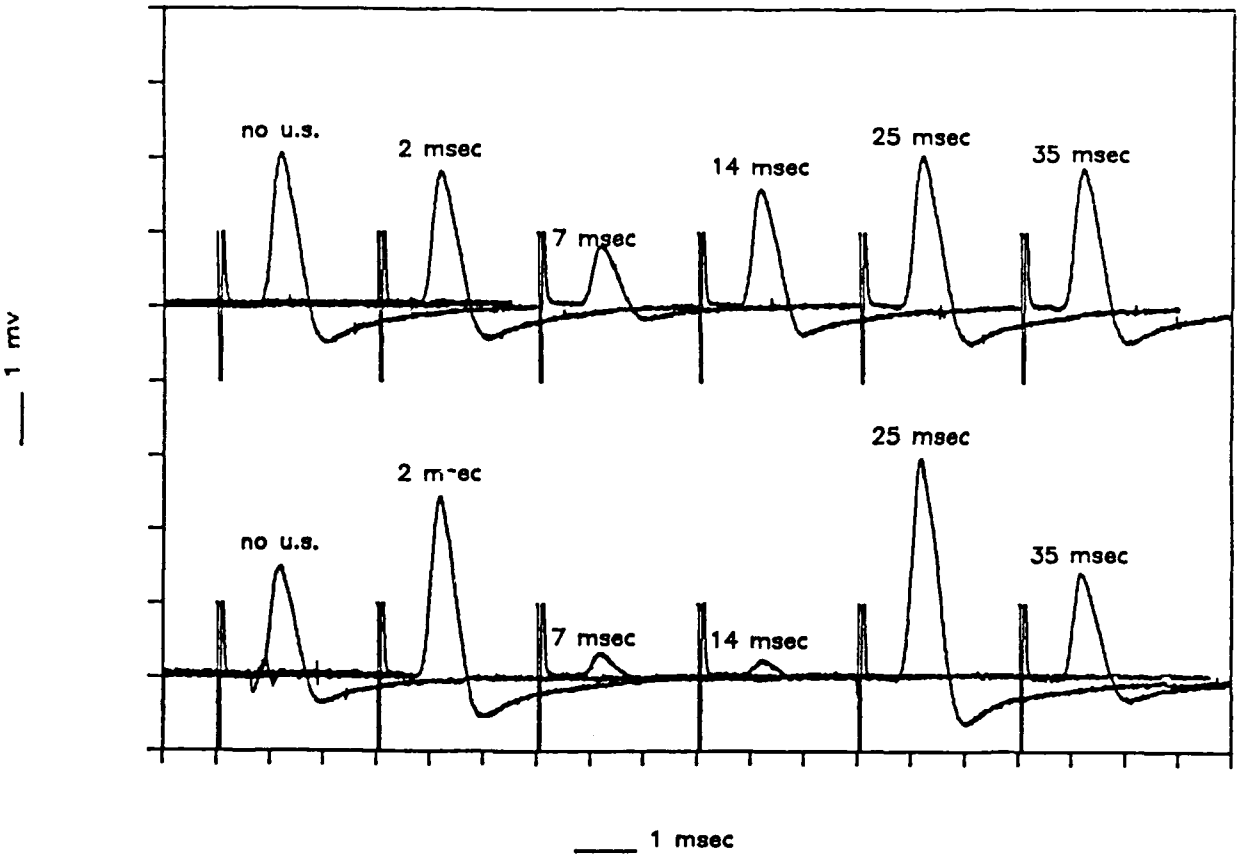

Figure 4. Compound action potentials (CAPs) recorded from the sciatic bundle at the indicated latencies following application of (A) 500-µsec , 150-W/cm^2 ultrasound pulse and (B) 500-µsec, 400-W/cm^2 ultrasound pulse, (both 2 MHz), suggesting a pre-stimulus intensity sensitivity of the response pattern. Recordings are from the same nerve, with identical electrical stimuli generating the CAPs, in back to back trials. Note that the response pattern in 3A follows that of the basic ES-type, while at a higher pre-stimulus intensity (B), the excitation at t= 2 msec and the inhibition at t= 14 msec suggests the emergence of a superimposed EE-type response. A complete transition into the EE-type response was not observed in this experiment at the maximum intensity available (800 W/cm^2) at the same duration.

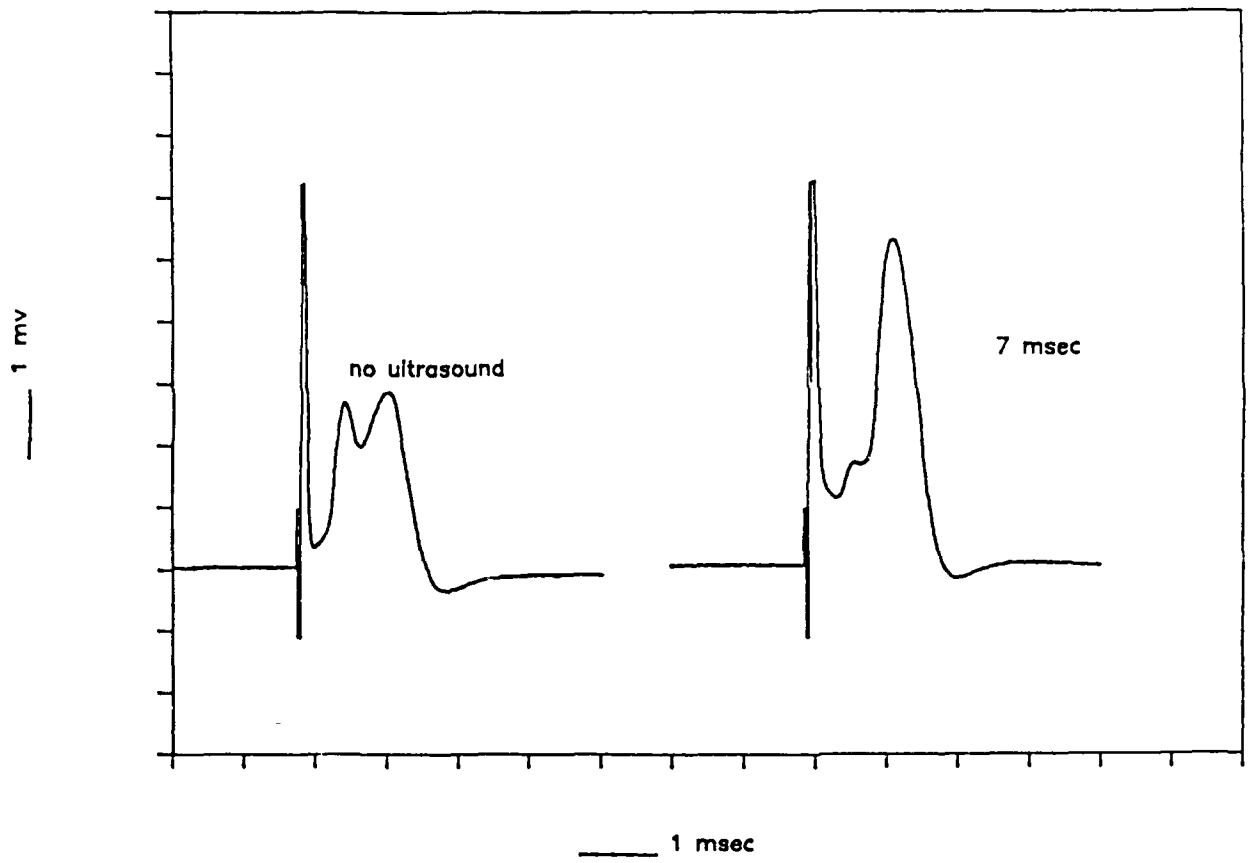

Figure 5. Response of different fiber groups to the ultrasound pre-stimulus. The two major classes of myelinated fiber can be differentiated by their relative diameters, resulting in different conduction velocities. Components of the total CAP contributed by the respective fiber groups will thus emerge as the slower component of the smaller B fibers lags that of the larger and faster A fibers during conduction along a length of axon. In the no ultrasound recording, amplitudes of the A and B fiber component are approximately equal. The subsequent recording shows the nerve response to a 500-μsec, 250-W/cm^2, 2-MHz acoustic pulse preceding the electrical stimulus by 7 msec. Note that the A fiber component exhibits a suppression of excitability, consistent with the ES-type response, while the B fiber component exhibits excitability enhancement, consistent with the EE-type response at this latency.

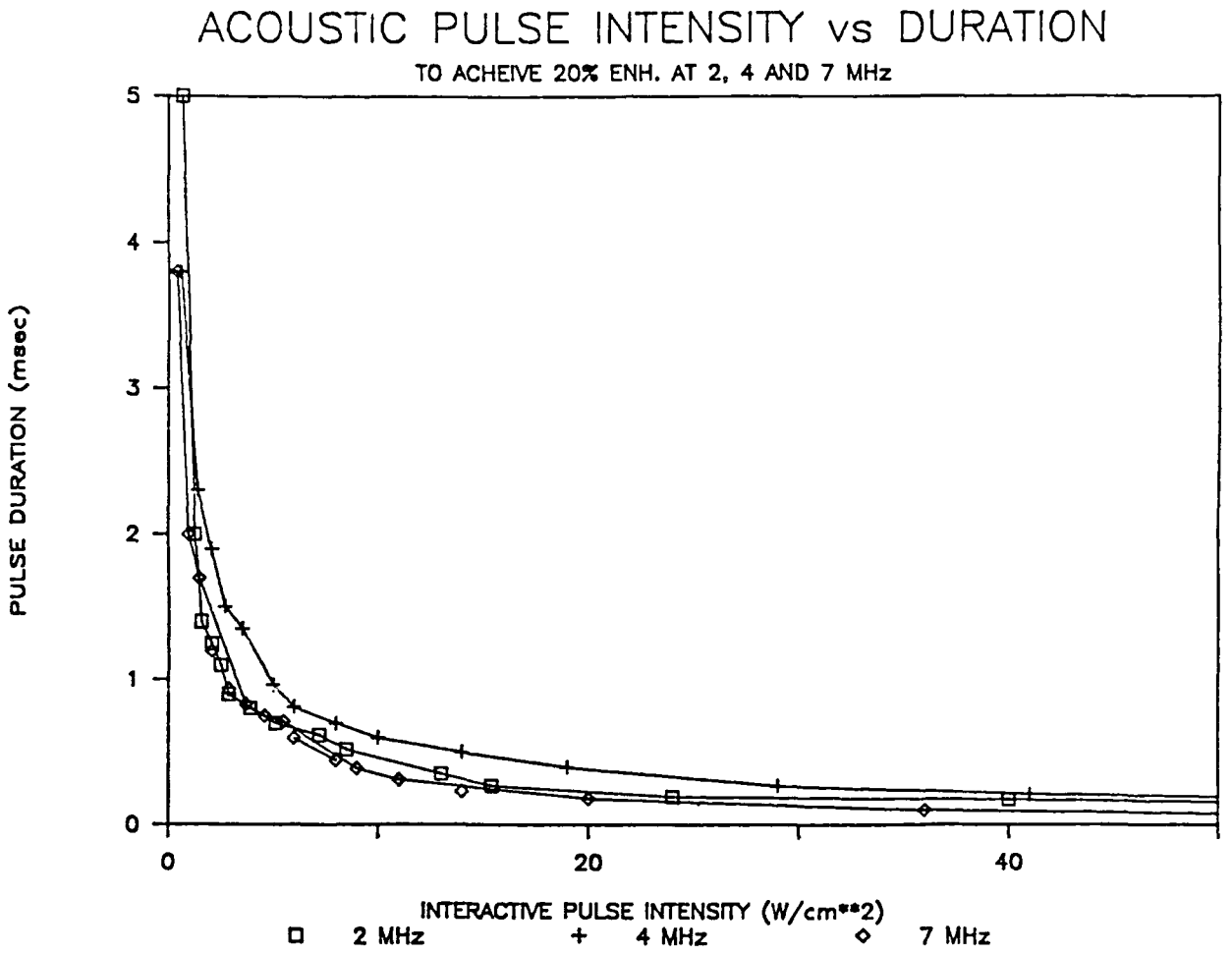

Figure 6. Ultrasound pulse intensity-duration plots for a predetermined effect at 2, 4, and 7 MHz. Each point on the curves represents the duration required at a given intensity to achieve a 20% suppression of the half-maximal CAP amplitude at the 7msec latency of an ES-type response. The acoustic spot size in each case was maintained at 2 mm. Note that the pulse durations are plotted as a function of interactive intensities rather than incident intensities to normalize for the different absorption and scattering coefficients for the different frequencies. The generally superimposed nature of the plots illustrate the relative frequency independence of the effect over a broad range of pulse intensities and durations.

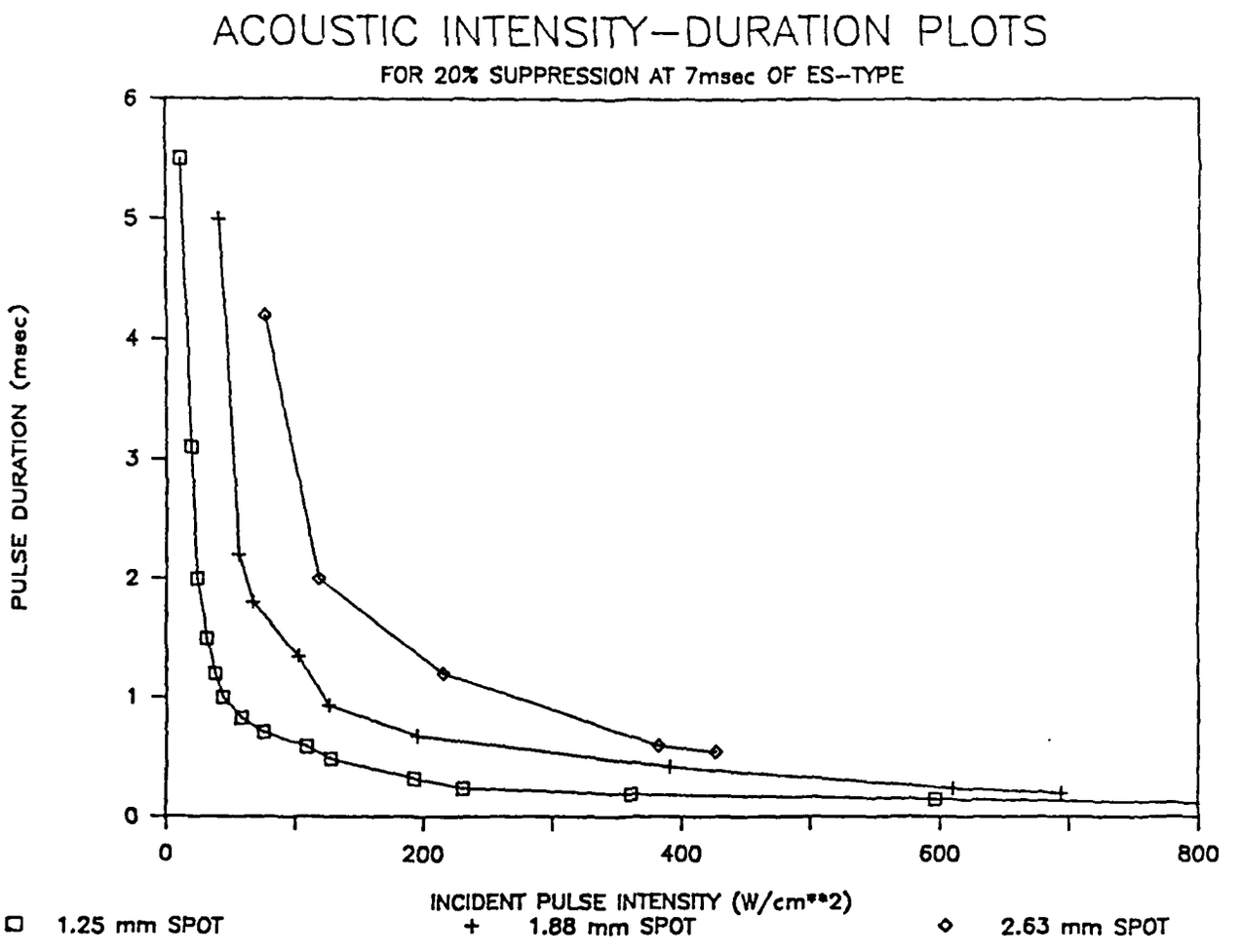

Figure 7. Effect of varying acoustic spot size while maintaining incident intensity. Each point on the curves represents the duration required at a given incident intensity to achieve a 20% suppression of the half-maximal CAP amplitude at the 7 msec latency of an ES-type response. The half-power field widths used were 1.25, 1.63 and 2.88 mm at a frequency of 2 MHz. Since peak intensities in each case were identical, the energy content of a pulse at a given intensity and duration decreases as the spot size is reduced. Note that over the entire range of pulse intensities studied, the smaller spot size is more effective at eliciting a given excitability modification even though the energy content of the pulse is less.

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