THEORETICAL RESPONSE TO TRAINS OF ACTION POTENTIALS OF A BIFURCATING AXON WITH ONE SHORT DAUGHTER BRANCH

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ABSTRACT It has been shown both experimentally (Stockbridge, N., and L. L. Stockbridge. 1988. *J. Neurophysiol.* 59:1277–1285) and theoretically (Stockbridge, N. 1988. *J. Neurophysiol.* 59:1286–1295) that the second of two closely spaced action potentials may be differentially conducted into a short daughter branch. Using numerical methods, the response to trains was examined in axons with a single bifurcation and uniform membrane properties. Short daughter branches conduct at higher rates of stimulation than do long branches. Under some conditions the longer daughter branch is always silent. Under other conditions, one or both branches will begin to conduct action potentials only when the stimulus frequency is high enough.

INTRODUCTION

Frequency-dependent differential conduction of action potentials at axonal bifurcations has been clearly demonstrated in several preparations (Parnas, 1972; Grossman et al., 1973, 1979*a*; Hatt and Smith, 1975) and is widely believed to be important in many other places (Krnjevik and Miledi, 1959; Van Essen, 1973; Farel and Thompson, 1976). It may be one of the principal ways in which neurons process information (Chung et al., 1970; Zucker, 1974; Raymond and Lettvin, 1978).

The best studied of axonal bifurcations that naturally exhibit frequency-dependent differential conduction occurs in motor axons of decapod crustacea (Parnas, 1972) which innervate two antagonistic muscles. Part of the reason for the development of conduction block in this preparation is the buildup of periaxonal potassium (Grossman et al., 1979*b*; Smith, 1983) but this cannot explain why conduction fails at the branch point per se rather than along the daughter axons (Parnas and Segev, 1979).

Frequency-dependent differential conduction can be experimentally induced in the squid giant axon (Stockbridge and Stockbridge, 1988) where it does not normally occur. The membrane properties are probably fairly uniform in this axon, but when one daughter branch is short, the second of two closely spaced stimuli will propagate only into the short branch. Preservation of conduction with short interstimulus intervals occurs because the threshold is lower in the shorter branch (Stockbridge, 1988).

This study was undertaken to investigate the response to long trains of action potentials of a model bifurcating axon with uniform membrane properties, and, specifically, to determine if such a model will demonstrate frequency-dependent partial block conditions in one or both daughter branches.

The membrane model used in this study was derived by Hodgkin and Huxley (1952) based upon relatively short voltage clamp experiments. The model fairly accurately reproduces the shape of the action potential in the squid giant axon, but is known not to be as accurate at long times. For example, accumulation of potassium in the periaxonal space (Frankenhaeuser and Hodgkin, 1956) is not included. The model of Hodgkin and Huxley can be modified to include such effects (Adelman and FitzHugh, 1975). Parnas and Segev (1979) applied such a modified model in a theoretical study of a bifurcating axon, but were unable to demonstrate differential conduction when the membrane properties of the daughter branches were uniform. In the study reported here, the membrane model contains no periaxonal ion–accumulating space. For this reason, and other deficiencies in the membrane model, this study cannot quantitatively describe differential conduction in the squid giant axon nor in any other neuron, but it does provide some insight into the factors which can give rise to differential conduction in axons.

Propagation through a bifurcation depends upon the relative loading of the membrane before and after the bifurcation. For long branches with uniform membrane and cable properties, it is useful to consider the geometric ratio (Rall, 1959; Goldstein and Rall, 1974), which is defined as

$$G.R. = \frac{\sum_i d_{d_i}^{3/2}}{d_p^{3/2}},$$

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where d_p is the diameter of the parent branch (upon which the action potential arises) and d_{d_i} is the diameter of the ith daughter branch. Goldstein and Rall (1974) showed that smooth propagation through such a branch occurred when the geometric ratio was equal to 1. A short daughter branch somewhat alters the significance of the geometric ratio in the simulations presented here, but still provides an appropriate way to compare various cases. Specifically, as long as the electrical length of all branches remains constant, changes in branch diameters that preserve the geometric ratio at the bifurcation will not change the pattern of propagation.

METHODS

The methods used in this study are basically the same as used previously in this laboratory (Stockbridge, 1988) and elsewhere (e.g., Parnas and Segev, 1979). Only details specific to this study are described here. The axon was considered to be composed of a total of 200 segments, each of which was short enough to be considered isopotential for the purpose of computing membrane ionic and capacitative currents. The membrane properties were described by the Hodgkin and Huxley (1952) equations and were uniform throughout the axon. A single bifurcation was present in the axon. The parent branch extended for at least six resting length constants (λ). The daughter branches were always the same diameter. The longer one extended for ~3 λ. The extent of the shorter daughter branch was usually <1 λ. The only temperature-dependent parameters in the model were the rate constants in the Hodgkin-Huxley equations. Unless otherwise specified, all simulations were performed with a model temperature of 32°C. The time step for integration was 10 μs. Regularly spaced suprathreshold current stimuli were delivered to the terminus of the parent branch distal to the bifurcation, and the membrane potential was monitored as a function of time at various places in the axon.

The extension of previous studies to long trains of stimuli necessitated a significant improvement in the speed of computation of the model equations. This improvement was achieved by performing most of the computation on an array processor, as will be described in detail elsewhere. These simulations required ~400 h of CPU time using the array processor hardware. Using the host processor alone (a 16-MHz Motorola 68020 with 68881 floating point unit), these simulations would have taken an estimated 500 d of CPU time.

RESULTS

Fig. 1 summarizes 1,200 separate branched cable simulations in which the parent axon experienced trains of action potentials for 1 s. The geometric ratio at the bifurcation was varied from 2.80 to 3.27. The length of the short branch was varied in units of 0.05 λ from 0.15 to 0.6 λ. The length of the long daughter branch was fixed at ~3 λ. For each of 40 frequencies of stimulation, the number of action potentials in the parent branch was counted 0.5 λ proximal to the bifurcation and the number of action potentials in the daughter branches was counted at their distal ends. The fraction of action potentials in the parent branch which successfully propagated into the daughter branch was used to compute the gray scale values shown in the figure. The response of the short branch is shown as the left bar of each pair and that of the long branch is shown on the right.

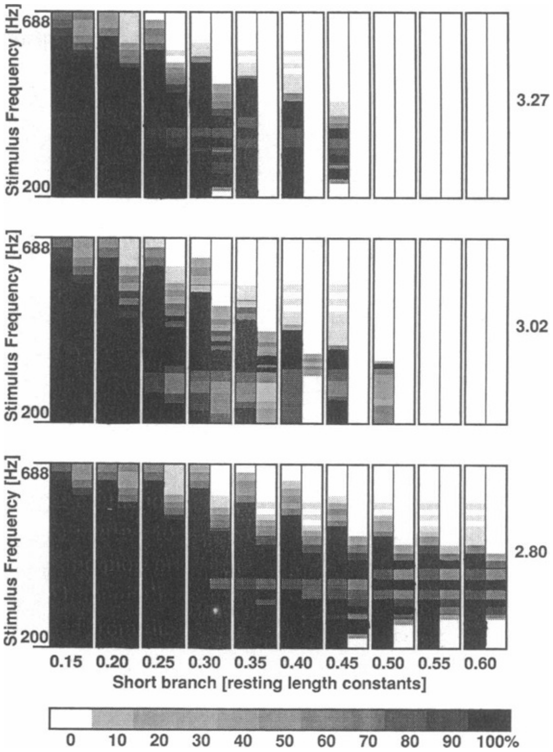

FIGURE 1 A series of simulations were run with branched cables in which the length of the short branch was varied from 0.15 to 0.6 λ. Each simulation run was a 1-s train of regularly spaced suprathreshold stimuli applied to the sealed end of the parent branch. The number of action potentials in the parent branch was counted ~0.5 λ from the bifurcation. Action potentials in the two daughter branches were counted at their distal ends. Bar on left corresponds to the activity in the short daughter branch, and bar on right to that of the long daughter branch. The number of action potentials in the daughter branch as a percentage of the activity in the parent branch was used to compute a grey scale value. As shown in the scale at the bottom, white areas correspond to complete conduction block and black areas to 100% propagation success. Values to the right of the figure give the geometric ratio at the branch.

Figs. 2–5 show examples of patterns of frequency-dependent differential conduction at bifurcations. In each case, the stimulus was a regular train of 100 ms duration. Recordings of the membrane potential are shown for five locations in the axon. The diagram to the left of each figure shows schematically the stimulation and recording sites.

Often, one or two stimuli following the first stimulus failed to propagate along the parent branch. Fig. 2 shows such an example. The first stimulus resulted in an action potential that propagated successfully into both daughter branches. The next two stimuli failed to propagate along the parent axon (E). After this, each stimulus resulted in a propagating action potential that successfully invaded the short daughter branch (A). Every second action potential successfully invaded the long daughter branch (B). Action potentials recorded from the ends of both daughter branches were larger than those recorded elsewhere (e.g., E) because of the effect of the sealed end. In other examples not shown, the establishment of such a 2:1 pattern in the long daughter branch only occurred after 10 or more action potentials successfully invaded the short daughter branch.

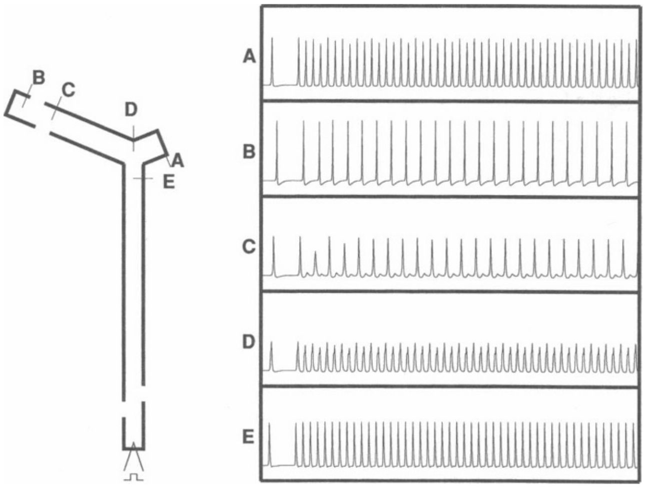

FIGURE 2 The geometric ratio in this simulation was 3.02 and the short branch length was 0.3 λ. The parent axon was stimulated at a rate of ~515 Hz for 100 ms. The second and third stimuli failed to produce propagating action potentials in the parent axon. All action potentials propagating in the parent axon propagated into the short daughter branch, but only half of them propagated in the long daughter branch.

Fig. 3 shows another example in which each action potential successfully propagating along the parent axon resulted in an action potential in the short daughter branch. This time, the pattern of response in the long daughter branch was more irregular, but was usually two successes followed by one failure.

In some cases, many or all of the action potentials in the parent axon successfully invade the short daughter branch and none invade the long daughter branch regardless of the rate of stimulation. In other cases, the long branch is silent at low rates of stimulation but begins to conduct as the stimulus frequency is increased. Such an example is shown in Fig. 4. All action potentials in the parent axon again propagated into the short daughter branch. The first action potential did not propagate into the long daughter branch. After the first action potential, the daughter branch responded to one-half to two-thirds of the stimuli. In other examples, the degree to which propagation into the long daughter branch was conserved as a result of activity in the short branch was either greater or less than in this example.

Fig. 5 shows an example in which fewer of the action potentials in the parent axon invaded the short daughter branch and fewer still invaded the long daughter branch. Intermittent propagation failure along the parent axon contributed to the erratic pattern of propagation in the daughter branches.

A series of simulations were run to find the maximum geometric ratio which allowed propagation of a single action potential into the daughter branches when one daughter branch was short. When both daughter branches were long, the maximum geometric ratio which allowed propagation through the bifurcation was found to be 2.60 at 32°C. This value is in good agreement with the critical geometric ratio of 2.59 found for an unbranched axon with a step change in diameter. When one of the daughter branches was shorter than ~1 λ, the critical geometric ratio for propagation into the two daughter branches was different. As shown in Fig. 6, the critical geometric ratio of both branches increased as one branch was made shorter, but the short daughter branch always conducted at a higher geometric ratio.

DISCUSSION

These results confirm and expand earlier observations (Stockbridge and Stockbridge, 1988; Stockbridge, 1988) that frequency-dependent differential conduction can be

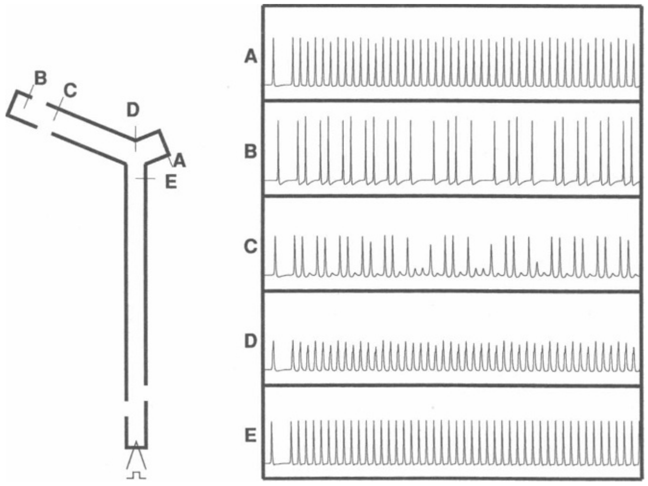

FIGURE 3 The geometry in this simulation is the same as in Fig. 2. The parent axon was stimulated at 500 Hz for 100 ms. The second stimulus did not propagate in the parent axon. A higher percentage of the action potentials which did propagate in the parent axon successfully invaded the long daughter branch.

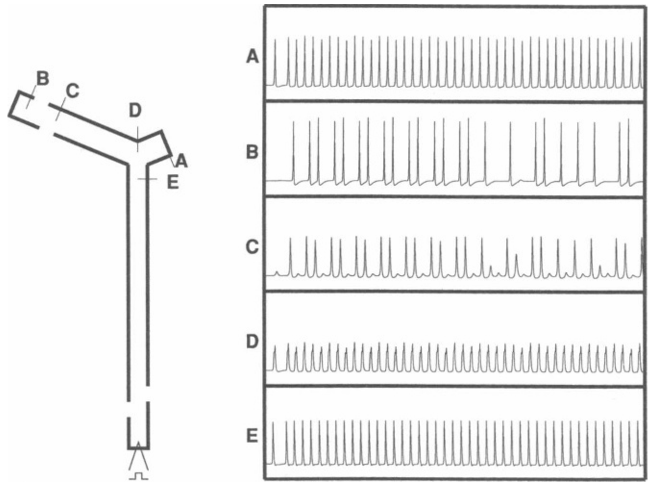

FIGURE 4 The geometric ratio in this simulation was 3.02 and the short branch length was 0.35 λ. The stimulus rate was 450 Hz for 100 ms. The long daugher branch begins to conduct after the first few action potentials successfully invade the short daughter branch.

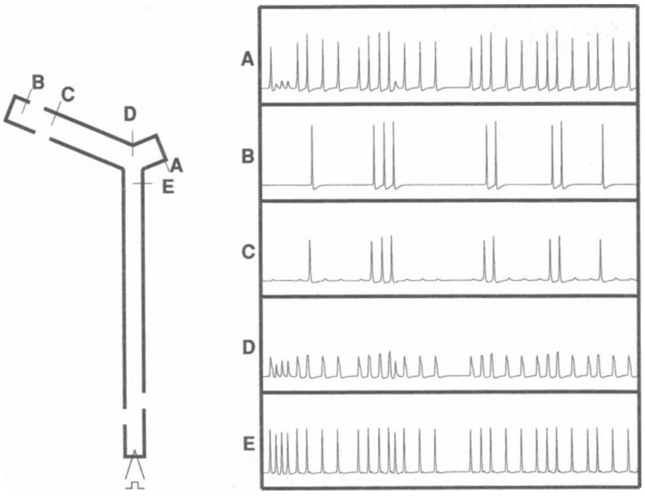

FIGURE 5 In this example, stimuli to the parent axon were too fast for the parent axon to follow (every 1.35 ms) and the result was an irregular pattern of action potentials encountering the bifurcation. The short branch tends to follow the activity in the parent axon when the interval between arriving action potentials is not too short. The long daughter branch tends to follow activity in the short branch when the interval between action potentials is not too long.

induced at bifurcations under conditions of uniform membrane properties. These results also confirm the suggestion that such differential conduction can occur in interesting patterns in response to long trains of action potentials. The spacing between action potentials necessary to cause frequency-dependent differential conduction in a long train is much lower than is the necessary spacing between two isolated stimuli. The range of stimulus intervals over which frequency-dependent differential conduction is obtained is also much broader in long trains. These new observations mean that it is more likely that this form of differential conduction plays a physiological role in the processing of information by axons.

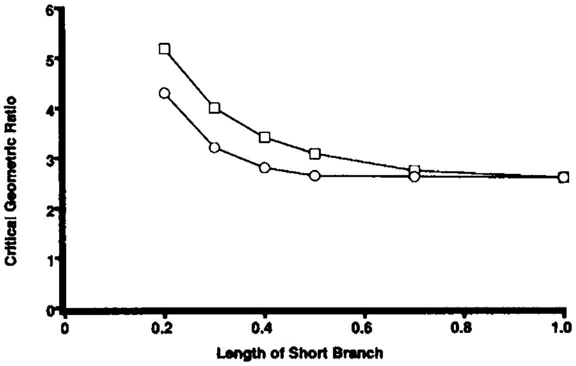

FIGURE 6 Shortening one daughter branch improves the safety factor for conduction into both daughter branches. Branched axons were modeled as in Figs. 2–5. The daughter branch diameters were the same and were held constant. The long daughter branch length was ~3.3 λ. The short daugher branch length was varied from 0.2 to 1.0 λ. The maximum parent branch diameter that permitted conduction of a single action potential into the daughter branches was used to compute the critical geometric ratio. Squares mark data from the short branch and circles mark data from the long branch.

Several factors contribute to whether or not conduction succeeds in one or both daughter branches. For the kind of differential conduction described in this study, the geometric ratio for conduction through a bifurcation must be greater than unity. How much greater than unity it must be depends upon the temperature, the lengths of the daughter branches, and the stimulus frequency. At high temperature, there is less charge movement underlying the action potential and the safety factor for conduction is lower (Westerfield et al., 1978; Stockbridge and Stockbridge, 1988). Short daughter branches send the axial current entering them out over a smaller area of membrane than do long branches, and consequently there is a higher safety factor at a bifurcation with one or more short daughter branches (Stockbridge, 1988). Bifurcations with a high geometric ratio also retard the propagation of successfully conducted action potentials. Thus, while high frequencies of stimulation do not result in action potentials with lower total charge movement along a long geometrically uniform axon, action potentials encountering a high geometric ratio bifurcation fall upon relatively refractory membrane.

Both daughter branches experience a higher proportion of propagation failures as the geometric ratio is increased. At the model temperature used in this study, the geometric ratio which causes propagation failure for an isolated action potential in a cable with long daughter branches is ~2.6. As shown in Fig. 1, geometric ratios near that value produce propagation failures at the bifurcation when the stimulus frequency is quite low. This happens at frequencies where one would ordinarily expect the membrane to be fully back to steady-state conditions between impulses. However, the geometric inhomogeneity slows conduction and widens the action potential, thus lengthening the refractory period following each impulse. The higher the geometric ratio, the longer the interval between action potentials must be to insure propagation through the bifurcation. When at least one of the daughter branches is short, the success rate is improved for both daughter branches, compared with the situation in which both daughter branches are long. The short daughter branch benefits because the axial current entering the branch depolarizes a smaller area of membrane. If this branch becomes active quickly enough, it begins to supply current to the long daughter branch and helps bring it to threshold. No circumstances were ever observed in which an action potential successfully invaded the long daughter branch but not the short daughter branch.

If threshold monotonically decreased as the interval between two stimuli increased, then the percentage of successes in the two daughter branches might be expected also to decrease with increases in stimulus frequency, but this is not the pattern shown in Fig. 1. Over a wide range of conditions, there is an apparent excess of action potentials propagating into both daughter branches when the stimulus frequency is ~400–500 Hz. Although the phenomenon has not been exhaustively studied, the cause cannot be differences in daughter branch lengths. This period corresponds roughly with the period of supernormal excitability following the preceding action potential. Although this effect is fairly small in the Hodgkin-Huxley model at a temperature of 32°C, the result of this minimum in the threshold is a considerable improvement in the midrange frequency response at the bifurcation.

Differences in the lengths of the daughter branches introduce different phase lags in the action potentials propagating into them. These phase differences cause further departures from monotonic behavior.

Some axons exhibit "tuning," conduction of action potentials particularly well over a restricted range of frequencies (Kocsis et al., 1979). The amount by which excitability is increased after an action potential will influence the sharpness of the tuning at the bifurcation, although it is clear from the current study that even small changes in excitability will produce large changes in propagation success rates. How long the period of supernormal excitability lasts will influence the range of frequencies over which the bifurcation will exhibit this tuning. Frequency-dependent alterations in conduction velocity (Kocsis et al., 1979) also affect such tuning.

Under some conditions, one or both daugher branches may begin to conduct only when the frequency of action potentials arriving at the bifurcation is high enough. Such a mechanism has been proposed to account for facilitation in the crustacean neuromuscular junction (Zucker, 1974). The simulations presented here offer a possible mechanism for such frequency-dependent activation of terminal daughter branches. The daugher branches begin to conduct when the spacing of action potentials entering the bifurcation is within the period of supernormal excitability. The small degree of this supernormal excitability produced by the Hodgkin-Huxley model membrane at 32°C is sufficient to cause this kind of facilitation.

In summary, the interaction of membrane excitability changes and phase lags caused by geometric factors produces a pattern of propagating responses in daughter branches which is a complex function of the frequency of action potentials in the parent axon. At bifurcations where the geometric ratio is not much greater than unity, quite complex patterns are possible. If, in addition, one daugher branch is shorter than a resting length constant, significant frequency-dependent differences in the responses of the two daughter branches will be manifest. To predict quantitatively the response patterns at a bifurcation, it may be necessary to have quite detailed knowledge of the geometric and membrane properties.

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REFERENCES

Adelman, W. J., and R. FitzHugh. 1975. Solution of the Hodgkin-Huxley equations modified for potassium accumulation in a periaxonal space. *Fed. Proc.* 34:1322–1329.

Chung, S., S. A. Raymond, and J. Y. Lettvin. 1970. Multiple meaning in visual units. *Brain Behav. Evol.* 3:72–101.

Farel, P. B. and R. F. Thompson. 1976. Habituation of a monosynaptic response in frog spinal cord: evidence for a presynaptic mechanism. *J. Neurophysiol.* 39:661–666.

Frankenhaeuser, B., and A. L. Hodgkin. 1956. The after-effects of impulses in the giant nerve fibres of *Loligo*. *J. Physiol. (Lond.).* 131:341–376.

Goldstein, S. S., and W. Rall. 1974. Changes in action potential shape and velocity for changing core conductor geometry. *Biophys. J.* 14:731–757.

Grossman, Y., M. E. Spira, and I. Parnas. 1973. Differential flow of information in branches of a single axon. *Brain Res.* 64:379–386.

Grossman, Y., I. Parnas, and M. E. Spira. 1979*a*. Differential conduction block in branches of a bifurcating axon. *J. Physiol. (Lond.).* 295:283–305.

Grossman, Y., I. Parnas, and M. E. Spira. 1979*b*. Ionic mechanisms involved in differential conduction of action potentials at high frequency in a branching axon. *J. Physiol. (Lond.).* 295:307–322.

Hatt, H., and D. O. Smith. 1975. Axon conduction block: differential channeling of nerve impulses in the crayfish. *Brain Res.* 87:85–88.

Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* 117:500–544.

Kocsis, J. D., H. A. Swadlow, S. G. Waxman, and M. H. Brill. 1979. Variation in conduction velocity during the relative refractory and supernormal periods: a mechanism for impusle entrainment in central axons. *Exp. Neurol.* 65:230–236.

Krnjevik, K., and R. Miledi. 1959. Presynaptic failure of neuromuscular propagation in rats. *J. Physiol. (Lond.).* 149:1–22.

Parnas, I. 1972. Differential block at high frequencies of branches of a single axon innervating two muscles. *J. Neurophysiol.* 35:903–914.

Parnas, I., and I. Segev. 1979. A mathematical model for conduction of action potentials along bifurcating axons. *J. Physiol (Lond.).* 295:323–343.

Rall, W. 1959. Branching dendritic trees and motoneuron membrane resistivity. *Exp. Neurol.* 1:491–527.

Raymond, S. A., and J. Y. Lettvin. 1978. Aftereffects of activity in peripheral axons as a clue to nervous encoding. *In* Physiology and Pathobiology of Axons. S. G. Waxman, editor. Raven Press, New York. 203–225.

Smith, D. O. 1983. Extracellular potassium levels and axon excitability during repetitive action potentials in crayfish. *J. Physiol. (Lond.).* 336:143–157.

Stockbridge, N. 1988. Differential conduction at axonal bifurcations. II. Theoretical basis. *J. Neurophysiol.* 59:1286–1295.

Stockbridge, N., and L. L. Stockbridge. 1988. Differential conduction at axonal bifurcations. I. Effect of electrotonic length. *J. Neurophysiol.* 59:1277–1285.

Van Essen, D. C. 1973. The contribution of membrane hyperpolarization to adaptation and conduction block in sensory neurones of the leech. *J. Physiol. (Lond.).* 230:509–534.

Westerfield, M., R. W. Joyner, and J. W. Moore. 1978. Temperature sensitive conduction failure at axon branch points. *J. Neurophysiol.* 41:1–8.

Zucker, R. S. 1974. Excitability changes in crayfish motoneurone terminals. *J. Physiol. (Lond.).* 241:111–126.