XI. NEUROPHYSIOLOGY*

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A. ACTIVITY DEPENDENT SHIFTS IN EXCITABILITY OF FROG PERIPHERAL NERVE AXONS

1. Introduction

In the 1930's, physiologists were interested in describing the relation between spike activity, after-potentials, and excitability in peripheral nerves. They believed that threshold was inversely related to the membrane potential, and that alterations in threshold following spike activity in axons were brief in large fibers.[1-3] Although the notion that the excitability of a nerve could be predicted from the sign and magnitude of the after-potentials came under severe criticism later by Lorente de Nó,[4] the concerns of physiologists had changed with the development of microelectrodes, and the issues raised by him were never dealt with seriously. Furthermore, the clarity of Hodgkin and Huxley's interpretation of threshold, published early in the 1950's, diverted attention from further exploration of some of the more curious phenomenological aspects of nerve threshold.

A complete account of the factors that determine the threshold of a membrane at any particular instant would be exceedingly complex. Nerve is in this respect similar to any other damped nonlinear oscillator. Exposed to stimulation near threshold intensity, its responsiveness will reflect thermal noise, 1/f noise,[5,6] and a host of relatively long-term and poorly understood effects following activity. For nerve, such effects would probably include changes in local ionic activity, space-charge variations near the membrane, "metabolic" shifts,[7,4,8] long time constants in higher order components of recovery of conductance mechanisms, and so forth. J. Y. Lettvin draws the analogy between nerve membrane and the old-fashioned thermionic valve that caused despair and hair-pulling among the builders of World War II function generators and frustrated the designers of the first modern computers such as Whirlwind. Among other things, excitation of these flip-flops heats the plate, as well as the glass envelope,

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cools the cathode and alters some of the fringes of the charge distribution near the cathode. Each of these changes has effects on threshold that generally far outlast the discharge (and seriously compromise the reliability of these circuits). Nowadays, reliability of flip-flops is insured by designing them so that second- and higher order effects are minimal with respect to trigger signals. Accordingly, the problem of near-threshold stimulation has virtually disappeared from electrical engineering. Almost no one is now concerned with the extended form of the van der Pol equations for a logic element. What has been successfully designed out of chrome-plated silicon fabrications, however, remains a basic problem with nerves and, indeed, may be intrinsic to the way a nervous system works.

The suspicion that such long-term threshold changes modify the invasion of axonal branches has guided the experimental work of our laboratory for several years. Thus far, we have confirmed the existence of zones of low safety factor in the central nervous system and have noted some of the ways in which nerve activity alters the conduction of impulses through such regions.[9] We have generalized these observations in a theory of nervous action,[10] parts of which have been described in previous reports.[11] The theory essentially holds that impulses do not always invade each branch of a terminal axonal arborization. A tree of such "fallible branches" where the invadability of each branch continuously changes in time can be shown to operate as a time-domain filter that can distinguish pulse interval sequences.

Our concern with the constraints that act to determine the propagation of impulses through a tree has led us to reinvestigate the classical problem of threshold variation with activity in nerve. Normally, the safety factor for conduction in unbranched axon is approximately 4:1. In other words, the time course and magnitude of currents generated by an excited stretch of axon are approximately 4 times those needed to excite the next stretch. At a zone of low safety factor, however, where propagation of the impulse becomes suddenly doubtful, essentially three inter-related parameters govern the outcome. A segment of nerve will be invaded or not, depending on the strength and time course of the incoming impulse, the threshold of the segment, and the coupling of the impulse to the segment. It is likely that all three of these measures vary with activity, but here we have only studied "threshold."

The purpose of this report is to show how the threshold varies as a consequence of activity. It is important to emphasize that the threshold alone does not predict the statistics of invasion of a branch, since threshold must be convolved with an unknown: the strength and time course of the driving impulse as seen by the membrane of the branch. This, in turn, depends in part on the precise nature of the coupling between the active segment and the driven segment. At the very least, the existence of long-term threshold effects sets a lower bound on the complexity of the dependence of the invadability of axon trees on activity. Furthermore, if activity leads to long-term

changes in threshold, it will almost certainly lead to equally long variations in pulse shape. The proof that axonal trees can discriminate pulse sequences does not depend on the exact form of invadability changes, but merely requires that such changes exist. We shall summarize here the main results of our work. A more detailed paper is in preparation.

Activity-dependent effects on the excitability of whole nerves were first noted by Waller,[12] who found an increase in excitability following activity. Later, Wedensky[13] claimed the existence of two phases, an early, short refractory phase followed by an "exalted phase" of excitability, which lasted for a considerable time following an impulse. Adrian and Lucas[14] found that the amplitude and duration of the exalted or "supernormal phase" depended on the freshness of the preparation and on the pH of the surrounding solution. They claimed that the supernormal phase was a transient return to normal threshold from the higher-than-normal resting threshold resulting from the unnatural conditions of the preparation. Graham disagreed,[15] for she found that veratrine simultaneously raised the resting excitability and both heightened and prolonged the supernormal phase. She interpreted the supernormal phase as an absolute lowering of threshold below a resting value and felt that it was a natural consequence of activity.

More recent investigations have been focused on the "noise" of excitability at single nodes and on explorations of metabolic consequences of tetanus. In order to explore steady-state threshold noise at nodes, investigators have found that stimulation rates above one spike every two seconds lead to "excess groupiness" as spikes begin to lose independence.[16, 17] D-C recordings of voltages proportional to the transmembrane potential on whole nerves show effects of severe tetanic stimulation that last for more than an hour.[18]

2. Methods

All of our experiments have been performed at room temperature (22°C) on the excised sciatic nerve of Rana pipiens. Initial experiments were performed in SuperGroob ringer solution with pH values near neutral (6.50 g/l NaCl, 0.25 g/l each of KCl, $CaCl_2$ and $NaHCO_3$). Adrian[14] and Lorente de No'[19] emphasize the importance of pH and $_pCO_2$ to various parameters of whole nerve activity, and recently we have used a modified Boyle-Conway[20] ringer tabulated as follows:

g/l	Molarity	g/l	Molarity
4.70 NaCl	80.5 mM	0.14 $MgSO_4$	1.17 mM
0.15 KCl	1.99 mM	0.094 Na_2SO_4	0.66 mM
2.10 $NaHCO_3$	25.01 mM	0.36 Na_2HPO_4	0.55 mM
0.25 $CaCl_2$ (Dihydrous)	1.70 mM	0.07 KH_2PO_4	0.52 mM
		0.60 $C_6H_{12}O_6$	3.3 mM

The pH was adjusted to 7.3 by varying the partial pressure of CO_2 in air constantly bubbled through the ringer throughout each experiment. Thus, the carbonate, pH, CO_2, O_2 and dissolved electrolyte levels were nearly identical to those of frog plasma.[21]

We have generally studied the threshold shifts occurring in single units rather than the responsiveness of the whole nerve. Single units were recorded from split distal branches of the nerve through suction electrodes. Platinum stimulating electrodes were placed 2-5 cm from the severed central end of the nerve in order to avoid effects of the injury potential.

To measure changes in excitability directly requires stimulation near threshold intensity. We can hold the stimulus constant and note changes in the probability of response, or we can note the variation in stimulus potency needed to maintain a constant probability of firing. We designed and built devices to use both of these methods.

a. The Hunter Circuit

This device "hunts" the 50% threshold level of a fiber by following a very simple scheme suggested to us by J. Y. Lettvin. It delivers a square wave to a light-coupled isolation stimulator (MetaMetrics Cooperative, Inc., Cambridge, Mass., SPS 1000). If the fiber responds, the next stimulus is briefer. If the fiber fails to respond, the stimulus duration is automatically increased. Approximate reciprocity of stimulus duration and strength holds for threshold stimulation over the duration range employed by the hunter circuit (25-325 μs).[22, 23] Increments and decrements of duration were generally between 1 μs and 3 μs, although when the threshold change was rapid, larger increments were used. A DC voltage proportional to the duration of the stimulus pulse was led to the vertical axis of an oscilloscope and gave a running index of the hunted threshold.

b. The Spike Probability Circuit

Because it was easy to operate, the hunter circuit was used most often for checking the time course of threshold shifts that occur after nerve activity. To gauge the importance of this measure of threshold variation, however, the relation between change in stimulus duration and the change in the probability of firing had to be learned. The spike probability circuit mapped threshold changes by determining the probability that a constant near-threshold stimulus would elicit an impulse as a function of delay after various conditioning spike trains. A series of conditioning stimuli were given, each followed by a test stimulus at a specific delay. At the end of 25 such pairs a bar with height proportional to the number of impulse responses was displayed on the vertical axis of an oscilloscope. A photograph of many such bars, each at its own delay, gives a graph of the probability of response as a function of delay after the conditioning influence.

3. Results

a. Threshold Variation Following a Single Impulse

Figures XI-1 and XI-2 are typical photographs showing threshold variation (change in the test stimulus duration in μs) observed during the first second after a conditioning impulse. The traces are composed of brightened dots, each denoting the duration of a test stimulus. They overlap, except where the threshold changes rapidly, immediately after the conditioning spike. Displacement along the horizontal axis from left to right is proportional to the time interval between the conditioning stimulus (coinciding with 0 on the abscissa) and the test stimulus. Stimulus pairs were presented once every 1.3 s for several minutes, with the test stimulus given at gradually increasing delays from the conditioning stimulus. Figure XI-1 shows that the excitability of the fiber was increased for more than 500 ms following a single impulse. (With a 1.3 s interval between stimulus pairs, successful responses more than 500 ms after the conditioning spike were clearly adding to the aftereffects of the next conditioning spike. Since this additional affect is small at long delays (see later experiments), we chose this fast repetition rate to reduce duration of the experiment and to produce a reasonably stable record showing the qualitative result.) The control level is indicated on the extreme left of the trace where the test stimulus preceded the suprathreshold conditioning

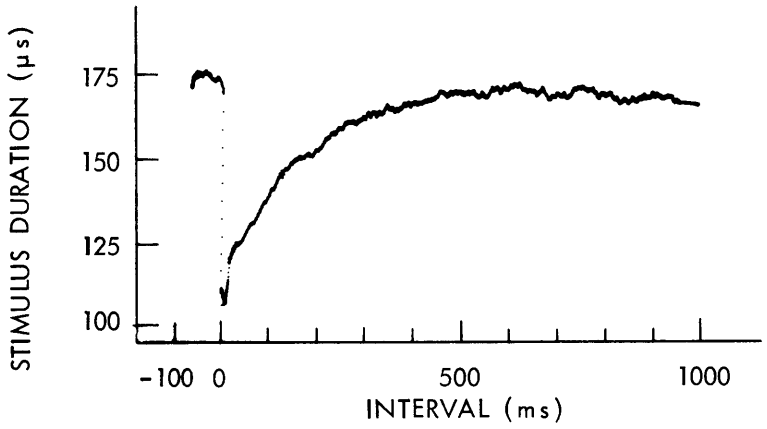

Fig. XI-1. Threshold variation following a single conditioning impulse. The abscissa shows the time interval between conditioning and test stimuli in ms. The ordinate gives the duration of the test stimulus in μs. The conditioning impulse occurred at 0 interval. Negative intervals indicate that the test stimulus preceded the conditioning stimulus. Short test stimuli imply low threshold. The drop in stimulus duration occurring after the dots at 0 interval is a real indication of lowered threshold because at that point the hunter circuit had begun to track the threshold of the test stimulus.

stimulus. As long as the conditioning and test stimuli are separated by more than 1 ms, the hunter circuit "hunts" only the threshold of the test stimulus and ignores the firing of the fiber in response to the conditioning stimulus. Thus it is possible to hunt the threshold during the later part of the relative refractory period (see Figs. XI-2 and XI-4), but we generally allowed the test stimulus duration to shrink rapidly (note dots in Fig. XI-1) during the few milliseconds when the hunter circuit responded to the conditioning spikes as if they were responses to the test stimulus. This practice allowed us to capture the peak of the supernormal phase.

Several minutes of repeated trials are required to complete threshold plots like those of Fig. XI-1. Slow, continuous drift of threshold during this period was generally minor. To verify the existence of the supernormal phase in the presence of drift, however, several series of pictures like Fig. XI-2 were taken. Figure XI-2 is from three consecutive experiments on the same fiber. Trace A is a measure of threshold variation similar to that of Fig. XI-1. During the control experiment (Trace B) which required 20 min to complete, the amplitude of the conditioning stimulus was lowered just enough to make it subthreshold for the recorded fiber. Some of the other fibers presumably continued firing, since impulses in response to the conditioning stimulus could be sampled from other twigs. No effects of such firings on the threshold of the test fiber were apparent. In comparison, the effects were profound when the conditioning stimulus was raised just enough to fire the fiber consistently. Trace C shows abrupt

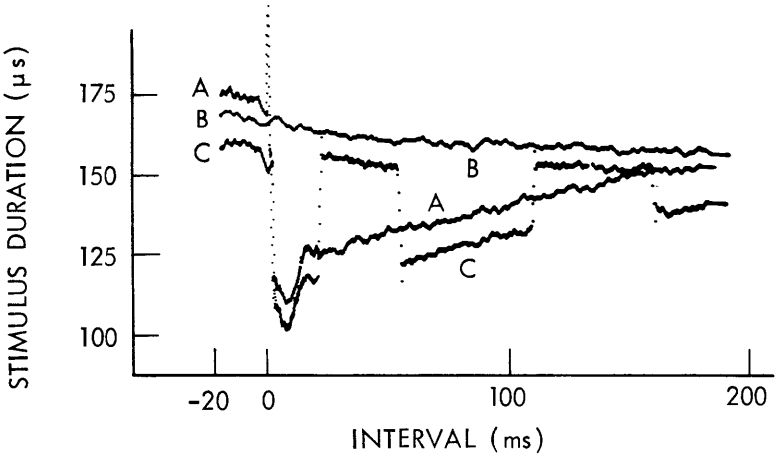

Fig. XI-2. Threshold variation with drift. The traces were obtained seriatim in alphabetical order. Trace A shows threshold variation as a function of the interval between test and conditioning stimuli. Trace B shows the threshold in the absence of conditioning spikes (control). Trace C shows threshold variation as the conditioning spike was alternately turned on or off. The stimulus was subthreshold for intervals 22-58 ms and 110-160 ms.

threshold changes as the conditioning stimulus was alternately raised and lowered above and below threshold level. The threshold jumped from supernormality to control level each time the conditioning stimulus was made subliminal.

Although the hunter circuit allowed us to monitor the time course of threshold change following an impulse, it did not show directly how firing probability of the fiber was affected. If threshold of a fiber falls after a spike so that stimuli 10 μs briefer than control stimuli generate spikes 50% of the time, how much has the probability of firing altered for constant stimuli? Figure XI-3 shows two spike-probability graphs that display the probability of responding to a near-threshold stimulus as a function of delay from a conditioning impulse.

Each vertical bar represents the percentage of impulses successfully generated in a series of 25 stimuli. The bars to the left of 0 give the control probability of firing to the test stimulus alone. As with the hunter circuit, a conditioning impulse was indistinguishable from a successfully conducted test impulse when the two stimuli were very close together. Thus the probability of the fiber firing appears to rise above control level near 0 interval. After a drop in probability of firing as the relative refractory period was sampled, the probability rose during the supernormal phase and returned slowly to control level. Much variation between fibers was noted in using this method of measuring threshold change. Figure XI-3a shows a fiber whose probability of firing rose nearly to 100% during the supernormal phase. The stimulus was sufficient to fire the fiber approximately 15% of the time without a conditioning impulse. After conditioning impulses, the fiber of Fig. XI-3b responded reliably to a test stimulus that was too weak to cause it to fire at all under control conditions.

b. Threshold Variation Following Short Bursts

The amplitude of the supernormal phase was consistently augmented following a burst of impulses. Figure XI-4 shows threshold variation following 1, 2, and 4 impulses. Figure XI-4b shows the supernormality observed in Fig. XI-4a after superimposing the control levels to correct for threshold drift. Peak supernormality increased with the number of conditioning impulses. Bursts of more than 6-8 impulses did not add to peak supernormality, however. The time course of the supernormal period after short bursts seemed roughly the same as that after single impulses.

c. Threshold Variation Following Tetanic or Prolonged Intermittent Activity

When the conditioning tetanus comprised more than 8 impulses (repeated every 2 or 4 s), the threshold rose slowly during the course of the experiment. It required several minutes or more following cessation of the intermittent tetanus before the threshold returned to the control level.

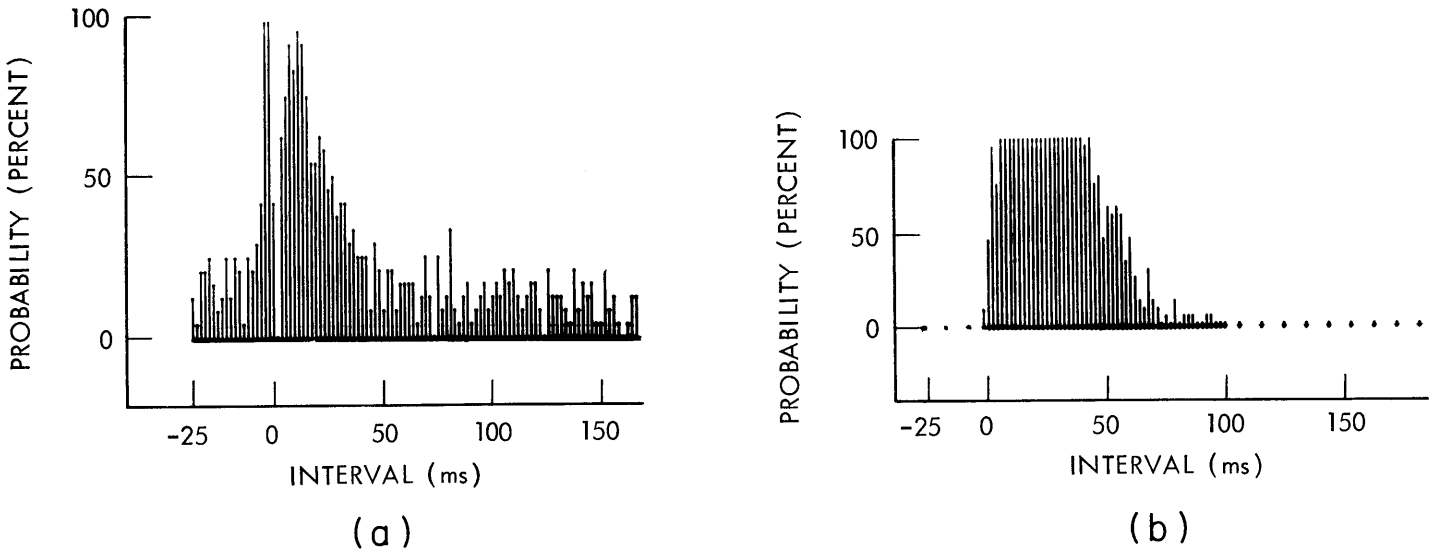

Fig. XI-3. Probability of firing as a function of the delay after a single conditioning pulse and a test stimulus. Conditioning and test pairs were presented once every 3 seconds.

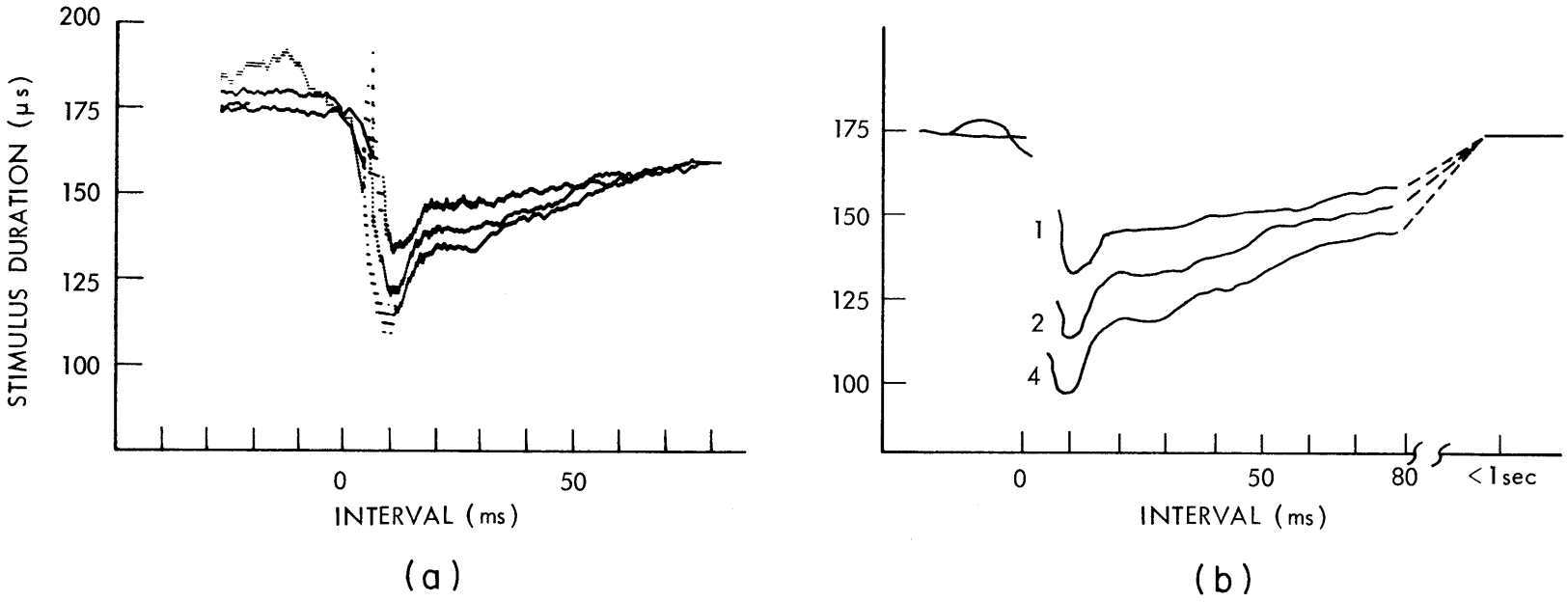

Fig. XI-4. Threshold variation following 1, 2, and 4 conditioning impulses. The traces were obtained consecutively from the same fiber. Zero on the abscissa marks the time of the last stimulus of the conditioning train. Trace 1 shows the threshold shift after a single impulse; Trace 2, after two impulses 3.2 ms apart; Trace 4, after 4 impulses at 13 impulses/second. Part B is a reproduction of the three traces in Part A corrected for threshold drift.

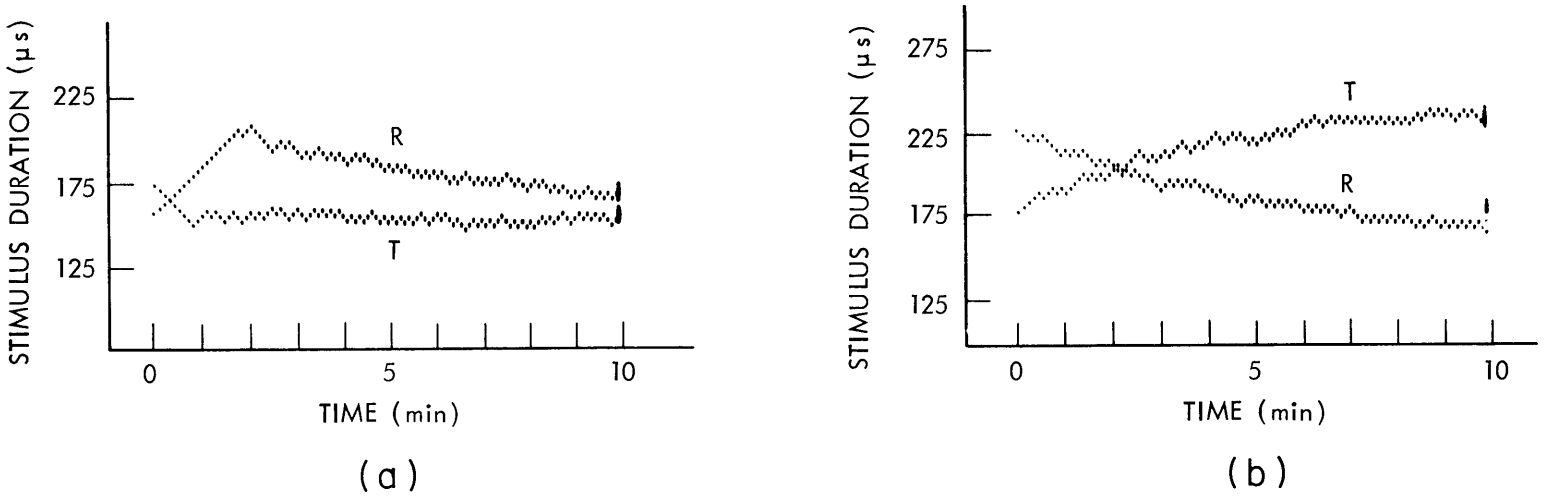

Fig. XI-5. Build up and recovery of long-term depression. The period of intermittent tetanus began at time 0 on the abscissa. Traces marked T show the threshold change with time at a 27-ms interval between the end of tetanus and the test stimulus (a) and at a 450-ms interval (b). Dots denote the duration of each test stimulus given after each tetanus (once every 5 s). Traces marked R are recovery traces. The test stimuli were again given at 5-s intervals but with no preceding tetanus. Recovery of the threshold to control level (approximately 175 μs) required ~9 min. Trace R in (a) began with an upward ramp because the test stimulus had occurred in the supernormal period during the development of depression, and consequently was far too brief to excite responses after the tetanus was abolished. Therefore a series of increments in test stimulus duration was required initially before the threshold of the fiber could again be monitored.

We have called the long-term rise in threshold after conditioning tetanus "depression." Depression of excitability increased in both amplitude and duration as the duration and frequency of the impulse activity increased. The conditioning stimuli used in the two experiments illustrated in Fig. XI-5 were 1-s bursts (at 50 impulses/second) repeated every 5 s (that is, an average frequency of 10 impulses/second). The elapsed time from the onset of this intermittent stimulation was plotted on the horizontal axis. The threshold was hunted for a stimulus at a particular delay after the last spike of each tetanus. As the experiment progressed the interval between the last spike of the tetanus and the test stimulus remained constant. The resulting graph gave the change in threshold at this fixed interval as a function of the duration of the period of intermittent tetanus. In Fig. XI-5a the test stimulus followed the conditioning tetanus by 27 ms, and the threshold remained below control level for the duration of the experiment. Depression was built up during this experiment, however, as shown by the results for identical conditioning in Fig. XI-5b, as well as by the recovery trace in Fig. XI-5a itself. The threshold of the test stimulus at 450 ms (Fig. XI-5b) began to rise almost immediately after the intermittent tetanus began, and attained a plateau after approximately 7 min. Recovery traces (marked "R") monitored the

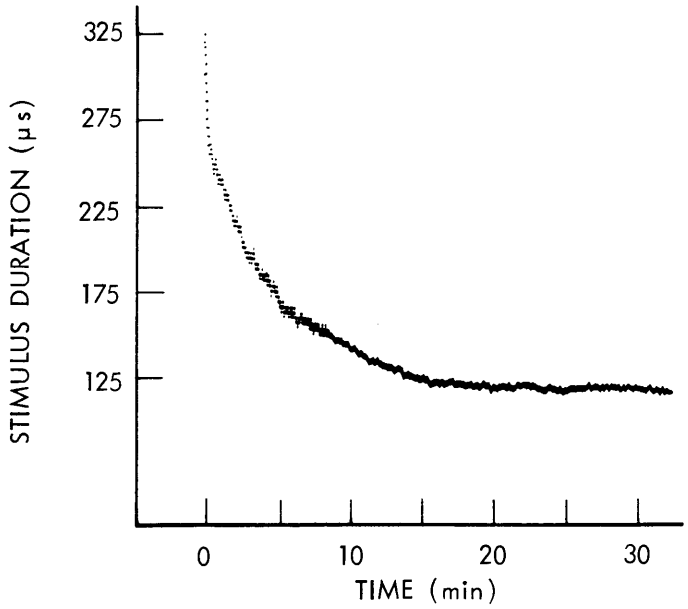

Fig. XI-6. Recovery following severe intermittent tetanus. The abscissa shows the time (in min) after the end of a period of repeated tetanus (1-s bursts at 50 impulses/second given every 3.3 s for 10 min). The test stimulus was given every 2 s during recovery. The initial depression level was slightly above the maximum measurable with the stimulator.

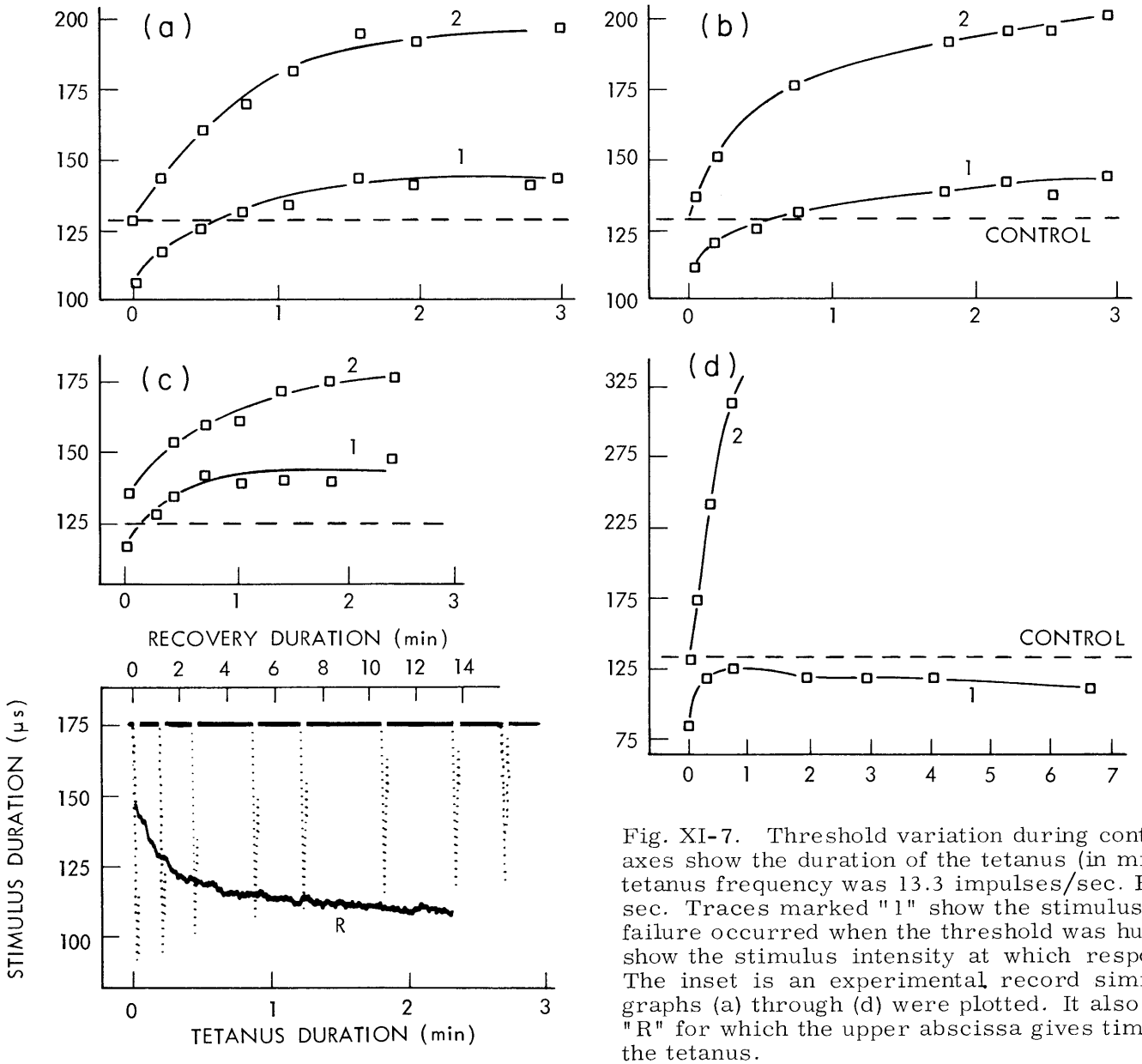

Fig. XI-7. Threshold variation during continuous tetanus. Horizontal axes show the duration of the tetanus (in min). For (a), (b), and (c), the tetanus frequency was 13.3 impulses/sec. For (d) it was 50 impulses/sec. Traces marked "1" show the stimulus duration at which the first failure occurred when the threshold was hunted. Traces marked "2" show the stimulus intensity at which responsiveness first returned. The inset is an experimental record similar to those from which graphs (a) through (d) were plotted. It also includes a recovery trace "R" for which the upper abscissa gives time (in min) after the end of the tetanus.

threshold after the termination of the period of intermittent tetanus. The plateau depression attained in Fig. XI-5b was essentially at the same level as the depression during the beginning of the recovery period, thereby indicating that very little, if any, further rise in threshold occurred at intervals greater than 450 ms. Thus the threshold shift after tetanus moved from supernormality to depression within the first second. It was clear from analogous experiments that this shift occurred more quickly if the tetanus was more severe.

Figure XI-6 displays a longer recovery period following a more severe intermittent tetanus (average frequency 15 impulses/second). The end of the continuous period of intermittent tetanus was marked at 0, and elapsed time since termination of tetanus was plotted on the abscissa. The depressed threshold did not return to the control level for more than 15 min.

Figure XI-7 shows the results of a series of experiments performed to determine threshold levels at short delays and at long delays after progressively longer conditioning tetanus. The tetanus was continuous for several minutes. Stimulus duration was held constant above threshold except at several times during the tetanus when the stimulus was allowed to hunt the threshold. As is illustrated by the inset, at these times the stimulus strength diminished continuously until it reached a level where it failed to excite the fiber. This level corresponded to the threshold at a short delay (1/tetanus frequency). The stimulus duration then increased continuously until it once again activated the fiber. The stimulus duration at which the fiber resumed firing marked a threshold level corresponding to a "long interval," since it usually occurred more than 500 ms after the fiber had initially ceased firing. It sometimes took between 1 s and 2 s for the stimulus intensity to increase from the "short-interval" value to the "long-interval" value, during which time the stimuli were below threshold for the fiber. Thus, strictly speaking, the tetanus was not continuous but was interrupted by a series of 1-2 s test periods. The duration of these interruptions was minor compared with the length of the tetanus. Since depression requires considerably more than one or two seconds to recover, these traces give a reasonably adequate picture of the development of depression as a function of tetanus duration and frequency. Longer tetanus was associated with greater depression. As seen in Fig. XI-7a, -7b, and -7c, however, the depression seemed to approach asymptotically a maximum value. The "long interval" threshold in Fig. XI-7d rose much faster and saturated the hunter circuit because of the higher tetanus frequency used in that experiment. The lower trace in Fig. XI-7d, analogous to a delay of 20 ms, remained below control threshold for the entire duration of the tetanus. Note that when the traces were extrapolated to 0 tetanus duration (a single conditioning impulse), the "short-interval" threshold levels lay below control level while the "long-interval" thresholds were at control level.

4. Discussion

a. Interpretation of Results

Figure XI-8 summarizes our results. The plot gives a qualitative indication of the magnitude and time course of threshold variation following different types of activity. We have chosen to consider threshold variation after activity in three phases: refractory, supernormal, and depression phases.

Refractory Phase. We have not studied the refractory period in detail. It is well known, however, that the time course of this period can be varied by changed experimental conditions.[24] See also Hodgkin[25] and Lorente de Nó[4] on this subject.

Supernormal Phase. Although the supernormal phase has been reported absent under certain experimental conditions in the whole sciatic nerve (high pH, Adrian,[14] low CO_2, Graham and Lorente de Nó[26,4]), it was invariably present in our recordings of single fibers. Our control excitabilities were determined by repetitive stimulation at intervals greater than 2 s. In most earlier work, recordings were made from whole nerves rather than from single units; hence, it is not always clear from earlier papers how "control" threshold level was determined in assaying the extent (or presence) of supernormality, and it is difficult to compare our work with theirs. Gasser[3] has claimed that B fibers do not show supernormality, and we are now checking for it in a variety of systems.

Figure XI-4 shows that adding more conditioning impulses to a burst increased the amplitude of the supernormal phase. Thus in Fig. XI-8 we show high frequency tetanus followed by larger supernormality peaks than those following low frequency tetanus. The most striking aspect of the excitability shift during the supernormal phase was that its amplitude was not particularly affected by depression. This is shown well in Fig. XI-5a, where the threshold at 27 ms remained consistently below threshold as depression built up. Thus the level which that threshold reached during the maximum of the supernormal phase depended only on the number of immediately preceding impulses. It was as if processes causing the enhanced excitability during the supernormal phase momentarily "shorted out" whatever depression was present. At extremely high frequency this may not be true, but since most axons do not naturally carry impulses at average frequencies exceeding 100 impulses/second for several minutes, we suppose that levels of depression occurring naturally have very slight effects on the peak of the supernormal phase, at least in large axons.

Although, in our experiments, the peak amplitude of the supernormal phase was not changed by the presence of depression, the time course was. The depression after a short burst of impulses was minor and did not change markedly the duration of the supernormal phase. The depression that was present during long tetanus dramatically shortened supernormality, however. In the absence of depression the threshold at 75 ms was not far from peak supernormality. Yet, as is seen in Fig. XI-7a, -7b, and -7c,

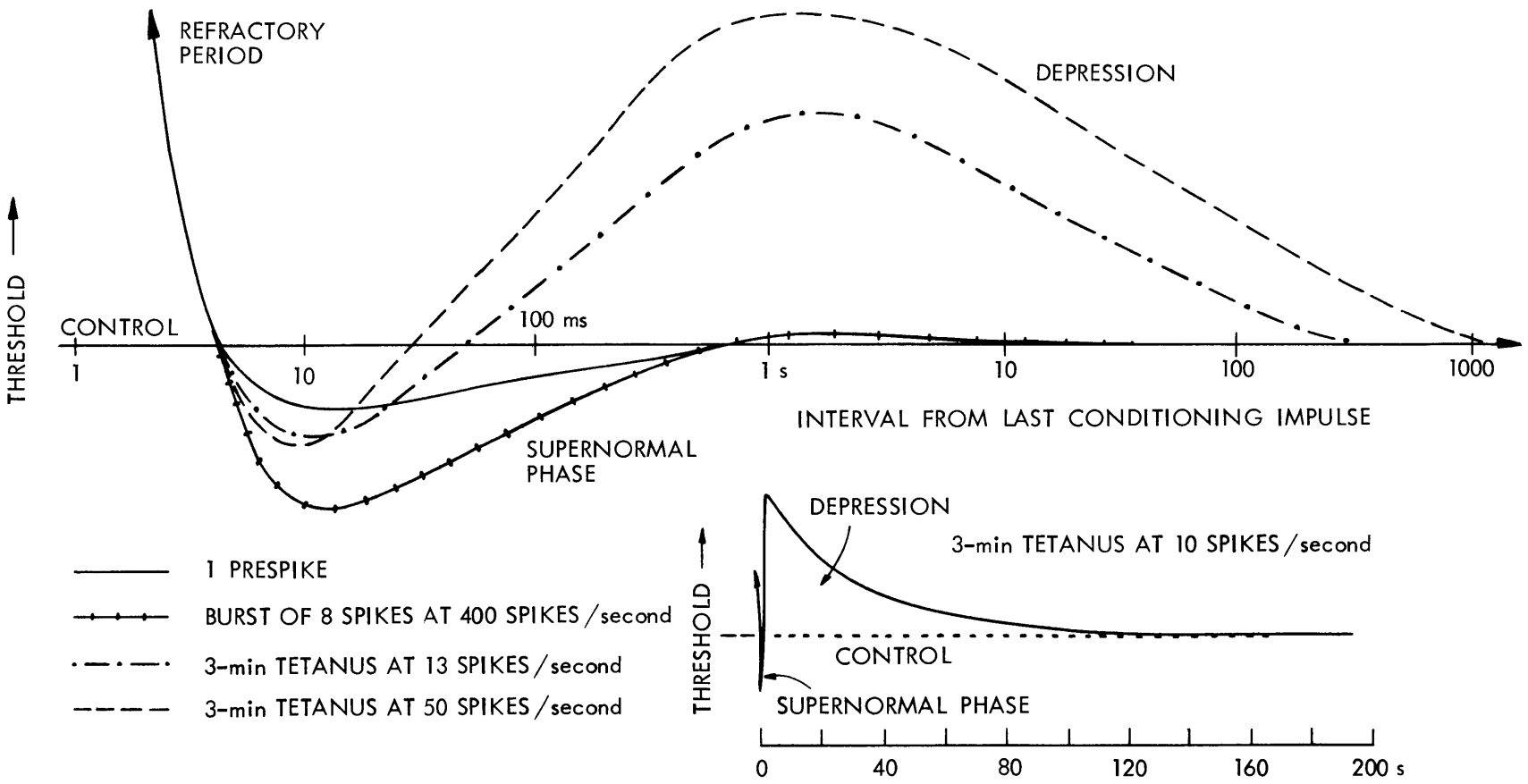

Fig. XI-8. Summary of threshold variation following neuronal activity. Consider the ordinate as an index proportional to the threshold of a single fiber relative to a control threshold established at slow stimulation rates. The abscissa shows the time since the last impulse of a conditioning train on a logarithmic scale. The inset shows how the threshold variation (same ordinate) would appear on a linear time scale after a mild tetanus of fairly long duration.

following a 3-min tetanus, the threshold at 75 ms was well above the control level. Similarly, the depression produced by an intermittent tetanus, as in Fig. XI-5, has nearly reached its maximum within 450 ms after the last spike – an interval that was part of the supernormal phase in the absence of depression. It is unlikely that the lower limit of supernormal phase duration is much shorter than 25-50 ms for tetanus within physiological range. For example, Fig. XI-7d shows an instance where a lowered threshold was present at 20 ms, even after several minutes of tetanic activity at 50 impulses/second. We have considered these results in diagramming the time course of the decline of the supernormal phase and the rise of depression as a function of tetanus in Fig. XI-8.

Depression Phase. Depression, unlike supernormality, was produced only by a series of impulses[27] and built up over several minutes. Experiments with intermittent tetanus and fixed test-spike delay, as in Fig. XI-5, indicated that the development of depression was incremental with each burst. The return to depressed threshold following the supernormal phase of a spike is shown on both linear and logarithmic time scales in Fig. XI-8. Compared with the duration of the recovery period, it is very rapid. It is also rapid in comparison with the rate at which depression builds up during activity, a rate that depends on the frequency of stimulation. Regardless of the level of depression, the threshold becomes supernormal, albeit only momentarily, after each spike. If threshold is measured after the end of the supernormal phase, depression may be seen to begin to develop immediately after the beginning of the tetanus.

Excitability during the depression phase resembles the behavior of products in some first-order chemical systems in equilibrium. The more "reactant" (depression), the more quickly "products" (recovery of excitability) are generated. The rate of recovery increases with depression. This is a precondition for the existence of dynamic equilibrium. At equilibrium (or plateau) depression levels, the rate of recovery will just match the depression generated by the extended tetanus; that is, depression at a particular frequency of stimulation will rise only to a level at which the rate of recovery has increased sufficiently to compensate exactly for the rate at which depression is being augmented. The equilibrium threshold level that is attained will thus depend on the frequency of stimulation as is seen, for example, in the curves of Figs. XI-5 and XI-7. The rate of rise in threshold was initially high and then asymptotically approached an equilibrium that was very high at higher rates of stimulation and lower at lower rates of stimulation. At rates such as those in Fig. XI-7d, the equilibrium threshold was above the maximum stimulus duration that could be provided by our device. This indicates that the degree of change in threshold stemming from processes operating during the depression and recovery phases is extremely significant in terms of the likelihood of firing for a nerve. The spike probability pictures showed that the much milder changes in threshold occurring after only one spike were, nonetheless, sufficient to change the probability of firing in response to a particular level of stimulation from zero to 100%.

b. Oddities and Paradoxes

The threshold of a nerve at any moment is determined by the interaction of all of the excitability changes occurring during each phase of the threshold function following a spike and by the history of its firing for at least the last 15 min. Consequently, somewhat unusual experimental circumstances can be devised leading to understandable but initially surprising or paradoxical results. Figure XI-9 shows two instances in which threshold functions play a dramatic role in the outcome of experiments. In Fig. XI-9a intermittent responsiveness is shown. This condition occurs if the nerve is stimulated continuously at a constant intensity that is just above the 100% control threshold, and several authors have puzzled over it.[16, 17] Initially the fiber will respond to every stimulus. The duration of this initial responsiveness depends on the frequency of stimulation and the degree to which the stimulus exceeds threshold. As depression builds up, the supernormal period shortens until a failure occurs that is long enough for the threshold to move from supernormality to depression. The threshold will then exceed the strength of the stimulus. Thus a long gap without spikes appears in the records and persists until the recovery process has lowered the threshold sufficiently that one of the stimuli is successful. The supernormality following this first response ensures that succeeding stimuli will also generate spikes, and continuous firing is established once again. By varying the stimulus rate, strength and duration periods of intermittent responsiveness and failure as short as several hundred milliseconds or as long as 10 min can be obtained. At slower frequencies depression develops more slowly, but the successive stimuli are also spread in time, and hence are farther from peak supernormality. The recipe for long periodicity seems to be to give tetanus stimulation at approximately 70 impulses/second so that succeeding stimuli occur just beyond the peak of the supernormal period of the preceding spike. This ensures that extremely high levels of depression will be required to bring about the first failure. Furthermore, the stimulus strength should be slightly greater than that which will fire the unconditioned fiber 100% of the time. These were the conditions used to produce the 7-10 min periodicity in Fig. XI-9a. If the stimulus strength is below 100% for the unconditioned fiber, it is possible to obtain clustering of responses and clustering of failures in a probabilistic sense, but no clear-cut periodicity of responsiveness such as that shown in Fig. XI-9a. We have observed this sort of pattern in single peripheral units when a partial conduction block has been created along the axon by cooling, pressure, or xylocaine.

Figure XI-9b illustrates a paradox during tetanus at what seems to be a supramaximal stimulus to the gross sciatic nerve. After tetanic stimulation for 1 min, which allowed considerable depression to develop, an increase in the frequency of tetanus (which might be expected to tire the nerve) led to no change in the number of fibers

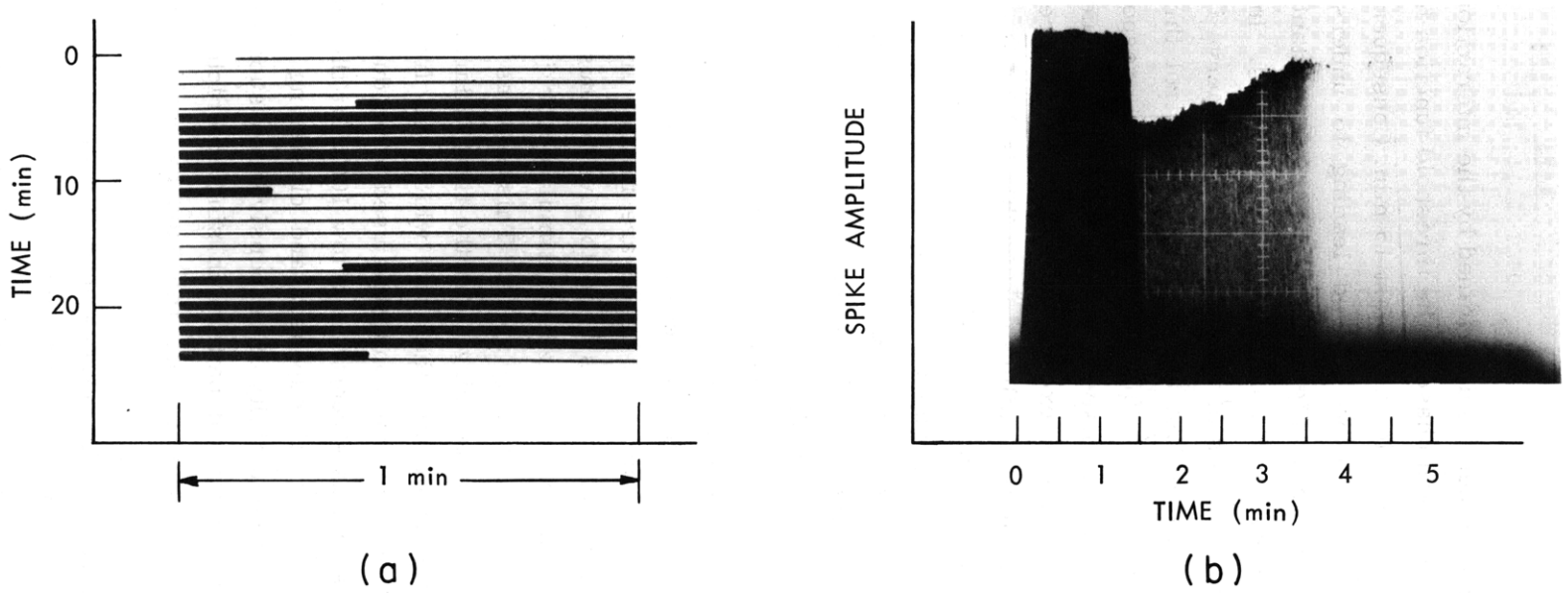

Fig. XI-9. Oddities and paradoxes. Part (a) is a record of intermittent responsiveness. Long periods of continuous conduction alternate with gaps of continuous failure. The sweep was continuous at 1 minute per sweep. The record reads sequentially as a book. The last period of no spikes ended 4.5 min after the finish of the record. Part (b) is a record of the tetanus paradox. A gross action potential was recorded with suction electrodes from the main stem of the nerve. A just supramaximal stimulus for fibers was delivered at 50 impulses/second. After more than 1 min of tetanus, the rate of stimulation was decreased to 5 impulses/second. Note the decline of the recorded potential and its slow return to the maximal level.

responding, whereas lowering the frequency of tetanus by a factor of ten (which rested the nerve) actually made the whole nerve less excitable. Our interpretation is that although depression rose to a high level during the initial tetanus, each stimulus occurred in the supernormal phase following the preceding spike. When the stimulus frequency was reduced, the stimuli fell within the depression phase, and the stimulus suddenly became subthreshold for many of the units. The recovery of excitability of the whole nerve after the drop in frequency took approximately as long as recovery of threshold takes in single fibers following depression.

c. Implications of Activity-Dependent Threshold Variation for Neuronal Operations

The existence of powerful aftereffects of activity on the excitability of axonal membrane has several important implications that bear directly on portions of our ideas about how the nervous system handles information in pulse-interval patterns.[10] The experiments that we have reported here were performed by using threshold-level stimulation, and their relevance to what occurs in the central nervous system depends on the existence in the CNS of analogous areas of near-threshold conduction. Various a priori theoretical arguments, as well as published and unpublished observations, suggest that nerve impulses encounter zones of conduction block, or near conduction block, in the vicinity of axonal bifurcations, particularly when activity rates are high (reviewed by Raymond[9]). Matthews discovered zones of low safety factor in what was probably an axonal link between dorsal roots of the cat[7]. Krnjević and Miledi[28] explored conduction failures in the terminal arborizations of the phrenic nerve of rats, and they felt that blockade occurred near branches. Recently, Van Essen[29] has been studying the effects of stimulus rates and polarization on conduction block in an identified sensory neuron in the abdominal ganglion of the crayfish. By careful latency measurements and intracellular records of the electrotonic depolarizations occurring after blocked impulses, he has located at least two separate zones of block near specific bifurcations, each of which has a different capacity for sustaining high rates of activity. In 1960, Ito and Takahashi,[30] using the dorsal root ganglion of the frog and toad, performed a similar analysis. They concluded that each node near the first branching of the primary sensory afferents was a potential locus of conduction block, but that the node at the bifurcation itself was the most sensitive. Asymmetries in conduction safety that depended on the direction of the propagated impulse were also observed, and an attempt was made to relate the sensitivity to blockade and the conduction safety asymmetry to the distribution of internodal distances and to fiber diameters. These experiments and other work have made us reasonably certain that regions of variable safety factor are common in single fiber trees all through the central nervous system.

Although our results allow us to describe activity-dependent changes in the threshold

of a fiber, they do not permit us to predict exactly the course of activity-dependent changes in invadability of the branches of an axonal tree, which depends also on changes in impulse shape and coupling. Since at least one of the important measures determining the invadability of a tree has been shown to vary strongly over much longer times than are generally taken into account, however, we can reasonably expect quite strong variation in invasion with activity.

These results suggest that conduction past regions of low safety factor will depend on the previous pattern of activity. Both the immediate history of a region, determining the magnitude of the supernormal phase, and the history of activity for as long as 15 min or more, determining the magnitude of depression and the duration of the supernormal period, undoubtedly influence the probability that a spike will propagate through the region. It is likely that the path of an impulse through an axonal arborization to the terminal branches will depend on the exact temporal relations of the previous impulses.

We still cannot predict, however, what aftereffect spikes will have on blocked membrane itself, because there are many ways to prevent conduction of impulses, and we are not certain that the membrane will at all times give threshold functions of similar time course and polarity. Nor is it clear that the tiny axon filaments which are most likely to block will show threshold curves like those of their parent axons. We do not know exactly what form the blocking of conduction will take when it occurs naturally in terminal axonal trees. Polarization and asphyxiation experiments on conduction block in cats[9] suggest that both anodal and cathodal blocks occur. Nonetheless, the presence of intermittent block (Barron and Matthews,[7] Krnjević and Miledi[28]) and the relation of changes in conduction probability to changes in stimulus pattern[31-33, 19] give some experimental support to the notion that activity-dependent changes in threshold influence conduction block which occurs naturally in various axons.

Most fibers of the CNS are continually carrying barrages of impulses. We have studied experimentally a form of this as "long-term conditioning tetanus." We tend to infer from our results, therefore, that many fibers in the CNS are in a maintained state of raised threshold induced by the impulses that they have carried. Presumably, each spike carries behind it a transient period of supernormal excitability that would have considerable local influence on the spikes immediately following it as it enters the regions of lowered conduction safety which we think are present in axonal arborizations.

Given the presence of blocks in axonal trees, and the similarity observed between such blocks and threshold stimulation, we can argue that the axon has a kind of threshold "memory" such that the distribution of threshold among the branches of a nerve depends on the past history of impulses in that nerve during the preceding 15 min. The exact pattern of thresholds in the bifurcations is likely to be a function of the spike distribution in time in the input train. With the kinds of information that can be recovered from spike trains of the parent axon,[10] it is conceivable that activity-dependent threshold

shifts are essential for information handling by the CNS. If so, even a complete knowledge of the connectivity of nerves will not be sufficient to reveal the way in which the nervous system handles information.

5. Conclusion

Nonsynaptic, activity-related influences have important effects on the threshold of peripheral nerve axons in the time zone varying from a few milliseconds to more than 15 minutes after activity. Threshold curves following conditioning trains have three distinct phases:

(i) The refractory phase, which lasts only a few milliseconds.

(ii) The supernormal phase, in which threshold is lower than control threshold, may last up to a second. Its time course is shortened by preexisting depression, but its maximum value, occurring ~15 ms after an impulse, remains the same for both rested and depressed nerves.

(iii) The depression phase, in which threshold is raised, is generated by activity rates well within physiological range (from 2 impulses/second to more than 100 impulses/second). Depression is momentarily abolished during the supernormal phase after an impulse, but it rises again to a maximum value within a second. In the absence of further activity a nerve may require more than 15 min to recover from depression.

We propose that these activity-dependent alterations in threshold are partly responsible for making the pattern of the distribution of nerve impulses among the terminal branches of an axonal tree depend on the temporal patterns of the impulses in the nerve.

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References

1. H. S. Gasser and J. Erlanger, "The Ending of the Axon Action Potential and Its Relation to Other Events in Nerve Activity," Am. J. Physiol. 94, 247-277 (1930).

2. H. S. Gasser and H. Grundfest, "Action and Excitability in Mammalian A Fibers," Am. J. Physiol. 117, 113-133 (1936).

3. H. S. Gasser, "Axons as Samples of Nervous Tissue," J. Neurophysiol. 2, 361-369 (1939).

4. R. Lorente de Nó, "A Study of Nerve Physiology," Studies from the Rockefeller Institute, Vol. 131, 1947. pp. 344-389.

5. D. J-M. Poussart, "Current Noise in the Nerve Membrane: Measurements under Voltage Clamp," Ph. D. Thesis, Department of Electrical Engineering, M. I. T., May 1968.

6. A. A. Verveen, H. E. Derksen, and K. L. Schick, "Voltage Fluctuations in Neural Membrane," Nature 216, 578-589 (1967).

7. D. H. Barron and B. H. C. Matthews, "Intermittent Conduction in the Spinal Cord," J. Physiol. 85, 73-103 (1935).

8. R. Lorento de Nó and H. T. Graham, "Recovery of Mammalian Nerve Fibers in Vivo," Proc. Soc. Exptl. Biol. Med. 33, 512-514 (1936).

9. S. A. Raymond, "Physiological Influences on Axonal Conduction and Distribution of Nerve Impulses," Ph. D. Thesis, Department of Biology, M. I. T., June 1969.

10. S-H. Chung, S. A. Raymond, and J. Y. Lettvin, "Multiple Meaning in Single Visual Units," Brain, Behav. Evol. 3, 72-101 (1970).

11. J. Y. Lettvin, "Research Objectives and Summary of Research," Quarterly Progress Report No. 100, Research Laboratory of Electronics, M. I. T., January 15, 1971, pp. 235-236.

12. A. D. Waller, "Observations on Isolated Nerve (with Particular Reference to Carbon Dioxide)," Phil. Trans. Roy. Soc. London, Series B 188, 1 (1897).

13. N. E. Wedensky, "La Phase Refractaire et la Phase Exaltée," Trans. Physiol. Labo. Universität St. Petersburg 3, 142 (1908).

14. E. D. Adrian, "The Recovery Process of Excitable Tissues," Part 1, J. Physiol. 54, 1-31 (1920); Part II, J. Physiol. 55, 193-225 (1921).

15. H. T. Graham, "Supernormality, a Modification of the Recovery Process in Nerve," Am. J. Physiol. 110, 225-242 (1934).

16. D. J-M. Poussart, "Measurement of Latency Distribution in Peripheral Nerve Fibers," S. M. Thesis, Department of Electrical Engineering, M. I. T., September 1965.

17. A. A. Verveen, "Fluctuation in Excitability," Netherlands Central Institute for Brain Research, Amsterdam, 1961.

18. C. M. Connelly, "Recovery Processes and Metabolism of Nerve," in J. L. Oncley (Ed.), Biophysical Science (John Wiley and Sons, Inc., New York, 1959), Chap. 51.

19. R. Lorente de Nó, loc. cit.

20. P. J. Boyle and E. J. Conway, "Potassium Accumulation in Muscle and Associated Changes," J. Physiol. 100, 1-63 (1941).

21. W. O. Fenn, "Electrolytes in Muscle," Physiol. Rev. 16, 450-487 (1936).

22. H. A. Blair, "Time Constant of Excitation and Velocity in Supernormal Phase of Nerve," J. Neurophysiol. 2, 249-255 (1939).

23. H. A. Blair and J. Erlanger, "A Comparison of the Characteristics of Axons through Their Individual Electrical Responses," Am. J. Physiol. 106, 524-564, (1933).

24. H. A. Blair, "On the Kinetics of Recovery during the Refractory Period in Frog's Nerve," J. Neurophysiol. 1, 127-143 (1938).

25. B. Frankenhauser and A. L. Hodgkin, "The After-Effects of Impulses in the Giant Nerve Fibers of Loligo," J. Physiol. 131, 341-376 (1956).

26. H. T. Graham and R. Lorente de Nó, "Recovery of Blood-Perfused Mammalian Nerves," Am. J. Physiol. 123, 326-340 (1938).

27. H. T. Graham, "The Subnormal Period of Nerve Response," Am. J. Physiol. 111, 452-465 (1935).

28. K. Krnjević and R. Miledi, "Presynaptic Failure of Neuromuscular Propagation in Rats," J. Physiol. 149, 1-22 (1959).

29. D. Van Essen, Private communication, 1971.

30. M. Ito and I. Takahashi, "Impulse Conduction through Spinal Ganglion," in Y. Katsuki (Ed.), Electrical Activity of Single Cells (Igaku Shoin, Ltd., Bunkyo-ku, Tokyo, Japan, 1960).

31. H. S. Gasser, "Recruitment of Nerve Fibers," Am. J. Physiol. 121, 193-202 (1938).

32. H. A. Blair and J. Erlanger, "Temporal Summation in Peripheral Nerve Fibers," Am. J. Physiol. 117, 355-365 (1936).

33. J. Erlanger, "The Initiation of Impulses in Axons," J. Neurophysiol. 2, 370-379 (1939).