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EFFECTS OF NERVE IMPULSES ON THRESHOLD OF FROG SCIATIC NERVE FIBRES

BY STEPHEN A. RAYMOND

From the Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, Massachusetts, U.S.A.

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SUMMARY

1. The firing thresholds of single myelinated fibres of frog sciatic nerves were monitored as a function of impulse activity in the fibre. The threshold was given by the number of coulombs in current pulses that excited a particular fibre half the time when delivered to the whole nerve. Threshold was tracked by a device that incrementally decreased the number of coulombs in the current pulse whenever the fibre responded and increased the pulse if it did not respond.

2. There was a pattern to the after-oscillations of threshold following activity. The fibres were briefly refractory, transiently superexcitable for about 1–1·5 sec and then entered a phase of raised threshold or 'depression' that lasted for many minutes.

3. Activity produced little change in the threshold curve during the refractory period. Strong depressions following prolonged activity prevented the threshold from returning to the base-line level within the time associated with the refractory period for the same fibre at rest.

4. After an impulse, superexcitability reached a maximum within 7–20 msec. This peak was larger as the number of impulses in a preceding burst increased and as the intervals between the impulses became briefer. Each successive impulse of a burst contributed less to the growth of superexcitability, and after the burst had 6–10 impulses additional impulses contributed nothing.

5. The depression phase was marked by the interaction between build-up, which depended on the activity rate, and recovery, which required as long as an hour or more for the threshold to be completely restored to resting level. These two mechanisms, one causing build-up and the other recovery, led to formation of dynamic equilibria. The threshold level at equilibrium increased monotonically with the activity rate.

6. The processes associated with superexcitability interact with those producing depression. In active fibres showing raised thresholds, impulses are followed by a relative superexcitability that persists for at least as long as an absolute superexcitability (with threshold below the *resting* level) can be measured in the same fibre at rest.

7. The duration of the superexcitable phase interpreted as a relative change in excitability was roughly the same regardless of the level of depression.

8. The magnitude of the oscillation in threshold was five to ten times larger than the grey region (the range of stimuli for which response is probabilistic). It is

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concluded that at regions of low conduction safety such as axonal branches, where weak forces can influence whether an impulse will pass, such pronounced and long-lasting after-effects of firing can be expected to modulate conduction of nerve impulses.

9. Two implications are drawn: (1) the static connectivity of an axon, as determined by its anatomy, will in general differ from its 'dynamic' connectivity as defined by the subset of its branches that conduct each impulse through the arbour to synaptic endings, (2) the temporal pattern of firing in the axonal trunk will produce throughout the teledendron a distributed time function of local thresholds that reflects the firing patterns, suggesting that the messages encoded in the pattern of firing may be resolved by variation of connectivity according to message.

INTRODUCTION

The threshold of a local region of nerve membrane is an important factor determining whether the region can be invaded by an approaching impulse. Recent direct work from several laboratories (Smith & Hatt, 1976; Parnas, 1972; Grossman, Spira & Parnas, 1973; Yau, 1976; Van Essen, 1973) supports earlier observations and suggestions that not all branches of an axon are invaded by each spike (Adrian, 1920, 1921; Lucas, 1917; Barron & Matthews, 1935; Krnjević & Miledi, 1959; Howland, Lettvin, McCulloch, Pitts & Wall, 1955, Raymond & Lettvin, 1969; Chung, Raymond & Lettvin, 1970), and that bifurcations constitute regions of low conduction safety (Dun, 1955; Ito & Takahashi, 1960; Lloyd & McIntyre, 1950; Wall, Lettvin, McCulloch & Pitts, 1956). Probability for invasion of a branch is neither constant nor independent of activity (Raymond, 1969; Grossman *et al.* 1973). Any influences acting to modulate conduction must be understood if it is to be possible to predict both the extent of invasion and the path that will be taken by an impulse travelling into the axonal arbour of the teledendron. One of the influences that modulates conduction is the firing threshold of the membrane. Since the threshold depends on activity, one may suppose that invasion of an arbour by an impulse will be contingent on past activity. As a step toward understanding the activity dependence of conduction of impulses in axonal arbours, this study is an examination of the activity dependence of threshold in fibres of peripheral nerves.

Several laboratories have investigated after-effects in peripheral nerves of amphibia (Adrian, 1921; Gasser & Erlanger, 1930; Lorente de Nó, 1947*a*) and mammals (Gasser & Grundfest, 1936; Lorente de Nó & Graham, 1936), and each of the principal phases of the after-oscillation of the threshold has been noted. The absolute and relative refractory periods, a supernormal or superexcitable period and a longer period of depressed excitability have each been observed. However, the interactions between these phases and the time course of their relations to each other have remained unknown. These previous experiments measured the excitability of whole nerve using the magnitude of the gross action potential as an indication of responsiveness to electrical stimuli. In the work reported here, the responses of single fibres were monitored for stimuli delivered to the whole nerve. A principal aim was to develop and test a method for measuring the threshold of excitable tissue over long periods. The method was then used to produce a more complete description of the

threshold following activity such that the threshold oscillations could be predicted during and after any pattern of activity. Single fibres of sciatic nerves from *Rana pipiens* were studied in *vitro*, and plots were made of threshold *vs.* time (threshold curve) after a variety of activity patterns.

METHODS

Care of nerve

Whole sciatic nerves from pithed, 3–5″ (snout to vent) *Rana pipiens* were placed in a double chamber having two independent circulations. Fig. 1 is a diagram of the experimental arrangement showing the features intended to minimize drift and to deal with the following problems.

(1) *Viability of the preparation.* The nerve is wet, submerged in circulating Boyle-Conway glucose Ringer solution (Boyle & Conway, 1941) aspirated with a mixture of CO_2, O_2 and air.

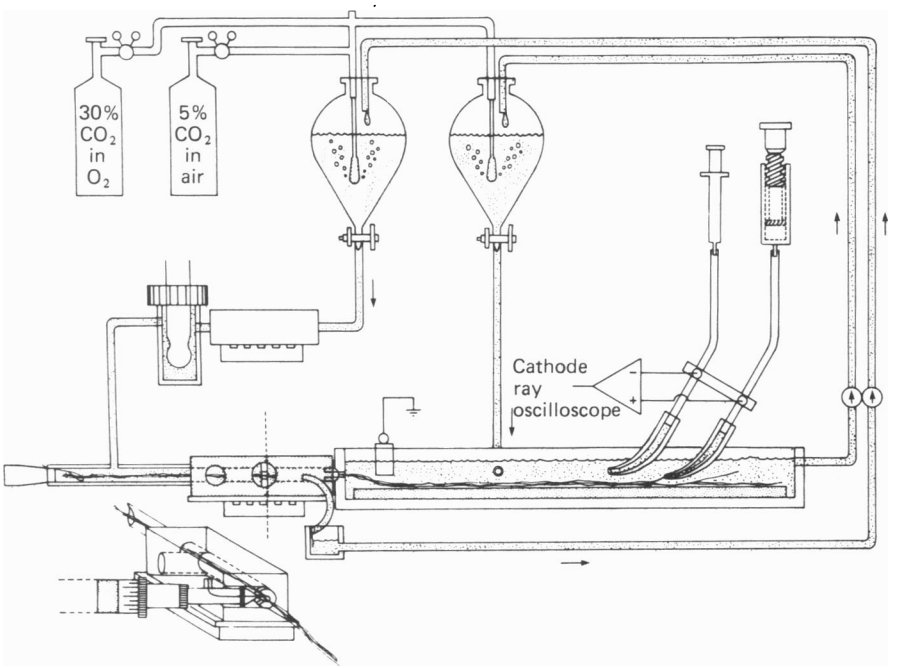

Fig. 1. System for maintaining nerve. Two pumps, symbolized by circles with arrows inside (far right), filled two 125 ml. separating funnels containing Boyle-Conway Ringer solution bubbled with a mixture of 30 % CO_2 in O_2, 5 % CO_2 in air and 100 % O_2 (not shown). The funnel on the left was the reservoir for the circulation through the stimulating chamber shown as a stippled block with the nerve running through a hole in it. The fluid left the reservoir and entered a heat exchanger made of two gold plates pressed against a plate of Teflon carved to form a long, sinuous channel. Under the heat exchanger and silver stimulation chamber were Peltier junctions referenced to flowing tap water. The electric current through the Peltier device was regulated by a thermostat having a manual set point. Next in line was a full-size pH electrode to guide the adjustment of gases. The fluid then entered a transparent plastic pipe connected to the silver block. A thermistor sensor for the temperature control system was screwed into the first hole. The stimulating electrode was driven into the next hole as shown in the inset. A plug of petroleum jelly ensured that fluid in the stimulating chamber exited from its third hole without leaking into the recording chamber, shown as a large open dish on the right. The distal end of the nerve was teased apart and recorded as illustrated. Nylon filters were placed at the outlets of each funnel.

The gas mixture was adjusted to ensure saturation of the solution with dissolved O_2 and a concentration of CO_2 sufficient to maintain a pH of 7·2–7·4. The Ringer solution flowed steadily from the funnels so that the volume around the nerve (1·2 ml.) was refreshed (flow rate 3–5 ml./min). Filters on the output of the funnels and at the pump (20–μm pore size) kept the solution free of precipitate or, in later stages of the experiment, from groups of cells, both of which were suspected of changing the local conductivity near the electrode as well as reducing the survival time of the nerve. In the recording chamber a separate filtered Ringer solution of similar composition bathed the distal end of the fibre. The temperature of the stimulating bath was regulated within 0·3 °C and was set at or near ambient temperature for these experiments.

(2) *Mechanical and electrical coupling.* A blue polyester sponge was attached to the floor of the Plexiglas recording chamber. By hooking tendrils of the nerve under the many irregularities of the surface of the sponge, the experimenter could assure that the nerve would not float to the surface, and that it would retain its position. An AC-coupled differential electrometer (Meta-Metrics, Carlisle, Mass., AK47) was connected as shown in Fig. 1, with a ground plate between the recording electrode and the stimulus, and a reference electrode in the recording bath. Impulses were recorded from a single unit in a dissected strand through flexible suction electrodes drawn by hand from 18-gauge polystyrene plastic hose. The electrode containing the fibre was placed so as to minimize stretching, and suction was applied through a threaded piston driven into an airtight cylinder rather than by a syringe. The piston afforded more control over the strength of the suction. The electrodes were 30–80 μm in diameter, permitting the experimenter to draw the strand of nerve at least 1 cm into the electrode.

The nerve was supported by the central hole of a Teflon disk 1·3 mm thick, that had its outside edge perforated to allow Ringer solution to flow through it. A hole was drilled at the centre of the disk for the nerve, and the support was sliced to produce a wedge-shaped entry for the stimulating electrode as it was driven through a hole in the silver stimulation chamber. The electrode was advanced until it just grazed the nerve at the centre of the support. At the beginning of an experiment the nerve was drawn through the chamber until the cut end was more than 7 cm from the Teflon guide and electrode, so as to minimize effects of the injury currents on the point of stimulation. The stimulating electrodes terminated in 1 mm spheres of either Ag plated with Ag/AgCl, or unplated platinum. The bore of the Ag stimulating block was plated with Ag/AgCl, and the entire block served as the reference electrode for the cathodal stimuli given to the whole nerve through the spherical electrodes via an optically isolated, gated current source (Meta-Metrics, Carlisle, Mass., SPS 1000).

Currents from adjacent membrane

Since stimulation was done by a gross electrode, neighbours of the unit being studied also fired in response to each pulse. The currents associated with firing and recovery of the neighbouring axons undoubtedly added to the stimulating currents. The method of testing for the influence of exogenous currents from neighbours was to adjust the duration of the conditioning stimulus over a wide range while monitoring the threshold of one fibre. The variation in the conditioning stimulus led to a corresponding variation in the currents contributed by neighbours as the number of responding fibres grew and diminished. However, the effects of such exogenous currents produced no discernible changes in the level of threshold. Any changes induced in the electrode by the stimulating currents would also be expected to produce a correlation between the duration of the conditioning stimulus and the threshold measured for the single fibre. However, the thresholds of the single fibres were observed to be independent of the variations of the conditioning stimulus, indicating that neither active neighbours nor electrode artifact were affecting the measurement.

Measurement of threshold

The threshold of a fibre can be viewed as a continuous function of time (Frishkopf & Rosenblith, 1958). The threshold depends on membrane conductances, membrane voltage, noise, and other dynamic parameters that determine the magnitude and duration required of a current pulse to fire the fibre. The threshold is a property of the fibre that changes in a noisy way from moment to moment and that also shows slower changes caused by impulse activity, temperature, and altered metabolism. The moment-to-moment threshold of a nerve fibre cannot be displayed

directly by any method that tests threshold by stimulating the nerve. Such tests themselves alter the variable they are intended to measure. Furthermore, if any stimulus exceeds the threshold, the fibre will fire, producing after-effects lasting a second or more that change threshold dramatically. Thus, threshold as a continuous function of time is an abstraction. It is not yet possible even to compute a continuous threshold as a function of continuous measurements of transmembrane voltage and currents. In active nerve such a computation must include as yet unknown relations giving the state of the fibre as influenced by its past activity, since the instantaneous membrane voltage is insufficient to determine threshold (Lorents de Nó, 1947*b*).

It is possible to approach an average value of the threshold by probabilistic operations. The first and most familiar method relies on the accumulation of the number of responses and failures for a series of N stimulations at a fixed stimulus intensity. As N becomes large, the statistical margin of error in the experimental measure of the probability of response at that stimulus level is reduced, but the time required to present N stimuli grows quite long, particularly when using long enough intervals to ensure independence of successive trials. In order to estimate the fixed stimulus associated with a 50 % firing rate, a number of trains of N stimuli are usually given, each train at a different stimulus level, and the results are plotted as probability of firing *vs.* stimulus intensity. The procedure yields an ogive curve intersecting the 50 % firing rate at some usually untried stimulus level. To track changes in average threshold by applying the preceding approach is possible but unwieldy (Newman & Raymond, 1971).

The second method is an adaptation of the 'method of constant response' (von Bekesy, 1947), since the stimulus is controlled to produce a constant probability of response. A device called a threshold hunter was developed to adjust a 'test' stimulus according to the outcome of every trial. If a test stimulus generates an impulse in the fibre being recorded, the threshold hunter reduces the number of coulombs in the next stimulus by shortening its duration. If a test stimulus fails to generate an impulse, the next stimulus is automatically made a bit longer. The output of the threshold hunter is a signal proportional to the duration of the hunting stimulus. Stimulus duration so measured constitutes an operational definition of the threshold of the fibre and is plotted in this work directly on the ordinate in μsec. Since the current was fixed, the stimulus duration was proportional to the number of coulombs transferred to the nerve. The stimulus durations used in these experiments (40–300 μsec) all fall within the region of the strength–duration curve where the product of current strength and pulse duration is essentially constant. Duration rather than strength was varied in these experiments simply because of the relative ease of measuring and controlling gate timings of an isolated stimulator.

The threshold was displayed as a function of elapsed time or as a function of delay from some previous conditioning activity. Elapsed times were measured from both a room clock and an HP 7100 B strip chart recorder. Delays were measured via a high-speed digital counter (HP 5248M) and a Tektronix time mark generator (Type 180-S1). They were measured accurately to ± 3 μsec. Except for the counters, all instrumentation was analogue. Voltages corresponding to the extent of delay and the value of threshold were stored in the capacitors of home-made, long-term integrators (drift < 1 % full scale/hr). Unless otherwise stated, the figures showing thresholds are photographs of the unretouched output of the threshold hunter as displayed on an HP 7035B X–Y plotter.

RESULTS

Convergence of the threshold hunter: Fig. 2 shows the form of the S-shaped curve relating stimulus strength to probability of response (Pecher, 1939; Verveen & Derksen, 1968). For all pulse durations less than some limit, L_1, the probability of a response, $P(R)$, can be defined as negligibly small. For durations greater than an upper limit, L_2, $P(R)$ approaches 1, and a response is essentially guaranteed. In between L_1 and L_2 response is probabilistic. The span from L_1 to L_2 is referred to here as the grey region. The duration, L_0, associated with a 50 % response rate is the long-term average threshold and occurs at the point of inflection in the ogive.

Given the S-shaped curve of Fig. 2, it can be seen that the threshold hunter must converge on L_0. The instrument operates to ensure that every response results in a

shortened test stimulus for the next trial. Thus the probability of shortening is the same as the probability of a response, $P(R)$, and the probability the new stimulus will be prolonged is identical to the probability the stimulus will fail, $P(F)$. The rate of decrease in the test stimulus is $P(R)\,\Delta/\tau$ where Δ is the size of the decrement in duration for each trial and τ is the cycle time between trials. The size of Δ is about 10 times smaller than the grey region. The rate of increase in the test stimulus is

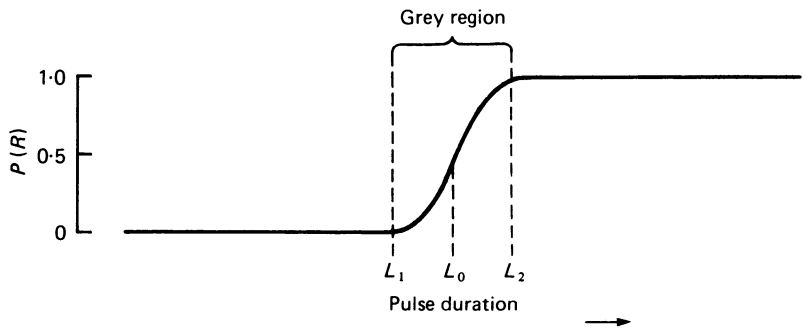

Fig.2. Relation between duration of a fixed current pulse and probability of a nerve fibre responding.

$P(F)\,\Delta/\tau$, since the sizes of the increment and decrement were equal. The net rate of change in the test stimulus is, therefore,

$$[P(R)-P(F)]\,\Delta/\tau.$$

This expression is 0 only at L_0, where the probability of prolonging is equal to the probability of shortening (0·5). All test pulses longer than L_0 are associated with a net probability that subsequent pulses will be shorter; pulses less than L_0 yield a net probability that subsequent pulses will be prolonged. For pulses outside the grey region in either direction, convergence is deterministic at a rate Δ/τ.

If the long-term average threshold should begin to rise or fall, the threshold hunter can be expected to begin a net change in the same direction. Since the grey region is a property of the nerve fibre related to the electrical noise of its membrane, it must travel with the threshold as it rises and falls. Thus, the values associated with the limits of the grey region L_1 and L_2 will migrate with L_0. The tendency for the threshold hunter to converge on L_0 will not be affected as L_0 changes, since none of the conditions used to establish the convergence at rest is altered if L_0 moves. However, after impulse activity, the nerve is no longer the same as in the resting state, and the width of the grey region may be substantially narrowed or widened. This aspect of the problem of activity-dependent variation in membrane has not been pursued in detail, but it has been noted during this study that the width of the grey region, measured after a period of tetanic stimulation when the threshold was elevated, shrank relative to the width in the resting condition.

Fig. 3 shows that the curve of the hunting stimulus converged toward the middle of the grey region even after it had been displaced by over-riding the threshold hunter. For a few minutes at the beginning of each trace the threshold hunter adjusted

the hunting stimulus in the usual fashion as the nerve fired and failed in response to stimuli given every 2 sec. Failures are marked by upward migrations of the plot, and successes move it down. After the initial control period, the threshold hunter was over-ridden several times in order to generate sudden reductions or increases in the duration of the hunting stimuli. The decrement was reduced as well to ensure that several stimuli would be given near the edge of the probabilistic region as the hunting stimuli approached it. The first failure after a string of successful stimuli marked the

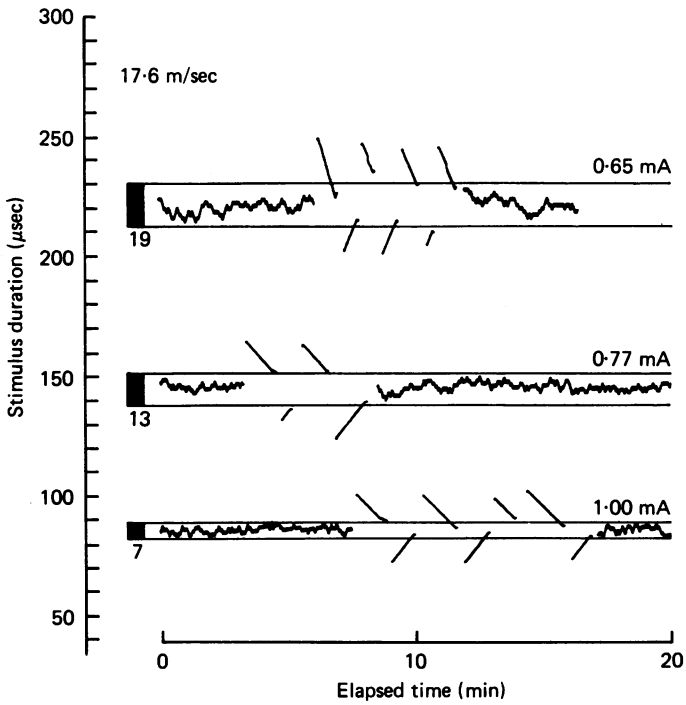

Fig. 3. Hunting thresholds at different current strengths. At the highest current strength, the hunting stimulus was shortest, resulting in the closest tracking and smallest grey region. At lower current strength, long hunting stimuli are required to fire the nerve. Tracking is noisier and the grey region is much larger. Conduction velocity, 17·6 m/sec. Temperature, 18 °C. Current strengths as shown.

halt of the descent. The hunting stimulus was then manually adjusted until it was too short to be successful. The stimulus would slowly increase trial by trial with a long string of successive failures. As before, when the first response signalled that the limits of the grey region had been entered, the ascent of the trace was halted, and the stimulus was suddenly prolonged more than enough to ensure 100% response and then allowed to hunt downward to the grey region. The procedure was repeated several times until it was clear to the experimenter where the perimeters of the probabilistic region lay.

Lines were drawn by eye, usually on the basis of the most extreme test, unless it seemed too inconsistent with the other tests. A bar spanning the grey region was then drawn to the left of each trace. After the tests for the boundaries of the grey

region were completed, the threshold hunter again tracked the fibre's resting threshold for a few minutes.

As would be expected, the extent of the grey region became greater as the duration of the 50% stimulus rose. At low current intensities, the duration of a liminal stimulus was longer, and the percentage of the total stimulus represented by the 1·5 μsec increment was much smaller. The reduction of proportionate value of Δ with increasing duration also accounts, at least in part, for the rather noisy hunting on the top trace.

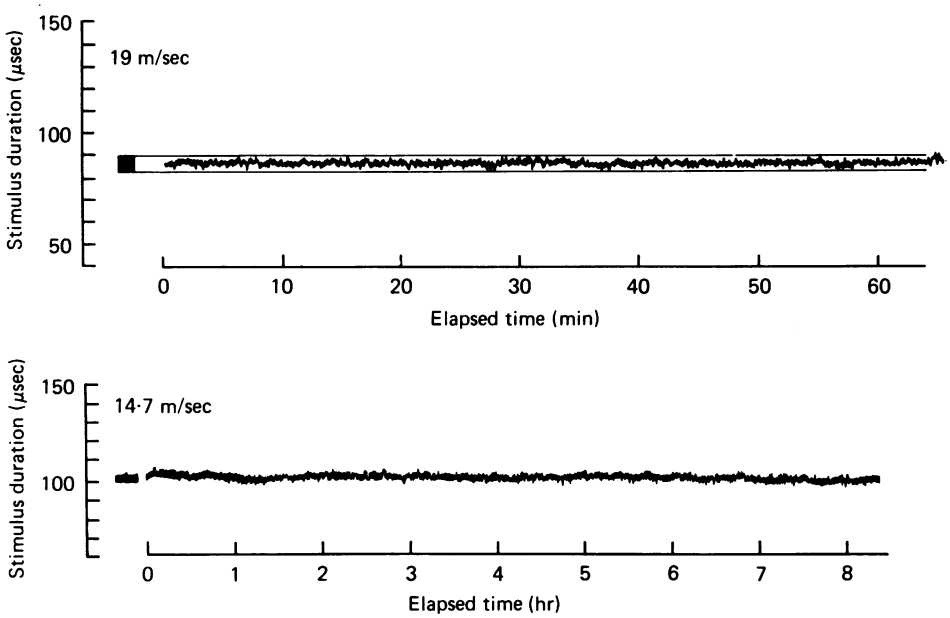

Fig. 4. Long-term stability of resting threshold in two fibres. The upper record shows the threshold hunted for about an hour at one test/2 sec. The limits of the grey region, as was measured before the record was done, were drawn to indicate the tendency to track the middle part of the probabilistic region. Temperature, 18·5 °C. Current strength, 0·30 mA. Conduction velocity, 19 m/sec. Lower record shows overnight tracking of the threshold of another fibre. Record was traced by hand from a chart recorder. Initial 20 min section was traced from threshold curve of the same fibre at rest 30 hr earlier. The 8 hr record was essentially flat, with the net change in threshold limited to less than 3 μsec over the entire period. Temperature, 18·5 °C. Current strength, 0·40 mA. Conduction velocity, 14·7 m/sec.

These experiments indicate that the threshold of the fibre can be measured by the threshold hunter. If the threshold should suddenly shift to a very different level, strings of successes or failures will occur, indicating that the hunting stimulus is outside the grey region and, thus, not near the threshold of the fibre. Conversely, the sporadic alternation between successful and failed stimuli is a sign that the instrument is tracking accurately enough that it is within the grey region.

Conduction velocities ranged from 3–30 m/sec, indicating that the population of fibres studied consisted of myelinated axons.

Stability of the system and preparation. With the validation of the technique of threshold hunting, it becomes possible to investigate the stability of the measure. Records made during early experiments showed slow drifts of threshold which seemed to be associated with the decline of the preparation and also exhibited some sudden jumps that seemed to be related to movement of the nerve or the presence of bubbles or detritus near the electrode (Newman & Raymond, 1971). However, in later experiments, once the problems of gas regulation, filtering of the solution and filling the system without producing bubbles were solved, the resting thresholds measured became flat, sometimes for several days. In Fig. 4 a 1 hr record of resting threshold is shown for a conducting fibre with 19 m/sec conduction velocity. The bottom trace shows an overnight tracking of the resting threshold of another fibre. The bottom plot was traced by hand from a section of the chart recorder records. Note that the resting threshold of the same fibre a day earlier, shown by the 20 min sample at the beginning of the record, was identical. These results are consistent with many other observations and show that in the absence of activity, the threshold of myelinated sciatic axons is essentially constant, and that there are no consistent long-term changes.

Impulse activity

The studies of the effects of impulse activity form a progession beginning with the threshold shifts that immediately follow an impulse and concluding with those that are most delayed and last the longest. Temperature has important effects on activity-dependence of threshold (Binder & Raymond, 1975), so the temperature during an experiment was held at a constant value.

Threshold after a single impulse. The threshold curve after a single impulse was derived by generating an impulse repetitively at a low rate ($< 0{\cdot}5$/sec) and hunting the threshold at different delays throughout a 1 sec interval beginning immediately after the impulse. The pulse programme is shown diagrammatically in Fig. 5*A*. Several hundred trials were run, each of which consisted of a conditioning pulse that was well above threshold and a single hunting stimulus. After each trial, the delay of the hunting stimulus with respect to the conditioning stimulus was increased slightly for the next trial. Since the increments in delay were small, there were sufficient trials in the neighbourhood of each delay to ensure that the threshold hunter continued to track the threshold as it changed incrementally. The sampling density was sufficient that a continuous record of threshold as a function of interval could be produced provided only that the threshold undergoes an identical swing after each conditioning spike.

Fig. 5*B* shows two such records of the threshold within the first second after the impulse. At first the nerve is absolutely refractory, and even maximal hunting stimuli are not effective. After 2–4 msec, the maximum stimulus generates an impulse, thus marking the beginning of the relatively refractory period. This is characterized by a steep drop in threshold that appears as a nearly vertical line on the threshold curves of Fig. 5*B*. Within 5 msec the relative refractory period ends as the threshold crosses the resting level. In all similar experiments the curve invariably continued to descend. It reached a minimum after 7–20 msec have elapsed following the conditioning impulse. This superexcitable or supernormal phase is characterized by a significant

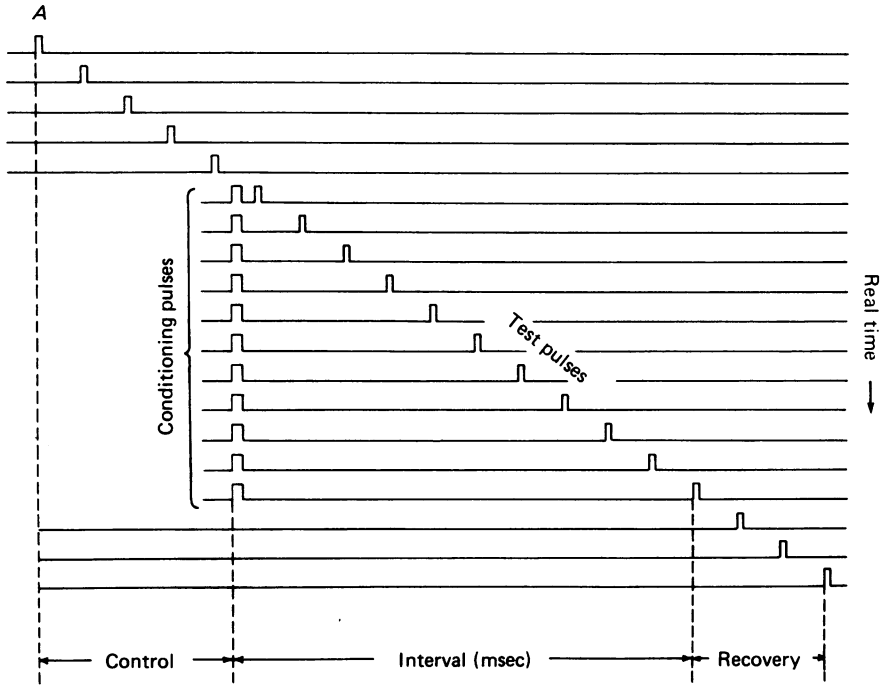

Fig. 5. *A*, the relation between timings of conditioning and test stimuli for the experiments on superexcitability. *B*, refractory and superexcitable phases following single impulses. The initial portion of both curves shows resting threshold level. The threshold was hunted at increasing intervals from a conditioning impulse generated every 2 sec at a time denoted by the mark at 0 interval. Trace (1): threshold during the first 200 msec following an impulse. The first response after the absolute refractory period occurs at 2·61 msec, the relative refractory period ends at 4·0 msec, and peak superexcitability is reached about 12·5 msec after the conditioning impulse. Trace (2): from the same fibre one day earlier, spans 1 sec. After conditioning stimuli were switched off (total presented 454 trace (1), 436 trace (2)), threshold is slightly elevated for a few minutes, as shown by the records marked recovery period at the tail of each curve obtained after the conditioning stimuli were switched off. Temperature 18 °C. Conduction velocity 17·2 m/sec. Stimulus strength 0·650 mA trace (1), 0·770 mA trace (2). Bars at left show extent of grey region (see text).

transient increase in excitability that wanes as threshold returns to the resting level over the course of 1–1·5 sec. For rested fibres (defined as fibres held at 18 °C and stimulated at rates slower than 0·5/sec), relative refractoriness lasts less than 3 msec. The threshold then overshoots to reach a minimum within about 10 msec. Similar results for the first few hundred msec after an impulse have been found on whole sciatic nerve action potentials using manual adjustments of the stimulus (Lorente de Nó, 1947*a*; Adrian, 1920 Gasser & Erlanger, 1930).

The amplitude of this threshold swing ranged far beyond the width of the grey region.

Short term after-effects of multiple impulses. In the case of multiple conditioning

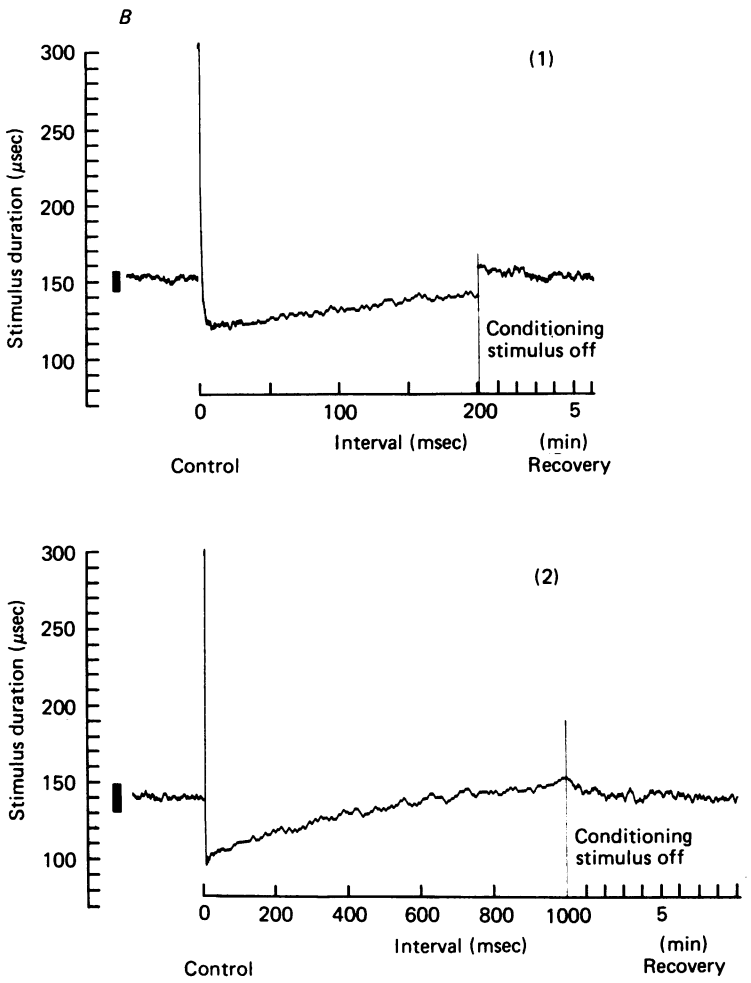

Fig. 5*B*. For legend see opposite.

impulses, the after-effects change as a function of the number of pulses and the intervals between them. Fig. 6 shows that a pair of impulses 400 msec apart is followed by a threshold curve having a slightly augmented superexcitable phase. If the two impulses are 10 msec apart, the superexcitable phase is much more pronounced. For interpulse intervals between 5 and 500 msec, the superexcitable phase becomes greater as the time interval between pulses shortens.

The superexcitable phase is also augmented as the number of preceding impulses is increased (Fig. 7). The first additional impulses generate large increments in superexcitability, but each successive impulse contributes a dimishing amount of additional superexcitability. A similar saturation effect after bursts of 6–8 impulses was reported by Gasser (1937, p. 130) for the after-potential of whole nerves. For the large individuals fibres studied here, a burst of 6–10 impulses leads to a maximum superexcitability about 7–20 msec following the last impulse of the burst. This

threshold appears to represent the excitable limit of the fibre, since any further increases in the rate of a conditioning burst or in the number of impulses produce no further increases in excitability. In fact, as seen in Fig. 7, bursts of 16 impulses were followed by smaller peaks during the superexcitable phases than bursts of 8. The apparent failure of longer bursts, such as the 16-impulse trains in Fig. 7, to generate threshold oscillations that reach maximum superexcitability appears to stem from a depression of excitability that builds up during the experiments as the long bursts are repeated.

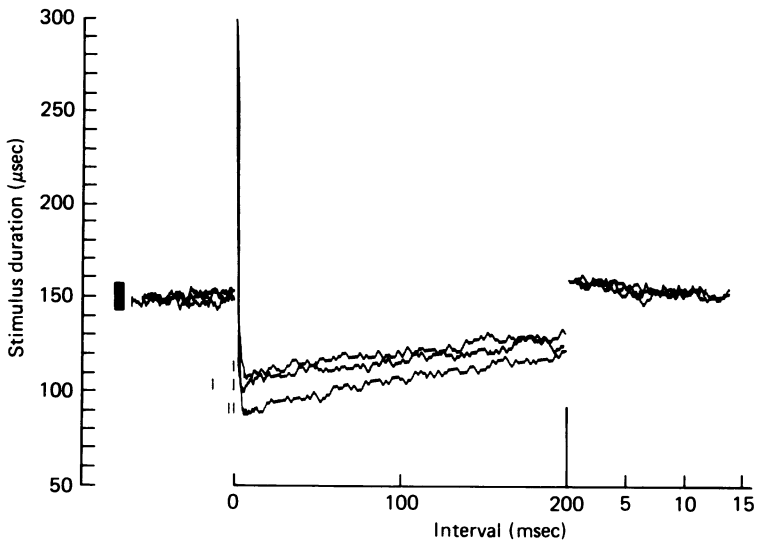

Fig. 6. Superexcitable phase as a function of interval between two conditioning pulses. Three threshold curves for 200 msec following conditioning pulse(s) are superimposed. The weakest superexcitable phase follows a single conditioning impulse, as shown by the single code mark to the left of where the curves descend to their minimum threshold level. A somewhat stronger superexcitable phase follows the second of a pair of conditioning impulses given 400 msec apart, as shown by the curve associated with the two widely spaced marks. The most dramatic superexcitable phase follows the second of two closely timed (10 msec) conditioning pulses as shown by the curve marked by two closely spaced lines. The line at 200 msec shows when the conditioning activity, repeated every 3·0 sec, was turned off. All three traces showed several minutes of slightly raised threshold caused by the low rate of stimulation (0·83/sec for the pairs and 0·5/sec for the single conditioning impulse). Temperature 18 °C. Conduction velocity 17·1 m/sec. Stimulus intensity 0·770 mA. Bar at left shows extent of grey region.

Some experiments were done to assess this explanation. Depression was minimized by using only a few trials having conditioning bursts of 16 impulses. The threshold was hunted at delays usually associated with maximum superexcitability (7–15 msec from the last impulse of the burst). The resulting measurements of minimum threshold were identical to the minimum levels reached in the same fibres when shorter bursts of 6 and 8 were used. If the longer bursts were repeated many times, the threshold measured at a fixed delay near the minimum began to rise after the first few trials, eventually changing by small amounts similar to the difference

between the peaks following bursts of 8 and 16 impulses in Fig. 7. These observations suggest that the mechanisms underlying superexcitability operate consistently after each long burst, but that the mechanisms underlying depression offset the full expression of superexcitability at the peak.

The time for the threshold to return to the resting level after the superexcitable peak is prolonged after multiple conditioning impulses. For long bursts this effect can be obscured, as it is in Fig. 7, where the threshold actually crosses the resting level

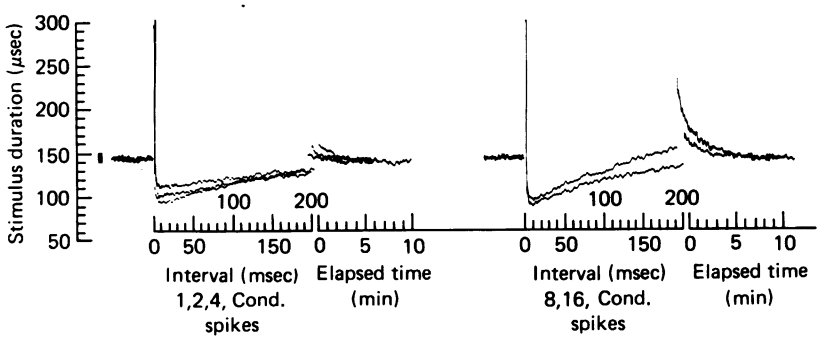

Fig. 7. Enhancement of the superexcitable phase following multiple impulses. Bursts of 1, 2, 4, 8, 16 impulses having an interpulse interval of 10 msec were given every 2 sec. Threshold was hunted for 200 msec after the last impulse in bursts of each category. The curve showing the threshold after bursts of 4 impulses was hunted first, the curves for 8, 2, 1 and 16 bursts were done in that order to offset possible gradual cumulative effects of successive experiments done at progressive burst lengths. Shown on the left are threshold curves for bursts of 1 (top), 2 (middle) and 4 (lowest) impulses, and on the right those for 16 (upper) and 8 (lower) impulses. After the approximately 30 min required to hunt each 200 msec interval, the bursts were turned off and the hunting stimulus was given every 2 sec in order to track the threshold for about 10 min as the fibre recovered from the activity of each experiment. Largest recovery transients followed the tests done with longer bursts. On the left, the top recovery curve (beginning a bit late) followed bursts of 4 impulses, the middle followed bursts of 2, and the bottom followed bursts of a single impulse. On the right the upper recovery curve followed 16 and the lower 8. The greater depression after bursts of 16 impulses was associated with a slight reduction of superexcitability and a steeper return phase. Conduction velocity 17·2 m/sec. Stimulus strength 0·650 mA. Temperature 17·9 °C.

within 200 msec. However, this apparent shortening of the superexcitable phase after bursts is illusory; it is a consequence of depression generated by the repeated activity. Experiments were done to estimate the persistence of superexcitability after a burst in fibres that were rested. Several brief runs of about ten trials were made. Each trial consisted of a conditioning burst and a roving hunting stimulus with its delay controlled manually. A delay was sought at which a run of ten or fewer trials produced slight but unambiguous superexcitability. This was assumed to reflect the last vestige of the decaying superexcitability, and the delay was noted. The same procedure was applied for nerves with single conditioning impulses. The persistence of superexcitability in the two cases was quite similar. Superexcitability after a few bursts lasted longer, but no more than about 30 % longer than it did after single spikes.

Long-lasting changes in threshold following long periods of activity. The depression of excitability, noted during the previous experiments with repeated long bursts, was detected by the effects it exerted on the peak and rate of return of the superexcitable phase. The following experiments were done to discover the relation between the phenomenon of depression and impulse activity in the fibre. The extent to which depression can be observed is a function of the interval between the last conditioning impulse and the time of testing. Thus, if conditioning bursts are repeated regularly every few seconds, depression of threshold is most dramatic after the processes

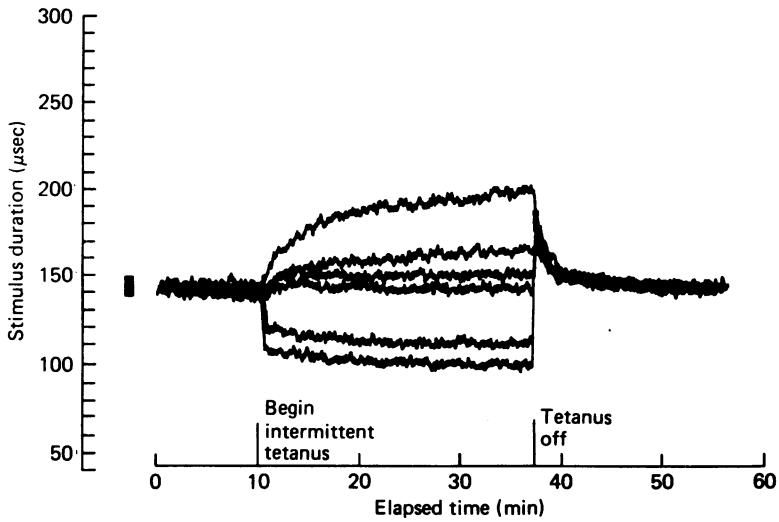

Fig. 8. Threshold hunted at fixed delays from repeated bursts of impulses. The same intermittent tetanic activity, consisting of bursts of 7 impulses with interpulse intervals of 10 msec repeated every 2 sec, was delivered to the fibre for all of the six curves shown. To the left of the line marking the beginning of the conditioning tetanus, only hunting stimuli (one every 2 sec) were given to measure the threshold of a 'resting' fibre. These stimuli were timed so that once the tetanus began, they would occur at a fixed delay from the 7th impulse of each burst. For example, the top curve shows the threshold hunted at a delay of 1·5 sec as a functon of elapsed time before, during and after the period of conditioning activity. In descending progression from the top curve to the lowest one, the associated delays were 1·5 sec, 600 msec, 350 msec, 275 msec, 75 msec and 15 msec. Thus the lowest curve, measured at a delay corresponding to maximum superexcitability, shows that even as depression builds up during the activity period, it exerts no detectable effects 15 msec after the bursts. When the tetanus was ended (after approximately 5,500 pulses), the recovery of threshold was identical for all curves, confirming that the same level of depression had been reached. Conduction velocity 23·5 m/sec. Stimulus strength 3·0 mA. Temperature 20·7 °C.

associated with superexcitability have died away. By hunting the threshold at several intervals from repeated bursts, it is possible to discover the earliest interval at which threshold reaches its maximum. Thus one can determine the interval associated with the peak of depression. Such experiments indicate that the maximum for depression is reached between 1·5 and 3·5 sec after an impulse, depending on the fibre. At earlier intervals some residual superexcitability can be observed, and it strongly influences the course of build-up of depression. Fig. 8 shows the build-up of depression as thres-

hold was monitored in the same fibre at several different fixed intervals from a repeated tetanic burst. At a brief interval (15 msec) after the last impulse in the burst, the fibre remained superexcitable for the entire 27 min period during which the bursts were repeated. For the curve taken with the hunting stimulus fixed at a delay of 1·5 sec, no superexcitable phase could be measured, and depression began to grow soon after the first bursts had travelled along the nerve. At a delay of 275 msec, the curve is flat; essentially no change in base-line threshold could be observed, despite the activity, until after the conditioning stimuli were switched off. The conditioning bursts were the same for each curve, and all the activity periods, therefore, could be expected to activate to the same extent the mechanism generating a depression. These mechanisms appear fully in the threshold records only for the curve measured at the 1·5 sec delay.

The experiment shows that over some range of delays no sign of the depression build-up from the activity can be observed, suggesting that within this range after-effects of one polarity offset those of another. Such experiments also show that even mild levels of activity produce a quite notable depression of excitability having its maximum after more than 1 sec, a period roughly equal to the maximum duration of the superexcitable phase observed in the same fibre. It appears that the processes or mechanisms that cause depression operate during the first second after an impulse, but that the mechanisms for superexcitability oppose and dominate during that interval, leading always to a relative superexcitability. At the peak of the super-excitable phase this dominance is strong enough that severe depressions may be needed to produce any change in threshold at all.

In order to assess the repeatability of the build-up to depression and to gauge its sensitivity to interspike interval, several experiments of the type illustrated in Fig. 9*A* were done consisting of repeated bursts and threshold tested at fixed intervals. It was generally observed that the level of depression attained at equilibrium was independent of inter-stimulus interval so long as the same average rates of conditioning activity were used. However, as shown in Fig. 9*B*, there were exceptions to this rule. When the interval between conditioning impulses in a burst was reduced to 5 msec, the level of equilibrium depression was not as high as it was for bursts having the same number of conditioning impulses but at an interpulse interval of 10 msec. Response of the fibre to all 5 impulses of conditioning bursts was monitored with oscilloscope and camera to assure that all conditioning stimuli were successful. The experiment shows that conditioning activities of the same average frequency resulted in consistently different levels of depression as a function of the interval between pulses in the conditioning bursts. Less depression was produced after bursts with impulses less than 10 msec apart.

The depressions shown in Fig. 9*B* are much like those observed in more than fifty similar experiments. The build-up starts quickly and noses over to a plateau. When the stimuli cease, the threshold recovers steeply at first, and then it slowly approaches the base line. The shape of such curves suggests that the severity of depression at equilibrium should vary monotonically with the rate of impulse activity. The recovery rate is evidently faster as depression becomes more intense. This should ensure that if the rate of build-up of threshold depends on activity rate and not on the level of depression, the rise in threshold after a step increase in activity rate will

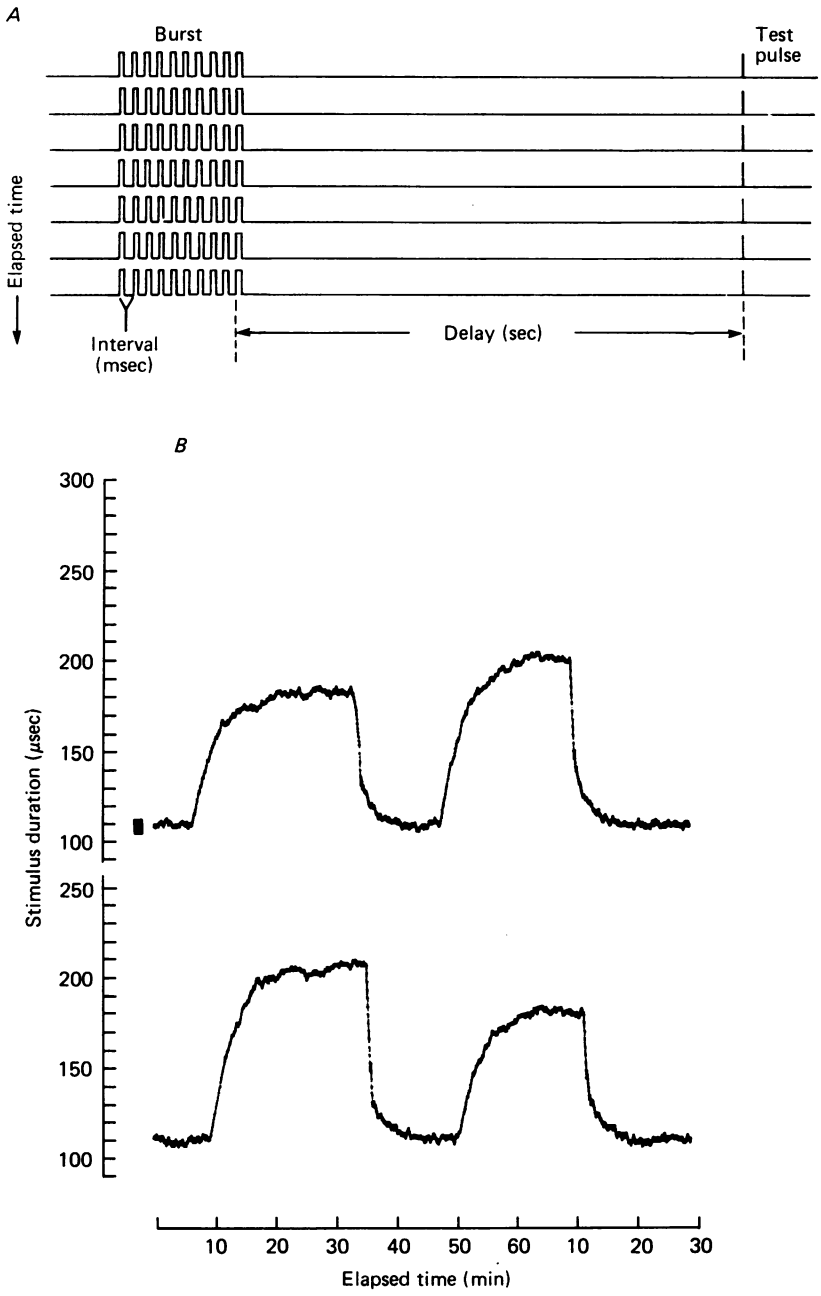

Fig. 9. *A*, relation of timings of conditioning burst and test stimulus for experiments on depression. *B*, build-up, plateau at equilibrium and recovery portions of the threshold curve during depression. The two traces were from one continuous record cut in half. When conditioning activity was begun, build-up of threshold commenced immediately. Conditioning activity consisted of 5 spike bursts given every 4 sec. Each burst was followed 2 sec later by a hunting stimulus. The drop in threshold during recovery began as soon as the bursts were turned off. During recovery and control periods, only hunting

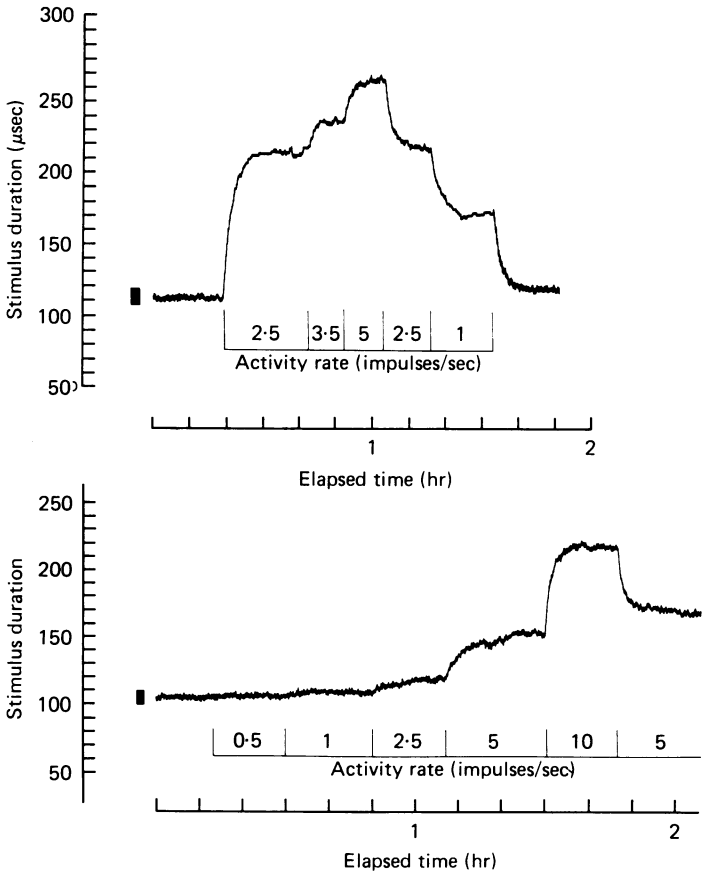

Fig. 10. Relation between threshold of the depression phase and activity rate. Upper record: threshold hunted 1·5 sec after bursts of conditioning impulses given every 2 sec so as to generate the average activity rates shown. For both traces the interval between spikes in the bursts was 10 msec. The level of threshold, once it reached equilibrium, increased as activity rates rose and decreased as they fell. Conduction velocity 13·6 m/sec. Stimulus intensity 0·75 mA. Temperature 15 °C. Lower record: threshold hunted 1·5 sec after bursts given every 2 sec. The two initial activity rates were quite low as indicated, yet slight rises in threshold can be discerned. The equlibrium threshold level during depression was monotonically dependent on activity rate. Conduction velocity 17 m/sec. Stimulus intensity 0·40 mA. Temperature 15 °C. Bar at left of each trace spans grey region.

stimuli were given, one every 4 sec. The interval between impulses of the burst was 5 msec for the first experiment (top trace, left), 10 msec for the second, 10 msec for the third (bottom trace, left) and 5 msec for the fourth. Threshold is given in μsec duration of the hunting stimulus as shown on the Y-axis. Its level at equilibrium was the same for both experiments using 10 msec interspike intervals in the conditioning bursts, and both experiments with 5 msec interspike intervals also reached the same level threshold even though they were separated by two other experiments and more than an hour. The average activity rate was the same for all four experiments, yet the 10 msec interspike intervals were associated with significantly greater depression. Conduction velocity 9·4 m/sec. Stimulus intensity 2 mA. Temperature 13·5 °C. Bar at left shows extent of grey region for rested threshold.

eventually be offset by the faster rate of recovery at the higher threshold level. The expected outcome is an equilibrium at a higher level of threshold.

Tests of this projection show that it is true. As a general rule, higher activity in the nerve produces greater depression. In Fig. 10 relatively low average rates of activity (0·5–10/sec) were established by varying the number of impulses in bursts given every 2 sec. As the activity rate increased, so did the threshold. As with the upper record in Fig. 10, the equilibrium value associated with any one rate was usually the same whether the level was reached by an increase or a decrease in average activity, indicating an absence of hysteresis. Sometimes the level reached after a reduction from a high activity rate declined very slowly during 'equilibrium' and never matched the level reached at the corresponding activity rate on the rise.

In Fig. 10 the change in threshold began at the rate of 1 impulse/sec. In other cases depression has been observed at 1 impulse/2 sec. Nerves that were tested during cooling of the Ringer solution to 12 °C showed definite depression at activity rates of 1 impulse/6 sec (Binder & Raymond, 1975).

Interactions between long-lasting and brief after-effects

A depressed fibre may require more than an hour of nearly complete rest before its threshold recovers to the resting level. Since activity rates of 10 impulses/sec or less may double the number of coulombs that must be delivered to a fibre to ensure a threshold level stimulus, it appears quite likely that if firing rates in excess of a few impulses/sec are associated with the daily life of frogs, the fibres of the nervous system will show prominent amounts of depression. Thus, single impulses and brief bursts ought not only to be studied in rested fibres. It seems that naturally occurring impulses will often propagate in the depressed membranes of fibres having some level of preceding or on-going activity. The question of how depression may alter refractoriness and superexcitability is, therefore, pertinent to the range of physiological experience of these fibres and not a theoretical curosity concerning the extreme limits of their operation.

During the recovery of a depressed fibre, the after-effects of a single conditioning impulse are indeed altered by the depression. In Fig. 11 the case of a single conditioning impulse presented during several levels of equilibrium depression is depicted. When the fibre was rested, the superexcitable maximum following the single spike was about 17 % below the resting level and recovery required 800 msec or more. During a mild depression of about 25 % above the resting threshold, the impulse resulted in a superexcitable phase that remained below the resting level for less than 50 msec. For this curve the minimum threshold was 26 % below the initial depressed level. For the more severe depression at the highest activity rate shown, the fibre never became absolutely superexcitable at all, since threshold never dropped below the resting threshold level. Nonetheless, the transient decrease in threshold during the relative superexcitable phase was substantial, 34 % below the initial depressed level. Such effects were consistently observed. In some fibres the superexcitable effects dominated the depression more substantially near the peak of the superexcitable phase so that the fibre was at least briefly superexcitable at equivalently strong depressions as those seen in the experiment of Fig. 10. It was observed that far from reducing the scope of the transient after-effects, depression consistently led to

pronounced relative increases in the superexcitable phase with respect to the initial depressed base line. Such changes made the after-effects more visible in the records and would seem to imply that depression should enhance whatever physiological consequences the after-effects of single impulses have.

The finding that superexcitability is augmented by bursts of impulses holds true for fibres depressed by on-going activity. However, the extent of the effect changed notably. As can be seen by comparing the superexcitable phases in Fig. 12 and Fig. 7,

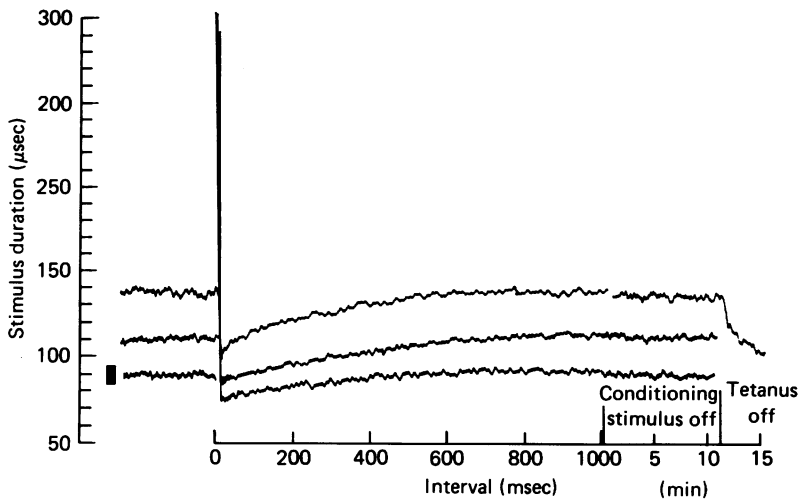

Fig. 11. After-effects of a single impulse interrupting depression as a function of activity rate. The three curves show threshold during a 1 sec interval following conditioning impulses generated every 3·6 sec and represented at 0 interval. Tetanic bursts of 20 (middle curve) and 40 (uppermost curve) stimuli at 10 msec intervals resulted in corresponding average activities of 5·5 and 11·1 impulses/sec. The last spike of the bursts ended 2 sec before the single conditioning impulse which therefore occurred near the maximum of the depression phase. No bursts were given for the lowest curve. During recovery periods after the 1 sec interval the single conditioning stimulus was turned off, but the conditioning bursts continued. The right end of the top curve shows the threshold recovering after the bursts were turned off as indicated. The 9 min control periods at the beginning of each curve show the threshold throughout the 300 msec interval preceding the single conditioning impulse. Stimulus strength 0·87 mA. Temperature 17·5 °C. Conduction velocity 16·3 m/sec. Bar at left spans grey region for the rested fibre.

superexcitable phases following closely timed multiple impulses add together during depression as they do for rested nerves. In Fig. 12 all of the curves were measured during equilibrium depression. The control level shown at the left was measured from a single trace obtained for the rested nerve before the intermittent bursts producing the depression were begun. The minimal threshold points measured after 1, 2, 4 and 8 impulses are also shown in Fig. 12*B*, but after the fibre had recovered from the depression. Note that during depression, a single impulse produced a large relative superexcitability that failed to descend to the resting level. At rest a single impulse in the same nerve was followed by a smaller superexcitability that lay entirely below the resting level. For the depressed fibre, two impulses produce strong augmentation of

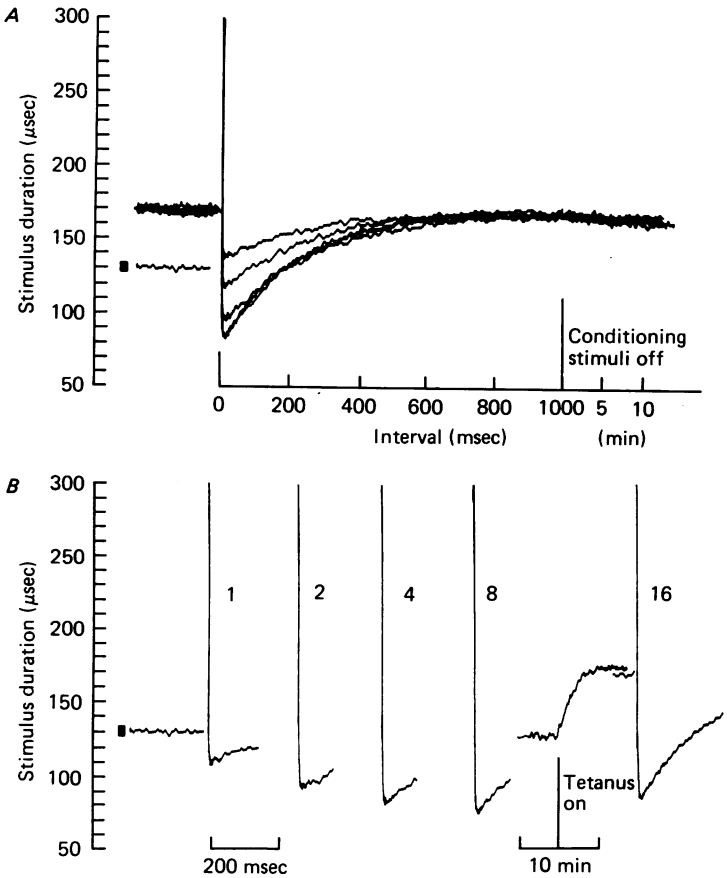

Fig. 12. Effects of multiple impulses on the superexcitable phase of depressed nerve. *A:* superimposed threshold curves following conditioning bursts of 1, 2, 4, 8 and 16 impulses for a depressed fibre. Depression of threshold was produced by tetani of 40 impulses ending before each conditioning burst. The cycle was repeated every 3·6 sec and the tetanus and conditioning burst in each cycle were adjusted so that exactly 40 impulses occurred, thus ensuring that the average activity rate remained constant at about 11 impulses/sec. The interpulse interval for both tetani and conditioning bursts was 10 msec. The last impulse in each conditioning burst, whether it had 1 or 16 impulses, occurred at the same point in the cycle and is indicated by the mark at 0 interval. Threshold was plotted for 1 sec after the last spike in the conditioning burst. The curve after 1 impulse has the weakest superexcitable phase and appears topmost of the set. Those associated with 2 and 4 impulses are successively lower. Curves for 8 and 16 are bottom-most and overlap. A single 7 min record of threshold for the fibre at rest is shown at bottom left. The control periods for the depressed condition overlap and measure threshold from 1·7 to 2 sec after the last impulse of the tetanus. The control periods at the right after the conditioning bursts were turned off show the threshold from 3·3 to 3·6 sec after the last impulse of the tetanus. *B:* threshold minima after conditioning bursts of 1, 2, 4, 8 impulses in the same fibre at rest. Threshold was hunted for an interval of about 200 msec after the last conditioning impulse in the burst. Relative refractory periods and the initial portions of the superexcitable phase are shown for bursts containing the number of impulses designated by the numbers to the right of the curves. The build-up to equilibrium depression is then shown as a separate inset. At the line marked 'tetanus on', 40 impulse tetani were presented every 3·6 sec. The curve marked for 16 impulses is from the depressed nerve and is shown to facilitate

superexcitability, as can be seen in Fig. 12*A*, and four impulses generate another sizeable drop in threshold. In fact, after four impulses the threshold reaches a minimum value that is reduced to almost half (56 %) of the threshold level associated with the equilibrium depression. In marked contrast to the case of rested nerve in Fig. 7, both 8 and 16 impulse bursts lead to threshold minima that are well below the minimum value reached by the threshold curve following four impulses.

The return phase is steeper than the return phase after shorter bursts, owing to the contribution of impulses in the bursts to the depression. The observation that there is a saturation of the increment in superexcitability within about eight impulses appears to hold during depression, since there is hardly any difference between the curve following a burst of eight and the curve following 16. The tests were made over the course of a 1 sec interval located near the maximum depression following the end of the conditioning bursts. The test period began 1·5 sec after the last conditioning impulse. As in the case of the rested nerve of Fig. 7, long bursts of 8 and 16 impulses after recovery from depression (Fig.12*B*) produced only a slight growth of superexcitable peak beyond that peak seen after 4 impulses. None of the threshold minima that followed bursts in depressed fibres ever reached levels as low as those reached by the threshold of the same fibres at rest, when they were subjected to similar conditioning bursts. However, superexcitability after multiple impulse bursts strongly counters the depression; so much so that the minimum value of threshold seen after bursts of eight impulses in Fig. 12 given during depression was only 3 or 4 percent higher than the level reached after an impulse burst given to the fibre at rest. This implies that the threshold after multiple firings in depressed nerve fibre descends very nearly to the minimum threshold reached by that fibre.

Depression also modifies slightly the course of threshold during the refractory period. As the threshold level rises with depression, the onset of the relative refractory period is delayed, and once the threshold has crossed its initial level, its further descent is slightly slowed. The thresholds always crossed the initial depressed levels and did so with less delay at greater depression. Threshold minima occurred essentially at the same time, indicating that the peak of the superexcitable phase is neither delayed nor advanced by depression.

As an additional effect of depression, it is worth noting that the fluctuations in the trace are considerably less noisy for the depressed membrane than for the membrane at rest. This observation is documented clearly in the top curve of Fig. 10, and shows in some of the other Figures as well. Control traces often indicate two or more failures or successes in a row in the excursions of the threshold hunter. Yet in Fig. 10 it can be seen that in the traces measured during depression the alternation between a success and a failure is quite regular. This indicates that the grey region of the membrane was effectively bracketed by the increment of the threshold hunter. However, the size of the increment of stimulus duration remained the same for all trials (1·5 μsec), including resting controls. The observation of close 'hunting' during depression thus

comparison of superexcitable peaks following long conditioning bursts in rested and depressed membranes. The records for 8 and 16 impulses overlapped each other whether the fibre was depressed or rested, though the minimum threshold after such bursts was lower in the rested than depressed condition. Conduction velocity 19·6 m/sec. Stimulus intensity 0·55 mA. Temperature 18°C.

implies that the threshold noise of the fibre is reduced by the processes producing depression. It has been demonstrated that the voltage noise of the membrane diminishes considerably with mild hyperpolarization (Verveen & Derksen, 1969), suggesting that depression originates in a hyperpolarization of the membrane.

Recovery from the long-lasting after-effects. The rate of recovery from the depression phase has already been seen to be a function of the level of the threshold. The recovery also varies with the duration of depression as given by the period a fibre is stimulated

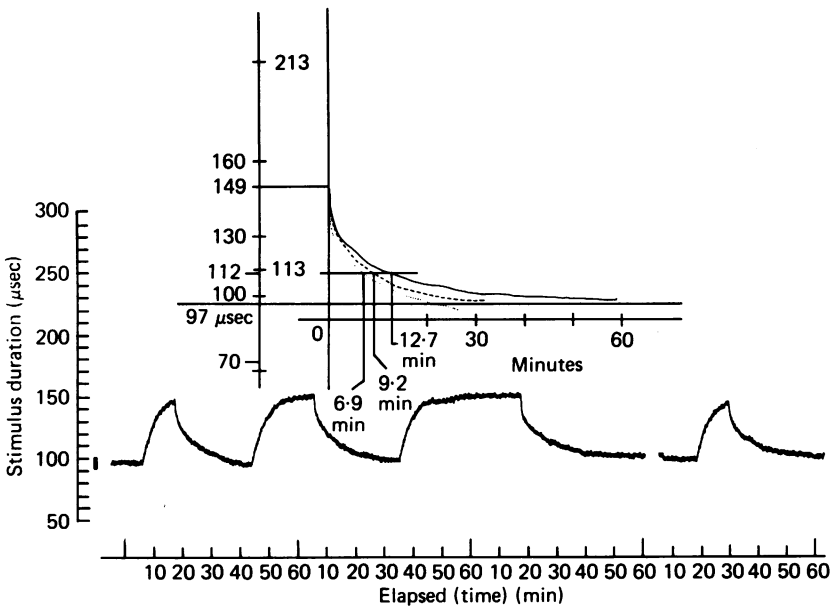

Fig. 13. Recovery as a function of duration of activity–induced depression. Threshold was hunted during four periods of activity generated by bursts of five impulses with interpulse intervals of 10 msec given every 2 sec. The hunting stimulus was given at a 1·5 sec fixed delay from the last impulse in the bursts. Average rate during periods of activity was therefore 5·5 impulses/2 sec or 2·7 impulses/sec. From left to right the first and fourth periods were 10 min long, the second lasted 20 min and the third 40 min. At the end of each period the 5 impulse bursts were turned off, marking the beginning of the recovery. The inset shows superimposed recoveries plotted by drawing lines through the centres of threshold curves hunted during the recovery periods; continuous line: 40 min period; dashed line: 20 min period; dotted line: first 10 min period. Recovery after the second 10 min period, not shown, resembled the recovery after the first. Activity of long duration was associated with a noticeably slower and prolonged recovery. Conduction velocity 13·7 m/sec. Stimulus intensity 0·630 mA. Temperature 16 °C.

at a constant activity rate. Even if the level of threshold does not change appreciably during a long period of steady activity, the recovery phase that begins when the activity is turned off may be prolonged. Fig. 13 shows the results of a typical experiment, measuring recovery as a function of the duration of the depression. At four separate times conditioning activity, consisting of repeated bursts of 5 impulses, was given, and the threshold was hunted at a fixed interval 1·5 sec after each burst. The rise towards an equilibrium level associated with each period of activity can be seen

clearly and serves to mark the beginning of the repeated bursts. The first and last periods lasted only 10 min, and the recovery from depression in each case is plotted in the graph. Once the bursts were switched off, the threshold recovered to the arbitrary level of 15 % above resting threshold after 6·9 min of rest. The intermediate period of depression lasted 20 min and it required 9·2 min of rest for the threshold to descend to the criterion level. The longest depression lasted 40 min and was associated with the longest recovery, 12·7 min to the criterion level.

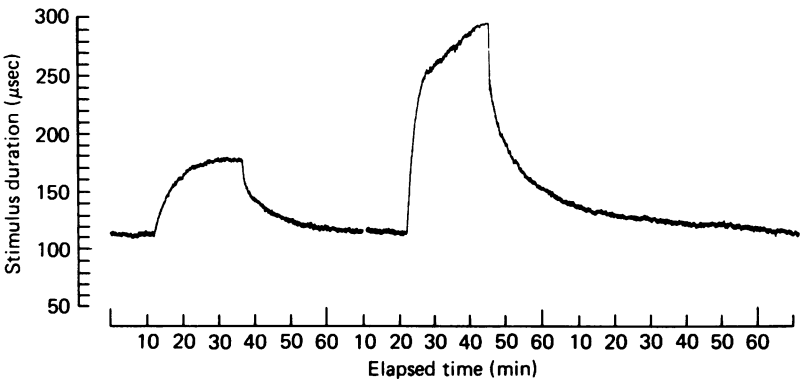

Fig. 14. Recovery of threshold following strong depression. The left curve shows the threshold measured 1·42 sec after bursts of 5 impulses given every 2 sec for 20 min and having an interburst interval of 10 msec, an average activity of 2·7 impulses/sec. Recovery of threshold to within 3 μsec of the initial control level as shown required 32 min. The right curve shows threshold for a 20 min period of similar bursts of 20 impulses that were then given to the same fibre, an average activity of ~ 10 impulses/sec. Recovery required 1½ hr to reach within 3 μsec of the initial control level. Strip chart records of the curve (not shown) revealed that the recovery continued for 4½ hr before stabilizing (for 7 hr) at 103 ± 2 μsec. The same level. 102 μsec, had been measured as the control at the commencement of the strong depression tests 2 hr before these records were taken, indicating that the base line of Figure 14 reflected a depressed threshold associated with long-term effects of prior tests. The shapes of the two recovery curves differ throughout their time course, so that the shorter can in no way be made congruent to any portion of the longer. Conduction velocity 14·7 m/sec. Stimulus intensity 0·570 mA. Temperature 18·5 °C.

The effects of the duration of depression are not as dramatic as the effect of its magnitude. Recovery time becomes increasingly extended, and recovery kinetics may even be altered as the magnitude of the preceding depression becomes greater. Recoveries lasting several hours can be measured following severe depressions. An example is given in Fig. 14, which shows that the recovery is prolonged not simply because the same mechanism operating at low levels of depression is displaced to a higher level and must, therefore, operate for a longer time in order to restore the system. At the left of Fig. 14 is a curve showing equilibrium depression associated with 20 min of 2·7 impulses/sec activity. The second curve was obtained on the same fibre subjected to an activity of 10 impulses/sec. The threshold of the fibre climbed to the saturation limit of the threshold hunter (300 μsec) just before the conditioning bursts were switched off. The period of recovery is

clearly very long. Furthermore, if the second recovery is superimposed upon the course of the first so that the highest level of depression of the first curve intersects the descending curve of the second at the same value, the subsequent course of the two curves is quite different. It requires almost three times as long for the second curve to recover over the same absolute threshold levels associated with the first. This shows that the kinetics of recovery are altered by previous displacement of the membrane to high levels of depression using activity rates. Unlike a first-order system, the rate of recovery is not a constant fraction of the instantaneous depression. Whatever the systematics eventually prove to be, both duration and severity of tetanus clearly figure importantly in determining the course of recovery.

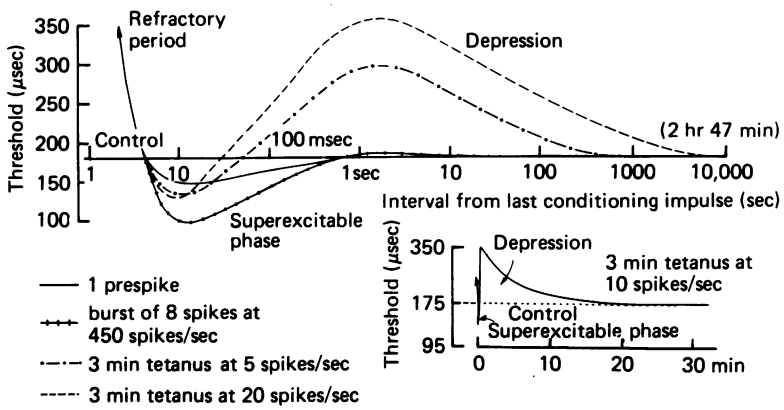

Fig. 15. Summary of activity–dependent after-effects on threshold. Four threshold curves following the last spike of single presentations of types of conditioning activity are depicted. The time course of each curve was estimated on the basis of the preceding experimental results. The abscissa shows time from the last spike, logarithmically compressed. Threshold values are estimates within the range of variation observed in experiments. Since the experiments required repeating the conditioning activity, they were not strictly analogous to the single presentations considered here. The inset shows how the threshold, after a mild tetanus, would appear on a linear time scale.

Continuous threshold curves. The preceding series of curves each present a particular aspect of what must be an essentially continuous process of oscillatory variation in threshold following each impulse in the on-going activity of nerve fibres. In order to envisage the shifts in threshold that occur, Fig. 15 was constructed on the basis of the experimental measurements. Although several other types of experiment were performed (Newman & Raymond, 1971), the ones presented here are sufficient to permit the construction of a general description of the threshold during the intervals between impulses as a continuous function of activity such that the threshold is altered by every impulse in a predictable way. In Fig. 15 the curves following the last spike in each of four patterns of activity are superimposed. Fibres varied sufficiently that the numbers used, while typical, would not apply to all. For example, the fibre of Fig. 11 would be expected to show a threshold that did not reach the resting level during the relative superexcitability following a 3 min tetanus at 20 impulses/sec. Yet the summary of the effects of activity would apply qualitatively to

this fibre and to all the others studied. It is obvious in Fig. 15 that each pattern of activity produces a quite different threshold curve. For instance, a 3 min tetanus at 5/sec results in a superexcitable phase with a more pronounced but briefer peak than the one following a single impulse. This is due to the cumulative effects of approximately the last eight preceding impulses. The curve recrosses the resting threshold level within 80 msec and proceeds to maximum depression within a second. In comparison to the 5/sec threshold curve, the curve after a 3 min tetanus at 20/sec has an even stronger, briefer, superexcitable peak, reflecting the cumulative effects of the last 8–10 more closely timed preceding impulses. Since the peak level of depression is higher, the cross-over at the end of the superexcitable phase can be expected to be earlier, and the recovery from depression will take longer. Note that the time axis is logarithmic to permit showing very brief phases simultaneously with the long ones. The inset shows a similar curve (after a 3 min tetanus at 10/sec) on a linear time scale.

Although the differences between the threshold curves after each pattern are striking, the similarities also show an important over-all consistency. Three phases follow every impulse: a refractory phase, a superexcitable phase and a depressed phase that is usually undetectable after single impulses because it is so small. Regardless of conditioning activity, the absolute and relative refractory periods change little. The duration and amplitude of the other phases after each impulse vary with temporal relations between the impulses conducted, but only one triphasic oscillatory function is necessary to summarize the relation of threshold to impulse activity (Raymond, 1974).

DISCUSSION

The threshold curves generated in these experiments are caused by mechanisms that depend on activation of conducted impulses in the fibre being studied. They do not appear to be caused by electrode artifacts associated with the delivery of stimuli or by changes in the currents or impedance of neighbouring fibres that are also activated by the conditioning stimuli. Variation in the number of coulombs in the conditioning stimuli produced substantial changes in the number of neighbours responding, but it had no effects on the threshold curves of the experimental fibre. Similar observations have been made for the superexcitable phase in other preparations (Gardner-Medwin, 1972, Swadlow and Waxman, 1976).

Mechanisms

Each phase of the threshold oscillation seems to be associated with different mechanisms. The refractory phase is altered very little by activity, and the results are consistent with the established interpretation that the refractory period stems from inactivation of the sodium conductance and from the transient high membrane conductance for K^+ associated with repolarization of the impulse itself (Hodgkin & Huxley, 1952). The dependence of such variables on activity does not seem to be very large, at least in squid giant axon.

The cumulative effects of several impulses on threshold levels during the superexcitable phase reported here, together with evidence of polarity reversal of the threshold curve during the superexcitable period in depolarized fibres (Raymond,

1976), and the observation that superexcitability vanishes under conditions of about 10 mM external K^+ concentration in crayfish axon terminals (Zucker, 1974) and dog heart Purkinje fibres (Spear & Moore, 1974), all suggest that a transient local increase in external K^+ concentration is generated by each impulse and persists long enough before diffusing to account for the period of lowered threshold that is observed. The superexcitable phase merits additional investigations concerned specifically with mechanism.

Ouabain eliminates the depressed phase in a manner that is consistent with the notion that depression arises from activity–dependent activation of an electrogenic pump for sodium (Raymond & Lettvin, 1978). An ion-pump mechanism for depression serves as part of an explanation of the observation that the threshold rises to different equilibrium levels for the same average rate of conditioning activity as interspike interval is changed (Fig. 9). The relation between impulse activity rate and depression suggests that as more coulombs are exchanged across the membrane, the rate of electrogenic pumping increases. The coulomb exchange associated with an impulse is contingent on the timing of the impulse in relation to other impulses, e.g. the second of a pair of impulses is often recorded as smaller than the first if the two occur close together (Frankenhaeuser & Hodgkin, 1956), and this may reflect a reduced transfer of sodium by the second impulse. Repeated bursts having closely timed impulses may thus exchange fewer coulombs, resulting in less depression. A test of this supposition remains to be made.

Ubiquity of threshold oscillations

After-effects of impulses on threshold of nerve and muscle fibres have been observed in many species. In the rabbit (Lorente de Nó & Graham, 1936) and cat (Gasser & Grundfest, 1936; Gardner-Medwin, 1972), the period of the oscillation appears shorter so that the phases are over more quickly. A period of ringing in threshold of mammalian nerves was reported by Gasser & Grundfest (1936) after long tetani at high frequencies. This could have been a desynchronization of strong triphasic after-oscillations in the separate fibres of whole nerve. A period of superexcitability is seen in leech (Yau, 1976) and crayfish unmyelinated nerves (Zucker, 1974), as well as in human saphenous nerve (Gilliatt & Willison, 1963) and muscle fibres (Buchtal & Engbaek, 1963; Adrian, 1921). Recent results on correlated threshold shifts and conduction velocity changes in myelinated and unmyelinated axons of corpus callosum of rabbit and monkey (Swadlow & Waxman, 1976; Waxman & Swadlow, 1976) also show a triphasic variation having the same form as the threshold and conduction latency variations for the frog nerve (Raymond, 1977), indicating that there is nothing unique about peripheral nerve that restricts such after-effects to axons of the periphery. Furthermore, when conduction velocity changes with activity, it is likely that there are correlated threshold changes, since such changes, distributed along the fibre, result in altered velocity of impulse propagation. In a variety of investigations, threshold changes are implicated from the conduction velocity measurements (Bullock, 1951; Gardner-Medwin, 1972; Kocsis, Vander Maelen & Kitai, 1977; George, 1977), and one variable can often be used as a measure of the other.

From the behaviour of other monostable oscillators one expects that some sort of

after-effect on threshold would be universal in all excitable fibres. Because of the similarity among species in the mechanisms of excitability, it seems reasonable to expect a triphasic after-oscillation of threshold to be widespread in the animal kingdom in excitable membranes of nerve and muscle. The results shown here on the frog demonstrate that such oscillations may be quite large, with the amount of charge transferred by a threshold level stimulus changing by a factor of three or more following bursts or high, sustained rates of firing. Even after single impulses, the threshold drops sufficiently so that for several hundred msec after the impulse, a stimulus that would be subliminal for the resting fibre would become 100 % effective. The threshold oscillations are also rather long-lasting, especially in comparison to the duration of the impulse or of synaptic potentials. These provocative features, ubiquity, high amplitude and long duration, have considerable bearing on the question of modulating impulse conduction at regions of low conduction safety.

Physiological role of threshold shifts

If the threshold were to drop low enough, the membrane would undoubtedly become spontaneously active, firing on its intrinsic electrical fluctuations. However, the threshold curves reported here never descended low enough to lead to the generation of nerve impulses. The role of such changes, if any, must be one of influencing impulses, not creating them. An area of membrane where conduction is marginal is also one where relatively small influences may determine whether an impulse will be blocked or be conducted (Raymond, 1969).

As noted, regions of low conduction safety have been associated with branches (Grossman *et al.* 1973; Dun, 1955; Ito & Takahashi, 1960; Yau, 1976), expansions and constrictions (Goldstein & Rall, 1976; Spira, Varom & Parnas, 1976) and other axonal specializations such as nodal distribution and demyelination (Waxman, Pappas & Bennett, 1972) or profound variation in the local density of Na^+ channels (Zeevi, 1972; Ritchie & Rogart, 1977). Such features may present no obstacle to conduction at low rates of discharge, yet they may become completely impassable as the activity rate rises (Krnjecić & Miledi, 1959), suggesting that the resulting growth in depression may be responsible for the conduction block. Alternatively, K^+ may build up with high frequencies of activity, leading to inactivation block (Parnas, Hochstein & Parnas, 1976).

At this point it is important to consider the relation between the triphasic threshold curves and the passage of impulses at any particular region of low conduction safety. It is well known that both anodal (hyperpolarization) and cathodal (depolarization) conduction block can be induced in axons (see Lorente de Nó, 1947*b*). Thus, one can expect two distinct types of regions of low conduction safety depending on whether inactivation or hyperpolarization is responsible for reducing the probability of conduction. The influence of threshold changes on conduction in either type remains to be determined.

The results of this study suggest that the superexcitable phase is associated with depolarization, and that depression is associated with hyperpolarization of the membrane. This leads one to anticipate that the processes producing the superexcitable phase would act to facilitate conduction through an anodal block, and that those processes producing depression would tend to render an anodal block more

severe. This proposition will hold true, however, only if these same processes do not also lead to offsetting variations of the strength of the arriving impulse. Thus, it is not yet possible to predict from a knowledge of the pattern of impulses and the threshold curves exactly what will be the effects on conduction. In a nervous system, the currents associated with activity in adjacent cells and fibres also influence conduction (Barron & Matthews, 1935; McCulloch, Lettvin, Pitts, & Dell, 1950; Dun, 1951; Wall *et al.* 1956; Decima & Goldberg, 1970; Chung *et al.* 1970). Slow potentials may also modulate conduction (Wall, 1964; Raymond, 1969). In experimental situations, control of conduction by the threshold has been demonstrated. Regions of low conduction safety that seemed anodal (Van Essen, 1973; Raymond & Pangaro, 1975*a*) and those that seemed cathodal (Smith & Hatt, 1976; Grossman *et al.* 1973; Spira *et al.* 1976) have been observed. As a final caution, it is possible for the type of region of low conduction safety to switch from anodal to cathodal and vice versa as the condition of the nerve changes (Raymond, 1969).

Switching of impulses through the tree as a function of the timing of impulses will occur regardless of the phase relations between conduction and threshold changes. All that is required is that following activity there be threshold variations that have high amplitudes and durations long enough so that the after-effects of different patterns of impulses will be different. The threshold curves described here meet these requirements, and an interesting result can therefore be proposed as a general rule: the connectivity between an axon and its post-synaptie elements is a dynamic property that depends on the temporal pattern of impulses it carries (see Chung *et al.* 1970).

Since the 'message' of an axon must be encoded by the temporal pattern of its discharge, the axon tree has the capability to act as a unique sort of 'spatio-temporal' filter to distribute different messages to different subsets of its terminals (Raymond & Pangaro, 1975*b*). This conclusion emerges as a direct consequence of the demonstration that axon membrane has a 'memory' of its recent discharges in the form of after-effects on threshold. Since such after-effects have quite different time courses for different activity patterns, and since the after-effects themselves must control conduction in the terminal branches of the axons, the distribution of impulses by the tree must vary with message. How such an apparatus might function to co-ordinate the behaviour of an organism is not yet obvious, but it seems quite pertinent to explore its possibilities and limitations.

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REFERENCES

ADRIAN, E. D. (1920). The recovery process of excitable tissues. Part 1. *J. Physiol.* **54**, 1–31.

ADRIAN, E. D. (1921). The recovery process of excitable tissues. Part II. *J. Physiol.* **55**, 193–225.

BARRON, D. H. & MATTHEWS, B. H. C. (1935). Intermittent conduction in the spinal cord. *J. Physiol.* **85**, 73–103.

BEKESY, G. VON (1947). A new audiometer. *Acta oto-lar.* **35**, 411–422.

BINDER, M. J. & RAYMOND, S. A. (1975). Some effects of temperature changes on the threshold of frog sciatic nerve fibres. *RLE Prog. Rep., Mass. Inst. Technol.* **116**, 281–287.

BOYLE, P. J. & CONWAY, E. J (1941). Potassium accumulation in muscle and associated changes. *J. Physiol.* **100**, 1–63.

BUCHTHAL, F. & ENGBAEK, L. (1963). Refractory period and conduction velocity of the striated muscle fiber. *Acta physiol. scand.* **59**, 199–220.

BULLOCK, T. H. (1951). Facilitation of conduction rate in nerve fibres. *J. Physiol.* **144**, 89–97.

CHUNG, S. H., RAYMOND, S. A. & LETTVIN, J. Y. (1970). Multiple meaning in single visual units. *Brain Behav. & Evol.* **3**, 72–101.

DECIMA, E. E. & GOLDBERG, L. J. (1970). Centrifugal dorsal root discharges induced by motoneurone activation. *J. Physiol.* **207**, 103–118.

DUN, F. T. (1951). The terminal arborization of nerve fibres as an important factor in synaptic and neuromuscular transmission. *J. comp. Physiol.* **38**, 133–135.

DUN, F. T. (1955). The delay and blockage of sensory impulses in the dorsal root ganglion. *J. Physiol.* **127**, 252–264.

FRANKENHAEUSER, B. & HODGKIN, A. L. (1956). The after-effects of impulses in the giant nerve fibres of *Loligo*. *J. Physiol.* **131**, 341–376.

FRISHKOPF, L. S. & ROSENBLITH, W. A. (1958). Fluctuations in neural thresholds. *Symposium on Information Theory in Biology* 1958, pp. 153–168. New York: Pergamon.

GARDNER-MEDWIN, A. R. (1972). An extreme supernormal period in cerebellar parallel fibres. *J. Physiol.* **222**, 357–371.

GASSER, H. S. (1937). The excitability cycle. In *Electrical Signs of Nervous Activity*, ed. ERLANGER, J. & GASSER, H. S. Philadelphia: Univ. of Pennsylvania.

GASSER, H. S. & ERLANGER, J. (1930). The ending of the axon action potential and its relation to other events in nerve activity. *Am. J. Physiol.* **94**, 247–277.

GASSER, H. S. & GRUNDFEST, H. (1936). Action and excitability in mammalian A fibers. *Am. J. Physiol.* **117**, 113–133.

GEORGE, S. A. (1977). Changes in interspike interval during propagation: quantitative description. *Biol. Cybern.* **26**, 209–213.

GILLIATT, R. W. & WILLISON, R. G. (1963). The refractory and supernormal periods of the human median nerve. *J. Neurol. Neurosurg. Psychiat.* **26**, 136–143.

GOLDSTEIN, S. S. & RALL, W. (1976). Changes of action potential shape and velocity for changing core conductor geometry. *Biophys. J.* **14**, 731–757.

GROSSMAN, Y., SPIRA, M. E. & PARNAS, I. (1973). Differential flow of information into branches of a single axon. *Brain Res.* **64**, 379–386.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **118**, 500–544.

HOWLAND, B., LETTVIN, J. Y., MCCULLOCH, W. S., PITTS, W. H. & WALL, P. D. (1955). Reflex inhibition by dorsal root interaction. *J. Neurophysiol.* **17**, 1–17.

ITO, M. & TAKAHASHI, I. (1960). Impulse conduction through spinal ganglion. In *Electrical Activity of Single Cells*, ed. KATSUKI, Y. Tokyo: Ikagu Shoin Ltd.

KOCSIS, J. D., VANDER MAELEN, C. P. & KITAI, S. T. (1977). Conduction properties of caudate efferent axons in the cat. *Neurosci. Abstr.* p. 40.

KRNJEVIĆ, K. & MILEDI, R. (1959), Presynaptic failure of neuromuscular propagation in rats. *J. Physiol.* **149**, 1–22.

LLOYD, D. P. C. & MCINTYRE, A. K. (1950). Dorsal column conduction of group 1 muscle afferent impulses and their relay through Clarke's column. *J. Neurophysiol.* **13**, 39–54.

LORENTE DE NÓ, R. (1947*a*). *A Study of Nerve Physiology*, vols. I, II. Stud. Rockefeller Inst. Med. Res. Vols. **131**, **132**.

LORENTE DE NÓ, R. (1947*b*). *Electrotonic Potential and Membrane Potential*. Stud. Rockefeller Inst. Med. Res. Vol. **131**, 344–389.

LORENTE DE NÓ, R. & GRAHAM, H. T. (1936). Recovery of mammalian nerve fibres *in vivo*. *Proc. Soc. exp. Biol. Med.* **33**, 512–514.

LUCAS, K. (1917). *The Conduction of the Nervous Impulse*. London: Longmans, Green & Co.

MCCULLOCH, W. S., LETTVIN, J. Y., PITTS, W. H. & DELL, P. C. (1950) An electrical hypothesis of central inhibition and facilitation. *Patterns of Organization in the Central Nervous System. Proc. Ass. Res. nerv. ment. Dis.* **30**, 87–97.

NEWMAN, E. A. & RAYMOND, S. A. (1971). Activity dependent shifts in excitability of frog peripheral nerve axons. *RLE Quart. Progr. Rpt., Mass. Inst. Technol.* **102**, 165–189.

PARNAS, I. (1972) Differential block at high frequency of branches of a single axon innervating two muscles. *J. Neurophysiol.* **35**, 903–914.

PARNAS, I., HOCHSTEIN, S. & PARNAS, H. (1976) Theoretical analysis of parameters leading to frequency modulation along an inhomogeneous axon. *J. Neurophysiol.* **39**, 909–923.

PECHER, C. (1939) La fluctation d'excitabilité de la fibre nerveuse. *Arch. Internat. Physiol.* **49**, 129–152.

RAYMOND, S. A. (1969). Physiological influences on axonal conduction and distribution of nerve impulses. Ph.D. Thesis, Department of Biology, *Mass. Inst. Technol.*

RAYMOND, S. A. (1974). Development of a model frog nerve axon showing threshold oscillation. *RLE Prog. Rep., Mass. Inst. Technol.* **112**, 129–130.

RAYMOND, S. A. (1976). The effect of ion pump poisons on threshold curves of frog sciatic nerve. *Neurosci. Abstr.* Vol. 2, p. 417.

RAYMOND, S. A. (1977). Changes in conduction velocity accompany activity-dependent shifts in threshold of frog sciatic axons. *Neurosci. Abstr.* Vol. 3, p. 223.

RAYMOND, S. A. & LETTVIN, J. Y. (1969). Influences on axonal conduction. *RLE Prog. Rep., Mass. Inst. Technol.* **92**, 431–435.

RAYMOND, S. A. & LETTVIN, J. Y. (1978) After effects of activity in peripheral axons as a clue to nervous coding. *Physiology and Pathobiology of Axons*, ed. WAXMAN, S. G., pp. 203–225. New York: Raven.

RAYMOND, S. A. & PANGARO, P. (1975*a*). Explanation of intermittent conduction based on activity-dependent changes in nerve threshold. *RLE Prog. Rep., Mass. Inst. Technol.* **116**, 273–281.

RAYMOND, S. A. & PANGARO, (1975*b*). Nerve Threshold and Intermittent Conduction. Color film, 17 min, Research Laboratory of Electronics, M.I.T. Distributed by MetaMetrics Corporation, Carlisle, Mass.

RITCHIE, J. M. & ROGART, R. B. (1977). The density of sodium channels in mammalian myelinated fibres and the nature of the axonal membrane under the myelin sheath. *Proc. natn. Acad. Sci. U.S.A.* **74**, 211–215.

SMITH, D. O. & HATT, H. (1976). Axon conduction block in a region of dense connective tissue in crayfish. *J. Neurophysiol.* **39**, 794–801.

SPEAR, J. F. & MOORE, E. N. (1974). Supernormal excitability and conduction in the His-Purkinje system of the dog. *Circulation Res.* **35**, 782–792.

SPIRA, M. E., VAROM, Y. & PARNAS, I. A. (1976). Modulation of spike frequency by regions of special axonal geometry and by synaptic inputs. *J. Neurophysiol.* **39**, 882–899.

SWADLOW, H. A. & WAXMAN, S. G. (1976). Variations in conduction velocity and excitability following single and multiple impulses of visual callosal axons in the rabbit. *Expl Neurol.* **53**, 128–150.

VAN ESSEN, D. C. (1973). The contribution of membrane hyperpolarization to adaptation and conduction block in sensory neurones of the leech. *J. Physiol.* **230**, 509–549.

VERVEEN, A. A. & DERKSEN, H. E. (1968). Fluctuation phenomena in nerve membrane. *Proc. IEEE*, **56**, 906–916.

VERVEEN, A. A. & DERKSEN, H. E. (1969). Amplitude distribution of axon membrane noise voltage. *Acta physiol. pharmac. neerl.* **15**, 353–379.

WALL, P. D., LETTVIN, J. Y., MCCULLOCH, W. S. & PITTS, W. H. (1956). Factors limiting the maximum impulse transmitting ability of an afferent system of nerve fibres. *Information Theory*, ed. CHERRY, C., pp. 329–344. New York: Academic Press; London: Butterworth's Publications.

WALL, P. D. (1964). Presynaptic control of impulses at the first central synapse in the cutaneous pathway. In *Progress in Brain Research*, ed. ECCLES, J. C. & SCHADE, J. P. Amsterdam: Elsevier Publishing Co.

WAXMAN, S. G., PAPPAS, G. D. & BENNETT, M. V. L. (1972). Morphological correlates of functional differentiation of nodes of Ranvier along single fibres in neurogenic electric organ of the knife fish, *Sternarchus. J. cell Biol.* **53**, 210–224.

WAXMAN, S. G. & SWADLOW, H. A. (1976). Morphology and physiology of visual callosal axons: evidence for a supernormal period in central myelinated axons. *Brain Res.* **113**, 179–187.

YAU, K. W. (1976). Receptive fields, geometry and conduction block of sensory neurones in the central nervous system of the leech. *J. Physiol.* **263**, 513–528.

ZEEVI, Y. Y. (1972). Structural functional relationships in single neurons: Scanning electron microscopy and theoretical studies. Ph. D.Thesis. University of California, Berkeley.

ZUCKER, R. S. (1974). Excitability changes in crayfish motor neurone terminals. *J. Physiol.* **241**, 11–126.