MECHANISMS OF BIOELECTRIC ACTIVITY IN ELECTRIC TISSUE*

I. The Response to Indirect and Direct Stimulation of Electroplaques of Electrophorus electricus

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INTRODUCTION

The electric discharge of electric fish was early recognized to be identical in nature with the less powerful electric activity of nerve and muscle and was, therefore, the subject of numerous investigations aimed at the analysis of bioelectric potentials. These studies primarily concerned themselves with the physical and physiological properties of the electric organ up to 1937, when Nachmansohn and his colleagues began to analyze the biochemical events associated with the generation of the electrical activity. The voluminous earlier literature on the physiological data was summarized by Rosenberg (18); a later discussion is by Albe-Fessard (1); while the biochemical findings were reviewed by Nachmansohn (17).

In recent years interest has largely centered on the responses of a few or single cells of the electric organ. Early attempts in this direction were carried out by Fessard and his colleagues (11). A new contribution is the work of Keynes and Martins-Ferreira (15), which has been paralleled by similar independent work in this laboratory. The present paper presents the first of a series of reports on our work, and confines itself to the results obtained with external electrodes on the responses of one or a few cells of the electric organ. Emphasis is put in this report on the responses of the cells to indirect stimulation through their nerves.

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Methods

Most of our experiments were performed on the caudal part of the electric organ, the bundle of Sachs. This portion is more suitable than is the larger organ for work with a few units since it is composed of only about 10 compartments per cm., each compartment containing one cell, the electroplaque. The compartments are essentially rectangles running from the midline to the periphery, about 5 to 10 mm. long, and 1 mm. in square section. Approximately every 3 mm. along the extent of the electric organ, nerves emerge bilaterally from the spinal cord to innervate the cells. In the bundle of Sachs the nerves run obliquely backward and downward, each nerve innervating a rostrocaudal segment up to 6 to 8 cells in width and up to 15 in depth. The nerve, therefore, supplies up to 120 or more cells enclosed in the volume determined by the rostrocaudal segment. Each cell is innervated only at the caudal face, which is also histologically differentiated from the rostral aspect. In *Torpedo marmorata*, Wagner (19) demonstrated that each cell receives a number of nerve fibers. The electrophysiological experiments to be described here show that in the eel, also, each plaque is multiply innervated. Couceiro and Akerman (9) calculated from the relation of the number of spinal motor cells to the number of electroplaques that each nerve fiber innervates about 2 cells. They have also reported that in the eel the nerve fibers have extensive end arborizations, making multiple contacts with the surface of the cell, as is also the case in *Torpedo* and the common ray (18).

The animals used in our experiments had been kept in the aquarium of the New York Zoological Society for periods from 6 months to several years. The fish varied in length, but all were longer than 90 cm. The experiments were carried out at room temperatures varying from 22–24°C.

The preparations consisted of transverse slices of the animal 2 to 3 cm. thick in the region of the electric organ. Hemorrhage at the cut surface of the fish was prevented by cauterizing the blood vessels. Under these conditions the animals survived for many months, and could be used repeatedly to provide a number of preparations.

Some of our experiments were performed on these slices perfused by the technique of Chagas *et al.* (8). Most of the experiments, however, employed a simpler preparation made by retaining only 1 to 3 layers of cells in the slice while preserving their innervation. The removal of the superfluous layers was done in either the horizontal (rostrocaudal) or the vertical (dorsoventral) plane. Each cell being innervated only at its caudal face, the arrangement of the cells differed in the two cases (Fig. 1). In the first type each layer presented the innervated face of one cell toward the non-innervated face of its neighbor. In the second type one surface of the layer was composed of the innervated faces of all the cells.

The nerves to the Sachs organ are extremely short and delicate. However, they can be dissected from the fatty tissue surrounding the swim bladder and made accessible to stimulating electrodes. The preparations were mounted in a plexiglass chamber, the cells being covered with a Ringer's solution similar to that of Hargreaves and Frota-Moreira (14), except that it contained a higher concentration of NaCl (0.18 M instead of 0.169 M). The segment of the spinal column and the immediately adjacent tissue including the dissected part of the nerve were above the level of the Ringer's solution, but so oriented in the chamber that they could be covered

with a layer of mineral oil to prevent drying. Stimuli were applied to the nerves by means of fine platinum or silver wire electrodes. Direct stimulation of all the plaques in a preparation was delivered through a pair of silver electrodes of rectangular cross-section, insulated except at the surface touching the cells at the margins of the preparation. Individual cells were stimulated directly by a pair of stainless steel needles insulated except at their tips. These electrodes were carried on a manipulator and positioned so as to excite any desired unit of the preparation.

The stimuli were rectangular pulses of 0.1 to 2.0 msec. duration and of controlled intensity. When paired stimuli were used each was independently controlled for amplitude and pulse duration, and the interval between them could be varied at

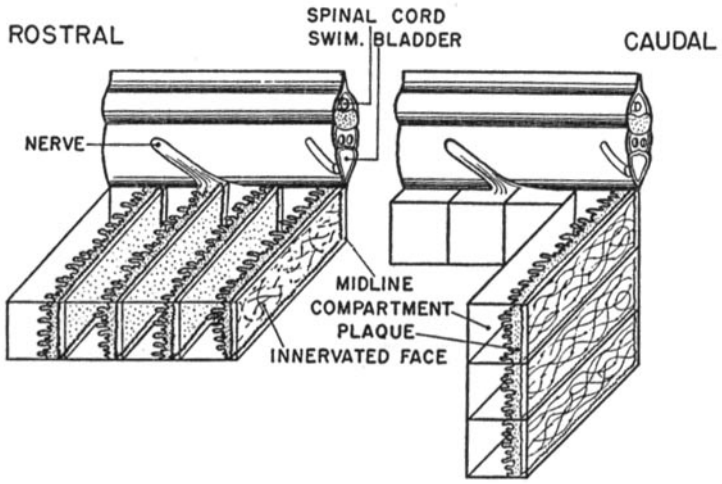

FIG. 1. Diagrams of the two types of layer preparations of the bundle of Sachs. On the left, horizontal layer; on the right, vertical layer.

will. The outputs of the stimulators were ungrounded with low capacity to ground, in order to minimize shock artifacts.

The electrical activity was recorded by means of stainless steel needles completely insulated except at the tips (13), or with saline-filled glass capillary electrodes with tips of about 2μ diameter. These electrodes were mounted on appropriate manipulators and positioned on the preparation as suited the needs of a particular experiment. Among the electrode arrangements those most common were: (*a*) One electrode inserted into each of the marginal cells of the preparation. (*b*) A pair of closely spaced electrodes applied so as to record activity across individual cells. (*c*) A "roving electrode" positioned along the row of cells and a fixed electrode in one or the other marginal cell. The diagram of Fig. 9 D shows these arrangements as used in one experiment.

The electrical activity was amplified by means of a differential amplifier coupled either capacitatively or directly, and led to a cathode ray oscillograph for photographic recording.

RESULTS

I. The Electrical Responses of the Electroplaque to Single Stimulation of One Nerve.—Stimulation of the nerve innervating a group of cells with a single pulse of 0.1 to 0.5 msec. duration produced responses after a latency which varied between 1.5 to 3.5 msec. Mapping of the potential field developed by these responses in the fluid surrounding the tissue demonstrated, as will be shown in section III, that activity was always associated with negativity of the innervated caudal faces of the active cells with respect to their rostral faces. This orientation corresponds with the polarity of the discharge in the intact animal.

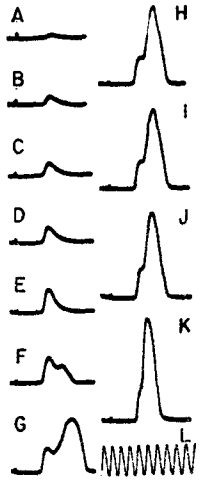

FIG. 2. Progressive development of the local response and then the spike, with increasing strength of the stimulus delivered to the nerve. Time is milliseconds, amplitude, 3 mv. Preparation containing 1 layer of cells, covered with mineral oil.

The response of the active cells has two components (Fig. 2). An early, small, graded response is evoked by weak stimuli to the nerve and increases as the stimulus is increased (Fig. 2 A to F). Eventually a second, much larger component is superimposed upon the first after a latency which is decreased with stronger stimuli. This activity is evidenced as a sudden, discrete, large increase of potential (Fig. 2 G to K) and is the spike response of the plaque. It remains stable for 3 or more hours, provided stimulation is not applied so frequently as to produce fatigue. This latter phenomenon is marked but has not been studied in detail.

The initial, graded response lasts approximately 3 msec. as recorded with external electrodes and at threshold for the spike it may be as high as 30 per cent of the latter. The spike lasts about 2 msec. In multi-unit preparations its

end is often distorted by later asynchronous responses, but when these are absent a positive phase is often observed which lasts about 15 msec. (Fig. 3). The amplitude of this positive phase is increased by summation during repetitive activity. Further details on the characteristics and nature of these components will be presented in a subsequent paper, describing the potentials recorded with intracellular electrodes.

II. The Response to 2 Stimuli Applied to the Same Nerve.—Whether in perfused slice preparations or in the layer preparations, a single stimulus to the nerve does not produce the maximal discharge of which the preparation is capable. This can be ascertained by the fact that a second stimulus to the nerve produces an increased discharge. This facilitation long outlasts the first stimulus and the electrical response associated with the latter (Figs. 4 to 9).

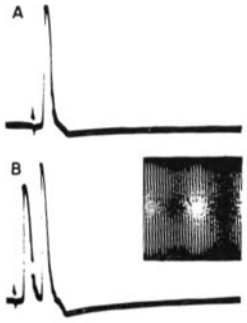

FIG. 3. Summation of the after-positivity observed in the neurally excited electroplaques. Inset is time and amplitude calibration in milliseconds and 30 mv.: 2 layers of cells, covered with Ringer's solution.

Fig. 4 illustrates facilitation in an experiment in which the intensities of the paired stimuli were independently varied, and the pairs were delivered at a constant time separation. Each column represents results with a constant testing stimulus and from above down the rows represent increasing conditioning stimulation. When the testing stimulus in isolation is just sufficient (column A) to produce a small initial response, the conditioning stimulus facilitates this, but does not elicit a spike. This is true of any strength of the conditioning stimulus. Both neural stimuli must produce the initial response for facilitation to occur.

When the test stimulus is increased slightly, but not to the level of a spike discharge (column B), the facilitation produced by the conditioning stimulus now may result in a spike (third and fourth rows, column B). When the testing stimulus is increased further, the amplitude of the facilitated response will depend on the strength of both stimuli, but primarily on that of the second (columns C and D). The experiment illustrated in Fig. 4 represents a prepara-

tion with only a few cells excited by the nerve volley, and therefore shows a sharp transition in the response height from very small to nearly maximal.

Gradation in the Amplitude of the Facilitated Response.—The stepwise gradation of the facilitated response is illustrated more clearly in Fig. 5 which shows the height of the testing response as a function of interval between two stimuli of constant amplitude. Even in this figure, however, which also represents an

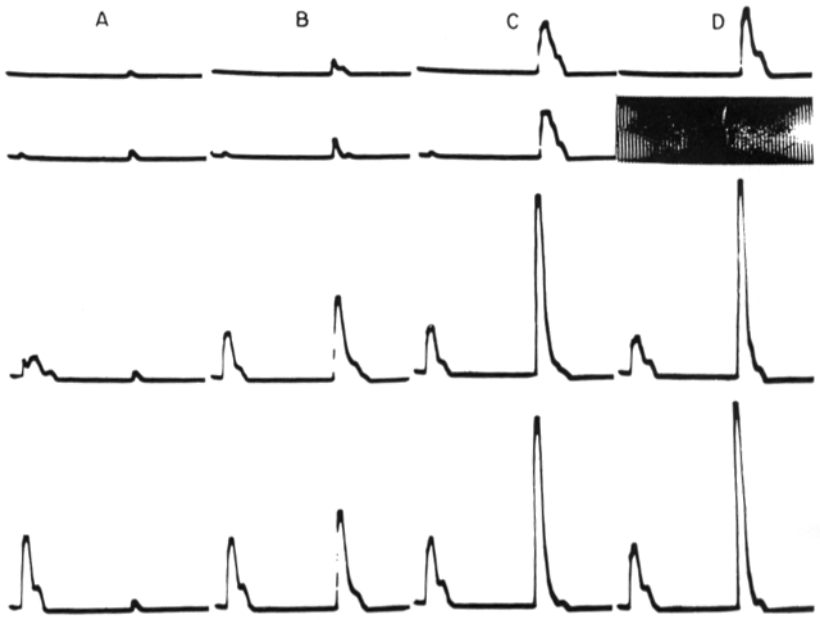

FIG. 4. Facilitation of one neurally evoked response of the electroplaques by a previous stimulus to the same nerve. The second, testing volley is constant for each column, increasing from A to D. The conditioning volley is constant for each row and increases from above down. Interval between stimuli, 28 msec. Time and amplitude calibration, 1 msec. and 30 mv.: 2 layers of cells.

experiment with only a few active units, slight gradations in the heights of the responses are seen. These gradations are usually well marked at the transition from one step to another. In most of our experiments, however, the units formed a complex series-parallel network in which up to 10 cells were discharging and others were producing prepotentials. In such a case it would be expected that the heights of the summated facilitated initial and spike responses should vary in a complex manner, and not necessarily exhibit stepwise changes. This condition is represented in most of our data and is shown in Figs. 6 and 7.

The Time Course of Facilitation.—Over 100 experiments have been performed to study the time course of the facilitation under a variety of condi-

tions. Fig. 6 represents the course of facilitation in the standard Ringer's solution at 22°C., when the conditioning and test stimuli are maintained constant (the former being maximal), with the interval between them varied. When the test stimulus is also maximal and follows the conditioning stimulus within 2 msec. the second response is smaller than the test response in isolation, or it may be absent. This is ascribable to refractoriness of the nerve.

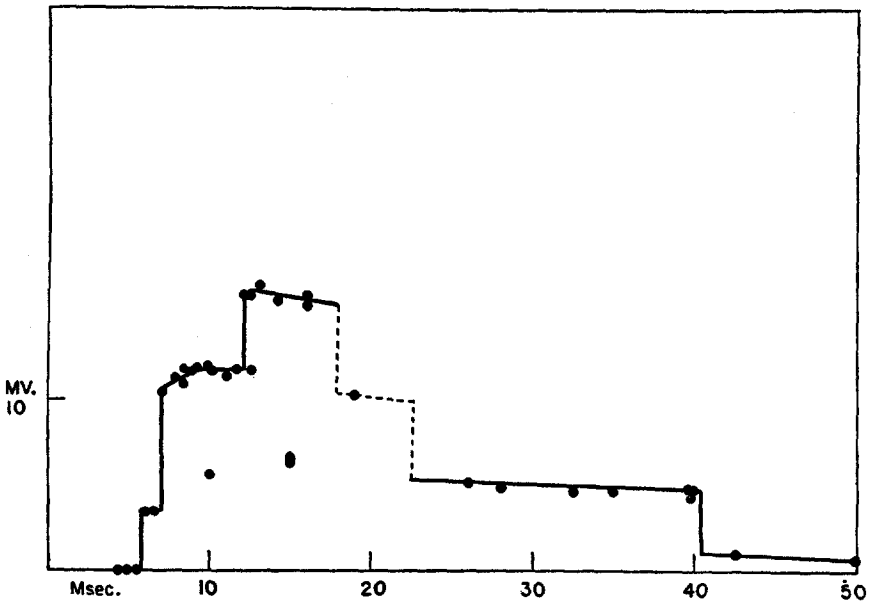

FIG. 5. Stepwise gradation of the facilitated response in a one layer preparation with only a few active cells. The conditioning and testing stimuli to the nerve were maximal. The height of the second response (in millivolts) is plotted as a function of the time interval (in milliseconds) between the two stimuli. The three deviant points represent failure of one and two cells to respond to the second stimulus. In this preparation a single stimulus to the nerve produced only prepotentials, as in the case of nerve I in Fig. 9.

The details of the early course of facilitation are better seen in Fig. 7. At longer intervals, the second response becomes larger than its test height, growing to maximum at 7 to 15 msec. and declining more slowly up to a stimulus interval of 100 msec. A remnant of facilitation declines still more slowly for an additional 700 msec. or more.

When the testing stimulus is submaximal, the early course of facilitation is delayed, as is seen in Fig. 7. This delay is probably due to the relative refractoriness of some of the nerve fibers which had been maximally excited by the conditioning shock.

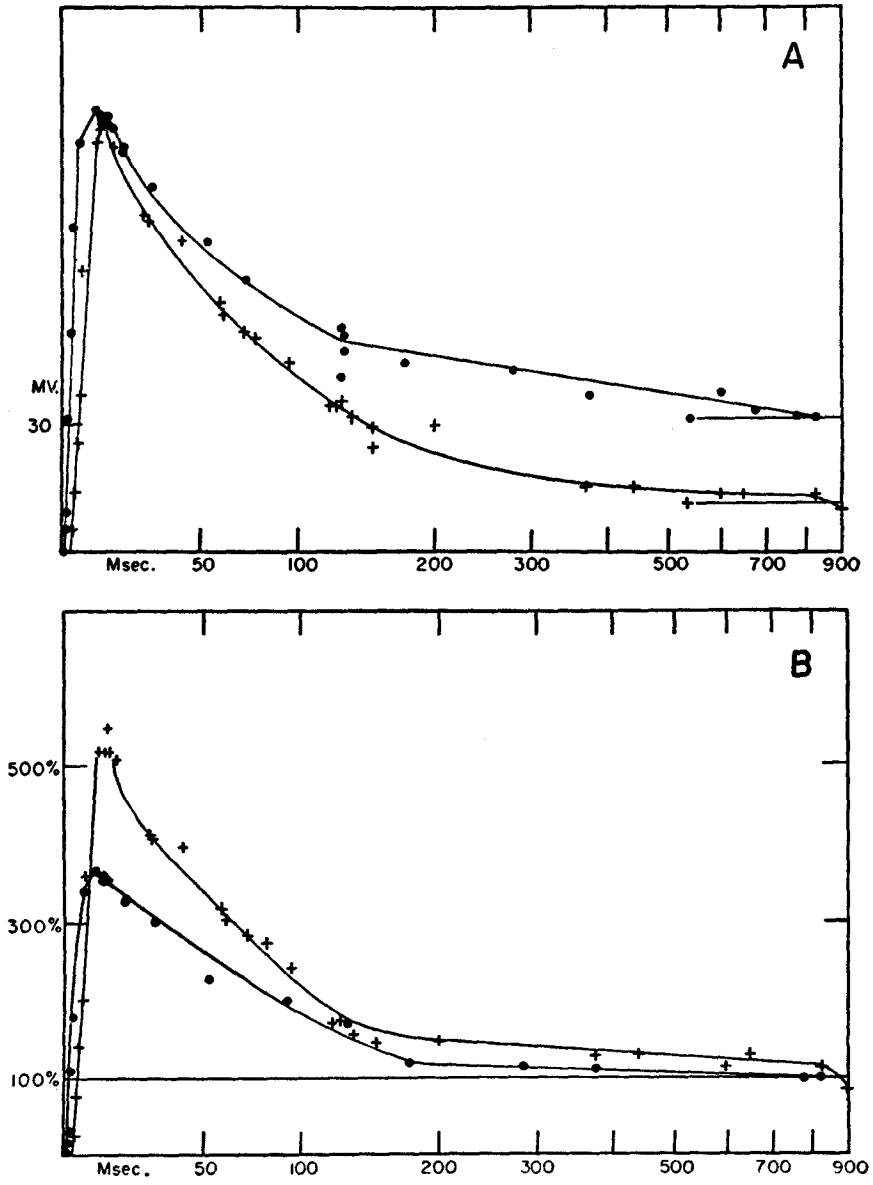

FIG. 6. The time course of facilitation of the response of the electroplaques on paired stimulation of one nerve, three layered preparation. The first volley was maximal. A, solid circles, the time course of facilitation when the test volley is maximal, and plus signs, when the test volley is submaximal. These curves show the recorded heights of the facilitated response in millivolts. The amplitudes of the test responses in isolation are also indicated in the figures as the horizontal lines under the curves. B, the time course of facilitation in terms of the percentage of the second response as compared with the testing response in isolation.

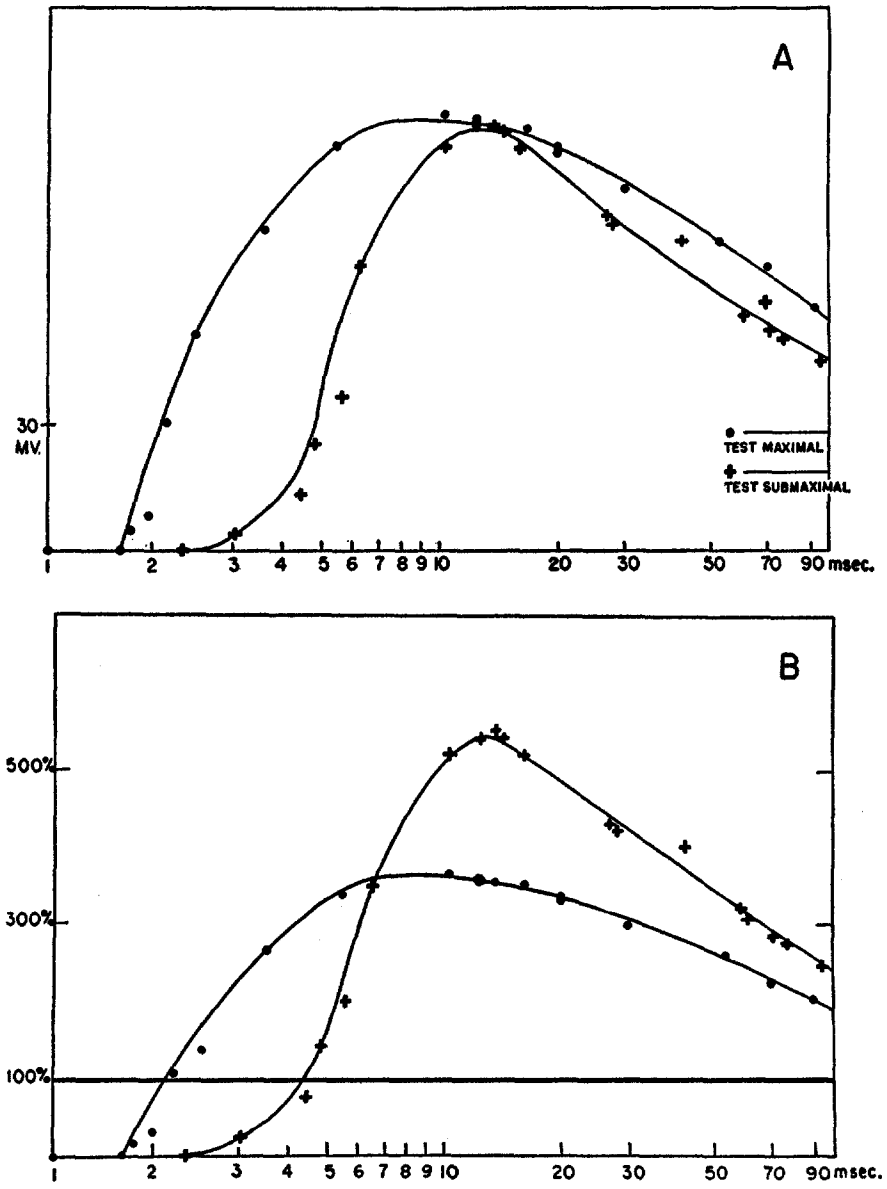

FIG. 7. The early time course of facilitation as a function of the size of the second neural volley. Same preparation as Fig. 6. A, solid circles, both stimuli maximal. Plus signs, the second stimulus submaximal. Heights of the testing responses in isolation are shown by horizontal lines under the curves. B, the facilitation in terms of percentage of the test response.

Except for weak stimuli, near threshold for the prepotential, when the period of facilitation is shorter (Figs. 5 and 8), the later time course is not significantly affected by varying the level of the testing response, as seen in Fig. 6 A, in which the absolute levels of the observed responses are shown. Fig. 6 B, in which the amount of facilitation is plotted as the percentage of the facilitated response with respect to the test response in isolation, shows the

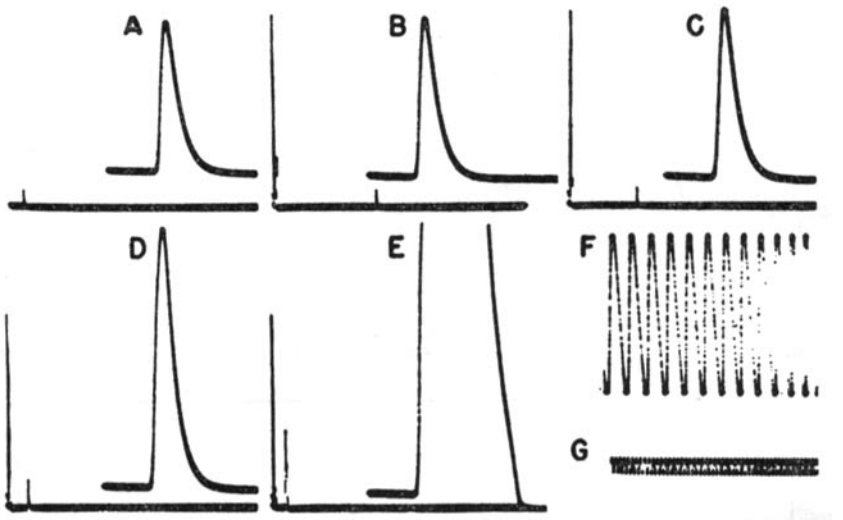

FIG. 8. The long lasting facilitation of the prepotential. The two beams of the oscillograph were operated at different sweep speeds and amplifications, as seen in the time and amplitude calibrations (milliseconds and 2 mv. in F, for the upper right hand trace; and 10 msec. at 2 mv. in G for the lower trace). The upper trace was initiated by the second testing stimulus (2 layers of cells). A, the prepotential in response to the weak testing neural volley in isolation. B, C, and D, the prepotential increased by facilitation from the maximal conditioning volley, the facilitation increasing as the interval between the stimuli decreased (intervals 250, 160, and 50 msec.). When the paired stimuli had a smaller separation (35 msec., record E) a spike resulted.

identity of the two curves more clearly. This figure also demonstrates the different rates of decay of the two components of facilitation as described above.

The origin of these different rates in the decay of facilitation is due to the involvement of two systems in the effect, those producing the prefatory graded response and the spike. Experiments illustrated in Fig. 8 demonstrate that the graded response is facilitated for a long time. However, only during the early part of the facilitation period is this graded response large enough to produce a spike, and this phenomenon, therefore, gives rise to the appearance of two distinct phases in the facilitation curve.

III. Activation of Individual Cells. The Discharged and Excited Zones of the Innervation.—Further details of the facilitatory process were studied at the unitary level in experiments in which the responses of individual cells in a single layered row were recorded under mineral oil. Fig. 9 is from an experiment in which 3 different nerves to the same preparation were stimulated individually with paired maximal stimuli. The potential difference across any two neighboring cells was recorded with a pair of roving electrodes (lead C of Fig. 9 D). One of the roving electrodes was also paired with an electrode at the left margin (lead A); and with another electrode at the right margin (lead B). The diagram of Fig. 9 D shows the cells involved, and the records of Fig. 9 A, B, and C, represent the responses obtained on stimulating nerve III, recorded with the different electrode combinations as the common roving electrode moved successively from cell 7 to 16. The innervated faces of the cells were proximal to the right margin of the preparation. When the common roving electrode was on cell 7 the response on lead B was maximal with the roving electrode positive to the fixed. As the roving electrode moved from cell 7 to cell 10 only small changes were observed in the height either of the first or of the second (facilitated) response. On passing to cell 11, though the first response is not changed, there occurs a considerable decrease in the facilitated response. This may be interpreted as activity of cell 10 during facilitation. In keeping with this interpretation is the simultaneous reversal of the second response on lead A and the previous appearance of a second response on lead C, in which again positivity on the common roving electrode is denoted by a downward deflection. The first response of B remains unchanged on moving to cell 12, but the second is further decreased. Cell 11, therefore, is also discharged during the facilitated second response. In C, however, the first stimulus produces a large response and in A there is a reversal, though small. Therefore, cell 12 was discharged by the first stimulus, as well as by the second. Similar analysis shows that cell 13 is also discharged by the first neural volley, while cells 14 and 15 respond by facilitation to the second neural volley. By means of the same procedure the effects of stimulating nerves I and II were also determined and the results are shown in Fig. 9 D. A single volley on nerve I in this experiment did not cause discharge of any of the cells. This result has been observed in other preparations (*cf.* Fig. 5), but it cannot be stated whether this is a normal occurrence in the bundle of Sachs or whether the preparation had been somewhat injured either at the nerve fibers or at the plaques. In other preparations it has been observed that one cell can be discharged by a single volley from either of two adjacent nerves.

The potentials recorded in Fig. 9 are determined by the E.M.F.'s of the generators of the spikes and of the prepotentials. They will be affected by electrical circuit conditions such as variation in electrode contact with the tissue, and the shunting. Both of the latter will alter the circuit resistance and the

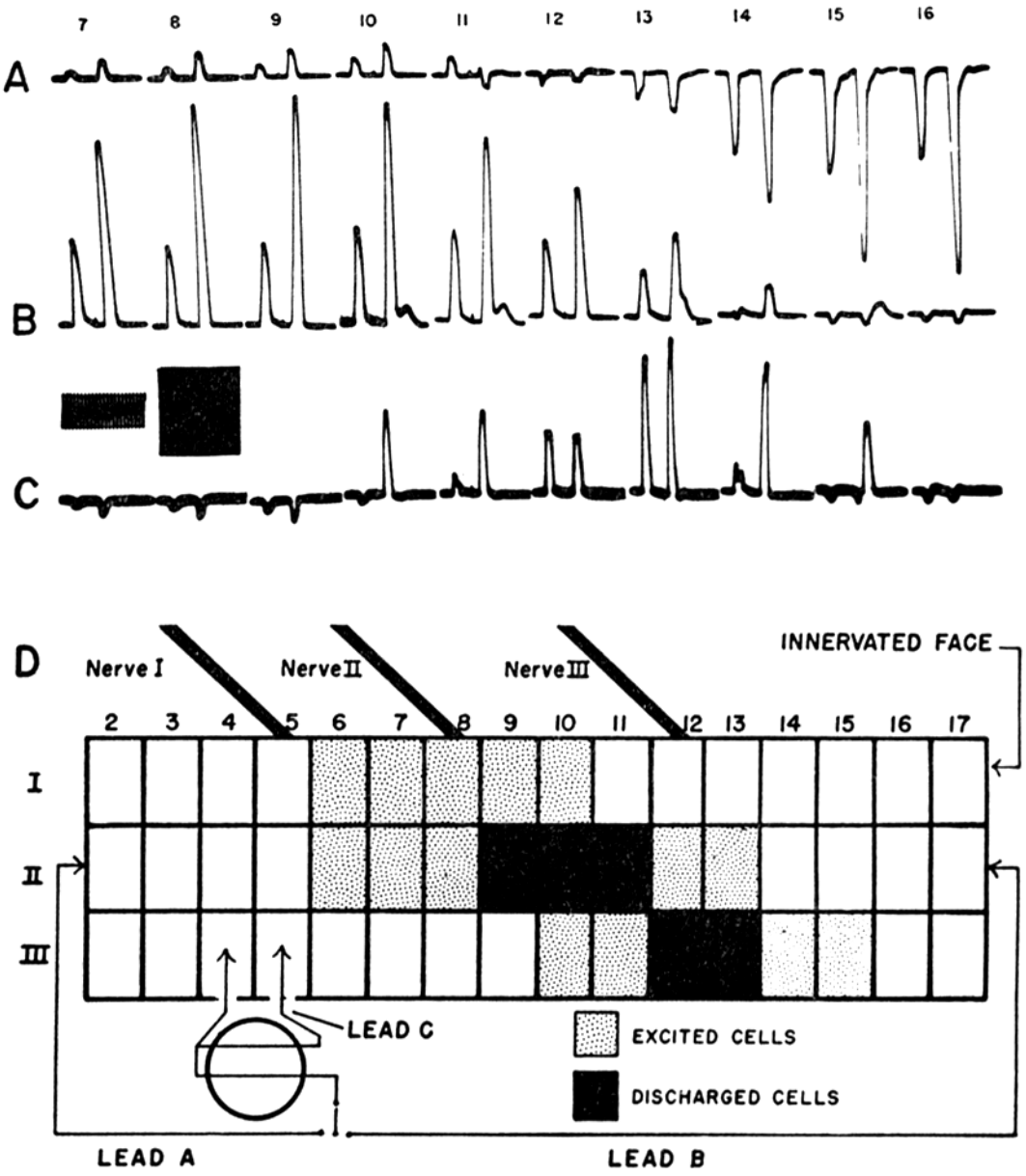

FIG. 9. Identification of cells discharged or excited by a single volley to each of 3 nerves (1 layer of cells under mineral oil). A, B, C, records for stimulation of nerve III, analyzed in text. D, diagram of the preparation containing 16 cells, indicating the leads for the records and the analysis of the effects of all 3 nerves. Time in milliseconds and amplitude of 30 mv., on the left for A and B, and on the right for C.

amplitudes of the recorded potentials. Hence, the records show some changes in amplitudes which reflect these variations, but are not significant for the analysis. An additional point which needs mention is the reversal in the first response at 11 C of Fig. 9. This represents the recording of the prepotential of cell 11 by the two roving electrodes, but cannot be observed by electrode combination A until the roving electrode is on cell 12.

The evidence presented in Fig. 9 demonstrates that cells are innervated by nerve fibers deriving from different trunks and that these different in-

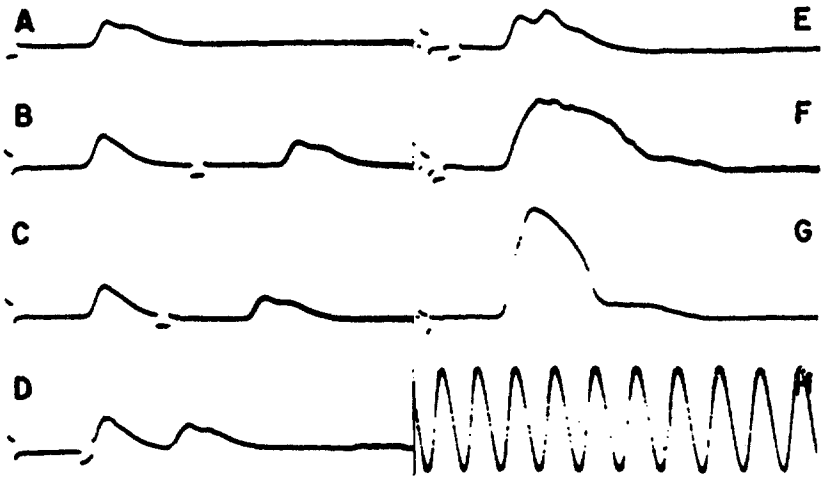

FIG. 10. Conditioning of the response to a threshold stimulus to one nerve by threshold stimulation of an adjacent nerve (2 layers of cells). A, test response in isolation. B–G, when this response is preceded by another prepotential caused by a different nerve there is no conditioning until the two volleys are less than 1 msec. apart (B to E). When the two volleys are almost simultaneous (G) a spike may result. Calibration in milliseconds and 10 mv.

nervating pathways have different effectiveness for the various cells. The records of Fig. 9 also demonstrate that activity of a cell is associated with negativity at the outside of its innervated face, and that the potential of the non-innervated side does not change. The possibility of this type of response was suggested by Bernstein (5), and was demonstrated with microelectrode recording by Keynes and Martins-Ferreira (15), and independently in this laboratory, with the same technique.

IV. Stimulation of 2 Adjacent Nerves in the Organ of Sachs.—When two adjacent nerves are stimulated in rapid succession with shocks which produce only the early graded response, the facilitation of the test (second) response by the activity consequent to the first stimulus lasts no more than 2 msec. (Fig. 10), in contrast to the prolonged facilitation produced by two stimuli to one

nerve. As described in a preceding section, if the facilitated graded response is large enough a spike may be produced (Fig. 10 G).

When the stimuli to both nerves are maximal, the effect on the second response takes the form shown in Fig. 11. At intervals less than 15 msec. (in this experiment 7 msec.) the second response is diminished, the greatest decrease

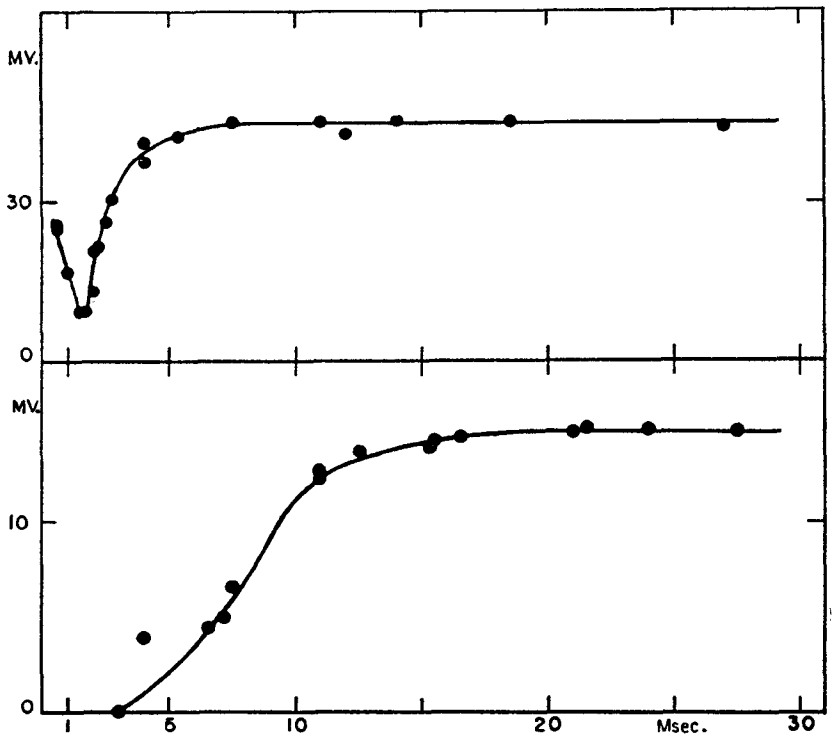

FIG. 11

FIG. 12

FIG. 11. Conditioning of the response to a maximal stimulus to one nerve by a preceding maximal stimulus to an adjacent nerve. Preparation consisting of one layer of cells.

FIG. 12. Recovery of excitability in the directly stimulated plaque, denervated for 67 days.

occurring at about 1.5 msec. For still shorter intervals the response rises somewhat, but when the two stimuli are simultaneous the total response is smaller than the sum of the responses to each volley, probably because of occlusion. The results are the same when the conditioning and testing nerves are interchanged.

The data presented in Figs. 10 and 11 describe the two extremes of possible experimental conditions with the stimuli to both nerves threshold or maximal. A variety of intermediate results are obtained when one or both of the two stimuli have intermediate values, but they will not be discussed here.

V. Responses of Electroplaques to Direct Stimulation.—Since there had been some question regarding direct excitability of denervated electroplaques (4, 12), we undertook a series of experiments on this question, independently of the work since published by Martins-Ferreira and Couceiro (16), and Albe-

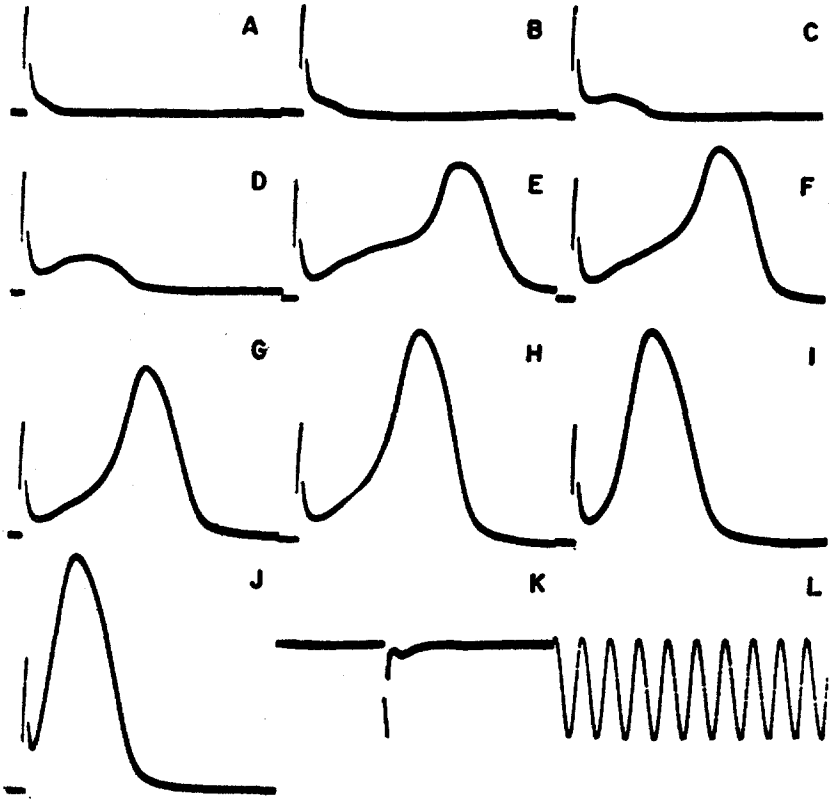

FIG. 13. Response of a single plaque denervated for 60 days to direct stimulation by increasing strengths of stimuli. At C, a prepotential appears and increases until the cell is discharged (E to J). With increasing intensities of the stimulus the spike arises earlier. Reversal of the direction of the strong stimulus used in J produces no activity in the cell (K). Calibration in milliseconds and 30 mv.

Fessard, Chagas, and Martins-Ferreira (2). Our results confirm the findings of these workers that the plaques can be stimulated directly in preparations denervated 7 weeks or more by careful and complete destruction of a segment of the spinal cord 10 to 25 cm. long. At the time they were examined the electroplaques no longer responded to stimulation of the nerves.

When individual cells are directly stimulated the response to stimuli of increasing intensity (Fig. 13) is comprised of a graded component which may be

analogous to the local response of cells or axons, and of a later spike. These components have durations similar to those of the components of neurally evoked activity.

The absolutely refractory period of directly stimulated single units is about 2 msec. and their relatively refractory period is about 15 msec. (Fig. 12). As stated earlier, it is the course of refractoriness of the cells which explains satisfactorily the effects of stimulating the electroplaques with maximal volleys on two different nerves (Fig. 11).

Noteworthy is the difference between the responses to paired direct stimulation of the electroplaques and to paired stimulation of one nerve. In the former case the large long lasting facilitation obtained on neural stimulation is not observed. Repetitive, direct stimulation, slightly subthreshold for the spike response, at a rate of at least 5/sec., after a few seconds causes the appearance of a spike. A small amount of residual excitatory state, therefore, persists for as long as 200 msec. after direct stimulation, but this facilitatory phenomenon which occurs in denervated as well as innervated cells differs markedly from the large facilitation caused by neural volleys. Another difference between neural and direct stimulation is the rapid fatigability of the neurally evoked responses, as observed by Albe-Fessard *et al.* (2), and confirmed by us.

DISCUSSION

1. We have confirmed the finding by Keynes and Martins-Ferreira (15) that the individual cell manifests its discharge by reversal of the membrane potential only at the innervated caudal face, as suggested by Bernstein (5). At the present time we can only speculate on the differences in the behavior of different regions of the membrane. Our experiments and others have shown that the denervated plaque can be electrically excited. Albe-Fessard *et al.* (3) demonstrated, and we have confirmed this, that stimulation of the plaque by direct currents is possible only when the direction of the current flow is toward the tail. Depolarization and consequent excitation, such as produced by this direction of current flow, therefore, takes place only at the innervated face, while depolarization of the non-innervated face, which would take place with current flowing in the opposite direction, either does not occur or does not excite. The difference between the two membranes may be inherent in their physicochemical properties or the inexcitability of the non-innervated face may be a consequence of the different anatomical structures.

Whatever the nature of this type of discharge its utility for the special adaptation of the electric organ is obvious. If both faces of the cell were altered, summation of voltages would not be possible. The reversal of the membrane on only one face of the cell permits the formation of a series circuit of the lengthwise array of electroplaques and consequently the high voltage discharge.

2. Two components of electrical activity are observed in the plaque on stimu-

lation of the nerve. The first, graded, smaller responses cannot be the pick-up of nerve impulses arriving in the terminations at the innervated faces of the electroplaques. As has been described above, this component is greatly facilitated by antecedent activity even when the nerve volley is maximal. Furthermore, it may be mentioned here that intracellular recording produces reversal of this potential as well as of the spike discharge of the cell. With intracellular recording the early potential is also larger.

In the records of Fig. 2 it is seen that for stimulation *via* the nerve the graded potential must be present before the spike discharge can occur. The former must also reach a threshold height before the latter can occur. The graded response, therefore, appears to have the characteristic of a prepotential or of a synaptic potential. Furthermore, on direct stimulation of the denervated electroplaque the spike is also preceded by a graded response (Fig. 13). The plaque, therefore, has the characteristics of an effector cell, exhibiting a prepotential and a spike. The first appears to be a partial response of a local region of the excitable membrane, the second being the all-or-nothing response of the cell. The duration of the local response is of the same order as is the duration of the spike, and the local response is, therefore, similar to the local response of nerve or to the synaptic potential in squid (6), and not to the longer lasting synaptic potential of the muscle endplate (10), or of vertebrate neurons (7).

3. The presence of an obligatorily intermediate graded response, which can be evoked by the impulses of a number of nerve fibers must result in complexities in the discharge of individual electroplaques upon nerve stimulation. This can be seen from the experiments of Fig. 9. One innervating pathway can discharge a given plaque while another evokes only the prepotential. Furthermore, since each cell is innervated by many nerve fibers, and since graded stimuli to a given cell produce first an increase of the prepotential, these complexities are to be expected. Thus, it must follow that changes in the excitability of the elements involved in the prepotential will produce 2 effects. A stimulus *via* the nerve, may increase the prepotential, but not sufficiently to discharge the plaque, or the prepotential may become large enough to discharge the cell. Therefore, the curves of the time course of facilitation should have two components. In the early part of its facilitation the prepotential will become large enough to produce discharge. The curves of Figs. 5, 6, and 7 show that this is the case. In any one preparation, the size of the prepotential necessary to cause discharge will be essentially constant. Therefore, changes in the strength of the stimulus will change the number of responding elements, but not the duration of the first phase.

The length of the facilitation period (*ca.* 900 msec.) is noteworthy in the light of the fact that no electrical concomitant has been observed. In the present stage of our work it need only be pointed out that large, long lasting facilitation is absent in directly stimulated plaques and is therefore apparently

associated with processes at the junction. Another important, but as yet unanalyzed difference is that the neural excitation of the plaques is subject to a high degree of fatigability.

The long period of facilitation discussed in the foregoing is always manifested on paired stimulation of a given nerve. It is analogous to facilitation produced by temporal summation.

4. A different type of result is obtained when each stimulus is applied to a different, adjacent nerve. When both nerve volleys individually cause only a prepotential, a short period of facilitation of the second response is observed. This lasts no more than 2 msec., as opposed to the very long facilitation period described above. The short facilitation appears to be analogous to spatial summation. The difference may be explained by assuming that the excitatory effects produced at the terminations of one nerve have only attenuated effects on the terminals of a neighboring nerve. This situation appears to hold only, however, for the cells of the organ of Sachs. Experiments in progress on preparations of the Large Organ show that in these units the excitatory effects from stimulation of different nerves can produce facilitation which is long lasting. Its duration, however, is not as long as that of the facilitation produced by paired stimulation of one nerve.

When maximal stimuli are applied to two adjacent nerves supplying cells of the organ of Sachs the response to the second stimulus is never facilitated, but is decreased for about 7 to 15 msec. The time course shown in Fig. 11 first exhibits progressive decrease of the testing response. The response reaches its minimum at about 2 msec. and then increases again. During the briefer time intervals, facilitation of the prepotentials of cells undischarged by the first volley can cause their discharge. But, as demonstrated with threshold stimulation, this facilitatory period lasts only about 2 msec. Subsequently, the curve exhibits the return of the ability of previously discharged cells to be again excited as they recover from refractoriness.

5. This view is confirmed by experiments on direct stimulation of the plaques. The cells have a relatively refractory period of about 15 msec. (Fig. 12).

SUMMARY

1. A preparation is described consisting of one or several layers of innervated cells of the electric organ of *Electrophorus electricus*.

2. Each plaque is multiply innervated and only at its caudal face. The nerve fibers may derive from two or more different nerve trunks.

3. During activity the innervated face becomes negative relative to the non-innervated.

4. The first electrical response of the cell to an increasing neural volley is graded and has the character of a prepotential. At a critical size of the prepotential the cell discharges with an all-or-nothing spike.

5. Both responses have durations of about 2 msec.

6. A neural volley which does not cause the spike discharge facilitates the discharge of the cell by a second subsequent volley in the same nerve (temporal facilitation).

7. The period of facilitation lasts *ca.* 900 msec. During the first 100 msec., the facilitation is large enough to cause a spike. In the later portion only the prepotential is facilitated. No electrical concomitant has been detected.

8. Neural volleys reaching the plaque from different trunks interact at the cell to produce a period of facilitation lasting only about 2 msec. This interaction is interpreted as spatial summation.

9. In a population of cells, simultaneous stimulation of 2 nerves causes a smaller discharge than the sum of the two isolated responses (occlusion).

10. Cells denervated for 7 weeks or more can be excited directly, but only by a current flow outward through the caudal face.

11. Weak direct stimulation causes a prepotential in the denervated plaque. On increasing the stimulus the prepotential increases to a critical size when a spike develops. The duration of both responses is about 2 msec.

12. The absolutely refractory period of the denervated cell is about 1.5 msec. and relative refractoriness lasts about 15 msec.

13. Direct stimulation causes slight facilitation lasting as long as 200 msec.

14. Repetitive stimulation of the nerve at low frequencies (2 to 3 per second) causes rapid "fatigue" of transmission. The denervated plaque, however, responds for several minutes to repetitive direct stimulation at high frequencies (25 per second).

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