The Electrophysiology of Electric Organs of Marine Electric Fishes

I. *Properties of electroplaques of Torpedo nobiliana*

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ABSTRACT Single electroplaques of *Torpedo nobiliana* have been studied with microelectrode recording. Direct evidence is presented that the only electrogenically reactive membrane of the cells is on the innervated surface and that this membrane is electrically inexcitable. Responses are not evoked by depolarizing currents applied to this membrane, but only by stimulating the innervating nerve fibers. The responses arise after a latency of 1 to 3 msec. This latency is not affected by large depolarizing or hyperpolarizing changes in membrane potential. Various properties that have been theoretically associated with electrically inexcitable responses have been also demonstrated to occur in the electroplaques. The neurally evoked response is not propagated actively in the membrane and may have different amplitudes and forms in closely adjacent regions. The maximal responses frequently are slightly larger than the recorded resting potential but the apparent small overshoot may be due to difficulty in recording the full resting potential. The responses are subject to electrochemical gradation and appear inverted in sign on applying strong outward currents across the innervated membrane. This membrane is cholinoceptive and shows marked desensitization. The membrane of the uninnervated surface has a very low resistance, a factor that aids maximum output of current during the discharge of the electric organ.

INTRODUCTION

Electric organ activity of *Torpedo* had been studied even before Galvani suggested the possible relevance of this material as a sample of electrogenic tissue (53). Du Bois-Reymond, stressing the importance of electric fishes in the study of electrophysiology, vigorously pursued comparative anatomical

and physiological work on all the then available forms of electric fishes (24, 25; complete bibliography in reference 11). It was he who first commented on a discrepancy between the properties of *Torpedo* and *Electrophorus* electric organs. When "fatigued" or curarized, the electric organ of the eel no longer responded to stimulation of its nerves, but could still respond to direct stimulation. This was not the case for *Torpedo*, the fatigued fish being unresponsive to electrical or mechanical stimulation. Much later, some of these observations were extended by Garten (50) who found that the curarized or denervated organ of *Raia* was unresponsive to electrical stimuli. Feldberg and Fessard (43), also confirming the above observations, showed further that the *Torpedo* organ responded to a chemical stimulus, the arterial injection of acetylcholine.

Fessard and his colleagues also confirmed and amplified Garten's finding that the elasmobranch organs discharged only after an appreciable latency in response to the strongest "direct" stimuli (*cf.* references 44, 45). An irreducible latency was also found in the "indirect" response of eel electric organs produced by passing a current toward the head. The direct response evoked by a current passing to the tail developed with virtually no latency when strong stimuli were used (6). Intracellular recordings from single eel electroplaques (7) then showed that the indirect response, like that which is evoked by stimulating the nerve fibers, was always prefaced by a postsynaptic potential (p.s.p.) which appeared only after a latency of some 1 to 3 msec. and was absent in the direct response even to strong stimuli. The latter is an electrically excitable overshooting spike. The spike could thus be produced by the depolarizing p.s.p. resulting from neural excitation or by depolarization with an applied current, while the p.s.p. could only be evoked by neural stimuli.

Fessard, on a number of occasions (44 to 46), had noted the different properties of the elasmobranch and gymnotid electroplaques. In the course of an extensive study of the electrical properties of *Torpedo* electric organs Albe-Fessard (2) observed that the sign of the response inverted when a strong current was passed outward through the ventral surfaces of the electroplaques. The inversion was ascribed to the reduced membrane resistance during the response.

The significance of the inversion as indicative of an electrically inexcitable response was not recognized, however, until later (53). Correlation of this finding with other data led to the conclusion that the recorded activity of *Torpedo* electroplaques arose solely from p.s.p.'s which are electrically inexcitable. If this conclusion were proved correct, then *Torpedo* electroplaques offered for the study of p.s.p.'s material uncomplicated by responses of electrically excitable membrane. Furthermore, the size of the cells, their multiple innervation from discretely localizable axons, and their geometry

also promised an opportunity for testing a number of deductions regarding the properties of the electrically inexcitable membrane.

Fessard and Tauc in *Torpedo marmorata* (47) and later Kao and Grundfest in *T. nobiliana* (53) had attempted to study electroplaques with intracellular recording, but succeeded only in obtaining resting potentials of 50 to 70 mv. However, in the latter experiments the material was a single large specimen which apparently had discharged to exhaustion during its capture. When brought to the laboratory its discharges to vigorous mechanical or electrical stimuli were less than 1 v. in amplitude. Most of the electroplaques of the organ therefore were inactive. The theoretical importance of the problem impelled another study, this time of the electric organs of the elasmobranch *Narcine brasiliensis* and the teleost *Astroscopus*. The preliminary data indicated that in both forms the electroplaques were electrically inexcitable and that their responses were p.s.p.'s (*cf.* references 53 and 71).

The present work represents a detailed study of the physiology and pharmacology of the *T. nobiliana* electroplaques. Subsequent papers describe similar studies on the electric organs of *Narcine* (17), particularly with reference to the properties of the accessory organ found in this form (71), and of *Astroscopus* (18). These studies confirm the conclusion that the electroplaques of marine electric fishes are electrically inexcitable. In several species of *Raia* studied in this laboratory (19, 57), however, the electroplaques exhibit marked delayed rectification, like that found in the frog slow muscle fibers (29).

The responses of marine electroplaques therefore must be p.s.p.'s, neurally evoked by release of some chemical transmitter agent (57). The pharmacological data which will also be presented confirm the earlier conclusion (43) that the systems are cholinergic. The occurrence of electrically inexcitable electroplaques in the only known teleost marine electric fish as well as in the elasmobranchs, while all fresh water electric fishes appear to have electrically excitable electroplaques, (*cf.* reference 57) points to the problem of the evolutionary relations of electric organs.

Anatomical Features

The following summarizes briefly earlier detailed studies on gross anatomy, histology, and innervation of *Torpedo* electric organs (*cf.* references 12 and 48). It also includes new data on the innervation of the electroplaques that are relevant to their mode of functioning.

The wafer-like electroplaques have large surfaces, about 5 to 7 mm. in diameter in *T. nobiliana* with a 1 m. diameter disc. They lie in the horizontal plane, stacked like a roll of coins one above the other in a column which occupies the full thickness of the fish. There are probably more than 1000

electroplaques in the thickest columns and between 500 and 1000 columns in each bilateral organ. An accessory organ, such as is found in *Narcine* (71) is absent in *T. nobiliana*. The electroplaques are thin, as they are in *N. brasiliensis* (7 to 20 μ; reference 71). Also like the electroplaques of *Narcine*, those of *Torpedo* lie spaced apart in histological and electron microscopic preparations (72). This is probably also true in living organs, according to electrophysiological evidence. During penetration of a column of electroplaques with a microelectrode there are considerable spaces in which no resting potential is recorded. At these sites, the microelectrode presumably lies between cells.

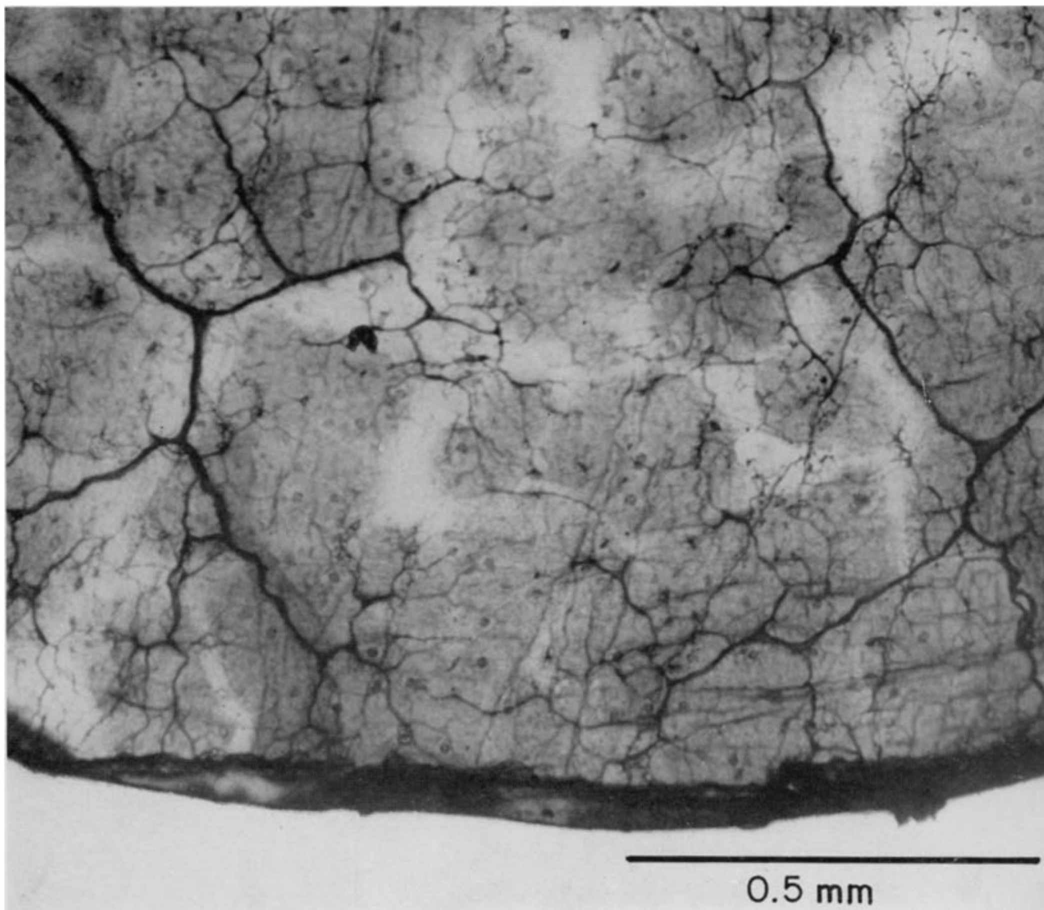

FIGURE 1. Innervation of *Torpedo* electroplaques. A segment is shown of a single electroplaque teased from formalin-fixed material and stained with methylene blue. Nerve fibers enter from the right and upper left and branch profusely over the cell. The areas innervated by the two fibers show little if any overlap, the line of demarcation passing approximately vertically across the middle of the figure.

The electroplaques are all innervated on their ventral surfaces by 5 to 7 axons which pass in trunks in the spaces between the columns. Each axon to a single electroplaque reaches the latter from a different point on its periphery. Each axon branches profusely, making numerous synaptic contacts which cover most of the surface of the electroplaque. Each nerve fiber tends to innervate a restricted area of the electroplaque (Fig. 1), overlap, if it exists, being small. The restricted zones of innervation have important consequences in the electrically inexcitable system, as will be described below.

METHODS

Four specimens of *T. nobiliana* were brought to the Marine Biological Laboratory in Woods Hole in each of the summers of 1958 and 1959. The largest of the fish were females and had discs about 1 m. in diameter. The smallest, a male specimen, was about half that size. Each of the fish lived for 3 to 4 weeks in captivity, although they had all refused food and were subjected several times to biopsy for removing parts of the electric organ.

Tissue was obtained after a fish had been anesthetized by pipetting tricaine (51) into its spiracles. Pieces of various sizes were removed. Bleeding was controlled by tying off the larger vessels in the wound. Excised tissue, kept refrigerated in elasmobranch Ringer's solution (27), was viable for over 24 hours. Small portions comprising 5 to 10 columns and about 1 cm. thick were used as needed.

These pieces were mounted in a paraffin block chamber, with either the uninnervated (dorsal) or the innervated (ventral) surface uppermost. As will be described below, the orientation is an important factor. Sometimes, the electroplaques used were those lying immediately below the skin. In other preparations, deeper tissue was used, and successive layers of electroplaques were removed under a dissecting microscope. Paired 125 μ silver wires, insulated except at their tips and applied close to the electroplaques, were used to stimulate the nerve fibers. In some experiments two pairs of electrodes were used. Because the axons innervate distinctly separate regions of the membrane (Fig. 1), separate stimulation of different nerve fibers was possible.

Glass microcapillary KCl–filled electrodes were used for recording from single electroplaques, either intracellularly or externally. In some experiments two or more microelectrodes were applied to the same cell with independent micromanipulators. Transistorized, neutralized capacity input amplifiers (10) were used for recording. Other experimental details have been described in previous publications (13, 15) or will be given below in connection with specific experiments.

RESULTS

A. *The Organ Discharge*

THE REFLEX DISCHARGE Rather vigorous prodding of the fish was necessary to evoke one or several trains of discharges. These occurred in

both organs (Fig. 2), at frequencies up to 100/sec. The amplitude varied in different fish and depended on the recording conditions, the largest responses attaining about 60 v., as measured between the dorsal surface in air and the ventral in a shallow tank. The pattern of the discharges differed somewhat

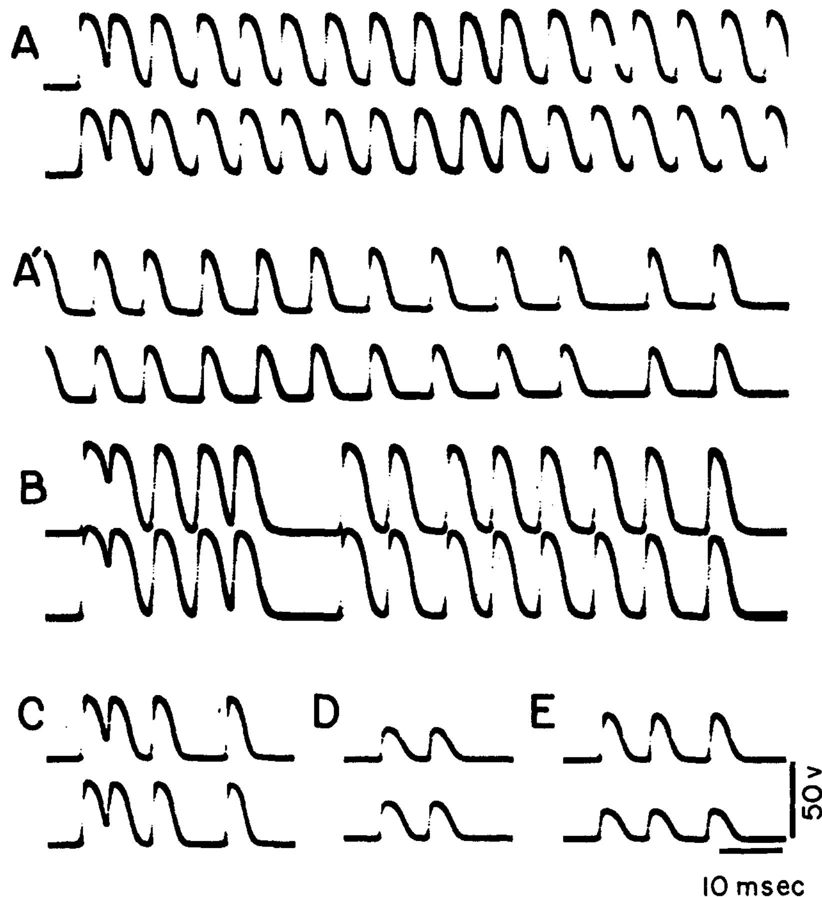

FIGURE 2. Reflex discharges in different fish, recorded simultaneously from both organs. The fish lay in a shallow tank with its dorsal surface out of the sea water. Recording was between probing electrodes on the dorsal skin above each organ and a ground electrode in the tank. Dorsal surface positivity up. The fish were prodded to produce the reflex discharges, which varied in frequency and duration in different fish. *A*, *A′*, beginning and end of one burst. Further description in text.

in different animals and in successive trains of responses in the same fish. On occasion the trains numbered only a few pulses (*C–E*).

There was little difference in the discharges of the two organs. The amplitudes of successive responses were remarkably uniform, but with continued activity the amplitude and the frequency tended to decline (*A*, *A′*). Pauses,

most striking in *B*, but also present in *A'* and *C*, must have been caused centrally at the command nucleus (5), since they occurred in both organs. The duration of each pulse, about 5 msec., remained relatively constant, but sometimes decreased slightly during a long train. The rising phase was steeper than the falling phase. Frequently (*A*, *B*, *C*) the two initial discharges were fused.

In two fish the current density over the center of the organ was measured while the animal was submerged in the sea water tank, by differential recording between two electrodes, one at the surface of the organ and one a centimeter above. The peak voltages so recorded were 1 to 1.25 volts. Assuming the current flow to have been perpendicular to the surface and taking the conductivity of sea water as 25 ohm-cm.^{2}, the current density during the peaks of the discharges was 40 to 50 ma./cm^2. Up to 75 ma./cm.^{2} was obtained from measurements made with the animal in air, using large metal electrodes. Since in these experiments there was still an external resistance which developed an appreciable IR drop, somewhat larger currents could probably be obtained on short-circuiting the discharges. The maximum peak power observed was about 600 watts per organ.

The electric organs appear to be effective in taking prey. Captured specimens contained remains of squid and rapidly swimming fish and it seems most likely that these animals were killed by the *Torpedo* discharges rather than taken alive or found dead. On one occasion a number of *Lophius*, 30 to 40 cm. in length, were inadvertently placed in the aquarium with the largest *Torpedo* and were either killed or stunned by its discharges.

RESPONSE TO STIMULATING A NERVE The response of the organ to a brief (0.5 msec.) stimulus applied to one of the electric nerve trunks was usually similar in shape to the reflex discharge, but somewhat lower in amplitude (Fig. 3*A*, *B*). However, its duration was never longer than the reflex discharge and could be considerably shorter suggesting that the whole electric organ might not be activated synchronously during the reflex discharge. In some preparations (Fig. 3*C*) stimulation of a nerve resulted in a more complex potential, the initial, large, elevation being followed by smaller peaks on a sustained level of depolarization. This complexity probably resulted from stimulation of the electric nerves by the discharges of the organ, since nothing comparable was seen in the single electroplaques excited by local stimulation.

B. *Activity of Single Electroplaques*

RESTING AND ACTION POTENTIALS OF INDIVIDUAL CELLS Microelectrode recording was used in this series of experiments. In the two experiments shown

in Fig. 4, the uppermost electroplaque in a column was excited by stimuli through fine electrodes closely applied to the surface of the cell. The potentials were recorded with an exploring microelectrode against a distant electrode in the bath. The position of the microelectrode, which is indicated in the accompanying diagram, was inferred from the successive appearance

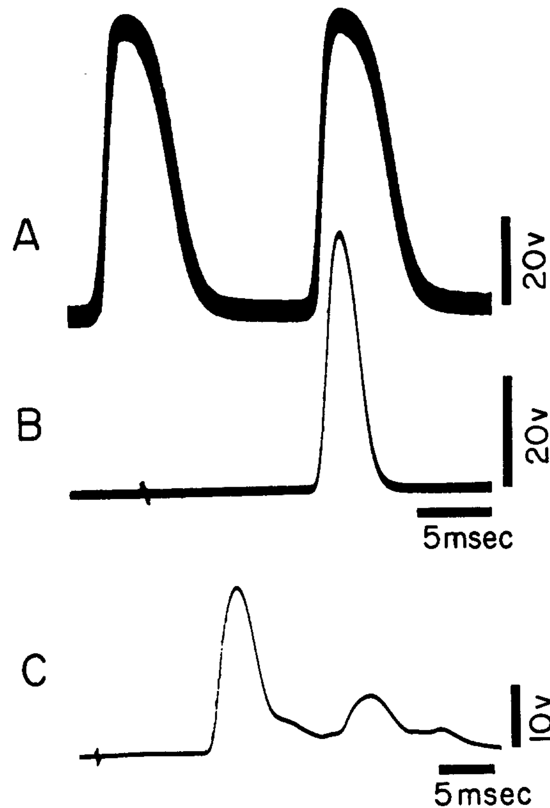

FIGURE 3. Comparison of reflex discharge and responses evoked by stimulating one of the electric nerves. Surface recording as in Fig. 2. *A*, part of a train of reflex discharges and *B*, a much briefer response evoked on stimulating a nerve in the same fish. *C*, another preparation. The response to stimulating a nerve had a number of elevations, which were probably due to restimulation of the nerve fibers by the discharges of the electric organ.

and disappearance of resting potentials as the electrode was advanced below the surface. The results differed depending upon the orientation of the electroplaque.

When the innervated (ventral) surfaces were uppermost (*A–H*), the electrode recorded a small negativity just above the active electroplaque. Advancing the electrode resulted in a resting potential of 50 to 70 mv., inside negative, and a positive-going response of up to 80 mv. which some-

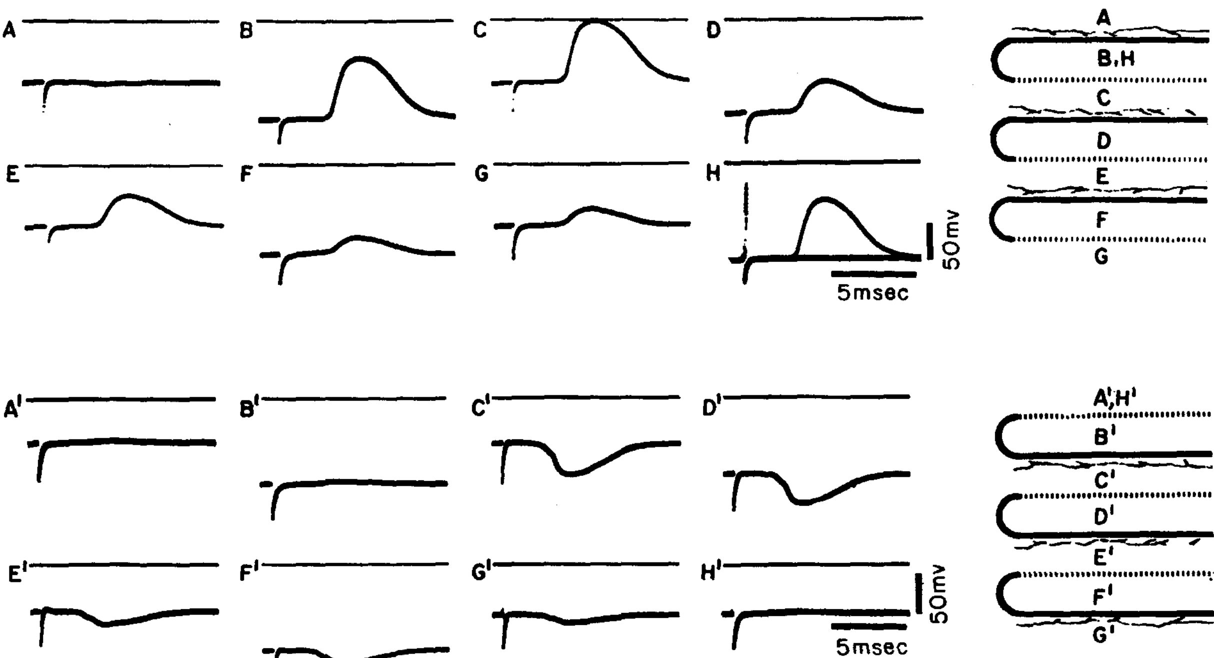

FIGURE 4. Potentials recorded from individual electroplaques during activity of the superficial cell. Two experiments. *A–H*, the preparation, a portion excised from the electric organ, was mounted with the ventral, innervated surfaces uppermost (diagram at right). *A'–H'*, another preparation mounted in its normal position, with the uninnervated, dorsal surfaces uppermost. The letters of the records correspond to the indicated recording sites as a microelectrode was pushed from the surface into the column of electroplaques. *H* represents return of the electrode to the interior of the uppermost cell, and *H'*, the return to the dorsal surface. *Upper trace* in each record is a reference line. *Lower trace* is the registration of the potential by the microelectrode. Zero potential is indicated by the distance of the potential trace from the reference line in *A* and *A'*. Further description in text.

times exceeded the resting potential by as much as 30 mv. These potentials indicated that the electrode had penetrated into an active cell. The response amplitude, in this case, was all-or-none, indicating activity of a single axon. No gradations in the potential were observed on varying the stimulus carefully between subthreshold and strong excitation (*H*). Although the fiber was stimulated on the surface of the electroplaque there was a latency of more than 3 msec. Latencies of this magnitude always occurred in the responses and could not be reduced appreciably by the strongest stimuli, or by other experimental factors, as will be described below.

When the electrode was advanced further, the resting potential was lost, indicating that the electrode had gone out of the cell, but the response remained practically at full magnitude (*C*). The persistence of the response at high amplitude indicates that the uninnervated surface of the electroplaque did not itself react to the stimulus. Furthermore, it shows also that this membrane had a very low resistance compared with that of the underlying tissue and bath, which together formed the remainder of the current path of the response. When the microelectrode was again advanced, there was return of a resting potential (*D*). The response, however, was approximately halved in amplitude. These two changes indicate that the electrode had penetrated another cell which was inactive and further, that the innervated membrane of that cell had a much higher resistance than the overlying uninnervated membrane. When the electrode was pushed deeper (*E*), the resting potential again disappeared, indicating passage of the electrode out of the cell, but the response amplitude did not change, showing that the resistance of the uninnervated membrane of this cell was also relatively low. When the electrode penetrated the next cell (*F*), denoted by the reappearance of the resting potential, the response again decreased. Again, there was no further change in the amplitude when the electrode was pushed out of the cell (*G*). The sequence of changes in the response height indicates that the resting resistance of the innervated membrane in each cell is considerably higher than is the resistance of the uninnervated surface. This difference in properties explains the responses observed in different recording situations, as shown in the remainder of this section.

When the dorsal, uninnervated surfaces of the electroplaques were uppermost in the experimental arrangement (*A′–H′*), a small positive response was recorded above the superficial cell. Penetration of the cell disclosed the resting potential, but the response did not increase (*B′*). The small size of the response in this recording situation is due to the low resistance of the unreactive, uninnervated membrane. On propelling the microelectrode deeper into the preparation the resting potential disappeared and a negative-going response was obtained (*C′*). This represents the activity recorded across the superficial electroplaque as in *C*, except that the potential was of opposite sign because

the cell was inverted. As the microelectrode was pushed further into the column (*D′–G′*) the sequence of potentials repeated in essence the sequence seen in *D–G*: penetrating the innervated high resistance membrane reduced the response, penetrating uninnervated low resistance membrane did not change the response. However, in this orientation, penetrating an innervated membrane was indicated by disappearance of resting potential and conversely penetrating the uninnervated membrane by its appearance. In the two experiments of Fig. 4 the forms and amplitudes of the responses were different. This matter will be dealt with below.

RESPONSES IN DIFFERENT ELECTROPLAQUES IN A COLUMN The foregoing data on recording conditions and events during activity of a single superficial cell also serve to analyze the responses evoked independently in two electroplaques of the same column. The two experiments shown in Fig. 5 also used the two orientations of the column. In the preparation of record *A–H*, two pairs of stimulating electrodes were applied to different regions of the exposed innervated surface of the superficial electroplaque. The second stimulus in each trace delivered through one pair of electrodes excited the superficial electroplaque. The sequence of changes recorded on penetration of the column was like those seen in Fig. 4*A–H*. The first stimulus, delivered through the other pair of electrodes, did not excite the superficial electroplaque since the response recorded intracellularly from that cell (*B*) and external to its uninnervated face (*C*) was negative. The response became positive on penetrating the next cell (*D*), indicating that the activity was produced at the innervated membrane of this cell. The sequence of changes in this response on further penetrations (*D–G*) paralleled the changes in the response of the superficial cell. The negative potential contributed to the superficial recording (*A*) by the deeper lying cell was much smaller than that caused by the uppermost cell. This difference is due to interposition of the high resistance, innervated face of the uppermost cell in the current path of the response of the lower.

The maximum response amplitude recorded monopolarly was different in the two cells. However, the total response amplitude is the difference of the potentials recorded from each side of the active membrane. The difference between the first responses in *C* and *D* is about the same as that between the second responses in *A* and *B*. Exceptionally in this experiment, the first stimulus excited the underlying cell at lower strength than it did the most superficial cell. A strong stimulus excited also the latter, and the responses of the two cells were of opposite sign in the space between them (*H*). Being somewhat asynchronous, they summed to produce a diphasic potential.

Series summation of potentials from electroplaques of the same column is illustrated in Fig. 5*A′–H′*. The dorsal surface was uppermost in this experi-

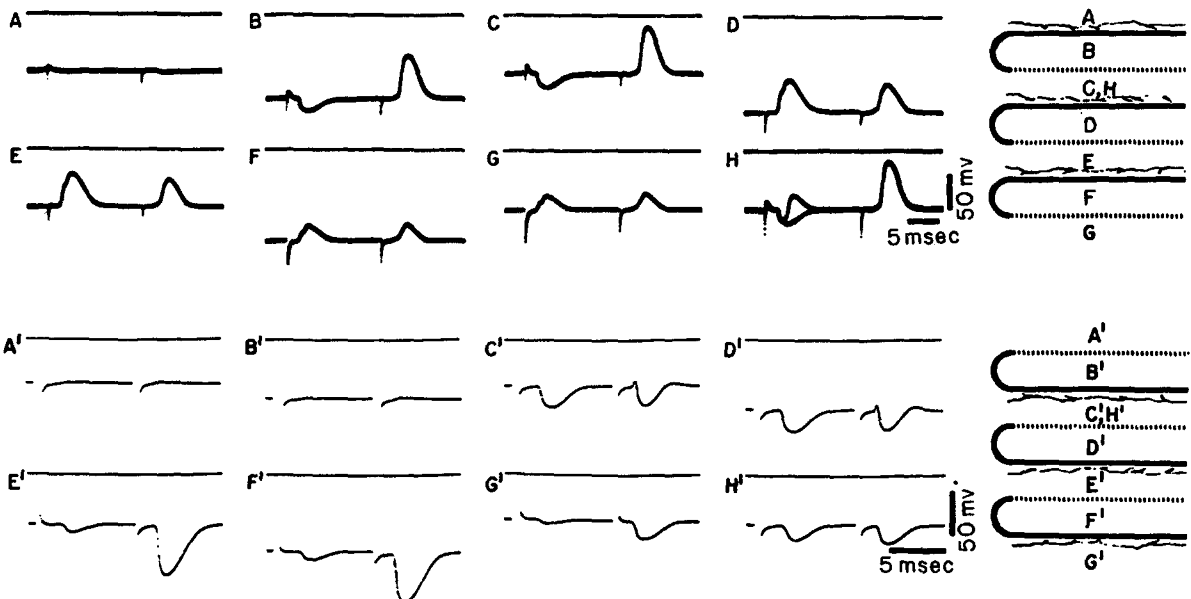

FIGURE 5. Potentials recorded when two electroplaques were active. Two experiments, with recording conditions the same as in the two of Fig. 4. A pair of stimuli were applied for each record. *A–H*, the stimuli were given through separate pairs of electrodes lying on the surface. The first stimulus excited only the second electroplaque, while the following stimulus excited the superficial electroplaque. In *H*, the first stimulus was made stronger during one of the two superimposed traces. Simultaneous activity of both electroplaques caused a diphasic potential, the difference between the two responses recorded at this site in opposite sign and out of phase. Further description in text. *A'–H'*, two stimuli were delivered through a single pair of electrodes, the second stimulus being stronger and exciting the second cell as well as the uppermost. In *H'* the second stimulus was made threshold for the first cell only, the absence of activity in the underlying cell being denoted by absence of the early positive deflection seen in *C'*, *D'*. The second response of the superficial cell was larger than the first because of facilitation. Further description in text.

ment. Two stimuli were applied through a single electrode pair. The first was weak and evoked activity only in the superficial electroplaque; the sequence of records is similar to that in Fig. 4*A′*–*H′*. The second stimulus was stronger and also caused activity in the next cell below. These responses were identified by their changes when the microelectrode was advanced into the column of cells. Inside the superficial cell, the microelectrode recorded only small positive responses for the reasons noted in connection with Fig. 4*B′*. When the microelectrode was between the innervated membranes of the two cells (*C′*, *D′*), the response to the second stimulus was distorted, showing an early brief and a late, longer lasting positivity. These positive phases were due to the activity of the second cell, as was disclosed when the microelectrode penetrated deeper (*E′*, *F′*). The second response now was purely negative and large. It decreased when it was recorded across the resistive barrier of another innervated, but inactive membrane (*G′*).

The potential recorded when both cells were active was not the algebraic sum of the potentials which would have been produced when the two were separately active, since the innervated membranes have different resistances at rest and during activity (*cf.* Figs. 6 and 7). Also, the response of the superficial electroplaque showed some facilitation after a previous conditioning stimulus (*H′; cf.* section D).

C. *Electrical Inexcitability of Torpedo Electroplaques*

EFFECTS OF CHANGING MEMBRANE POLARIZATION Neither depolarization nor hyperpolarization of the innervated face produced a response. In experiments like that of Fig. 6, responses of the superficial electroplaque were evoked as in Figs. 4 and 5 and were recorded with a microelectrode external to the innervated surface (E, in diagram). Polarizing currents were applied between another nearby microelectrode (I, in diagram) and a distant electrode. Polarization in this way rather than with electrodes across only the innervated face, was possible because of the relatively low resistance of the uninnervated membrane (*cf.* Fig. 4). Almost all the voltage drop of the applied polarizing current would have been developed across the innervated, higher resistance face. The advantages were that the electrode position was more stable between the thin electroplaques than in them, and that damage to innervation was less likely when the electroplaque was exposed from the dorsal surface.

Strong depolarizing stimuli, negative-going in the experimental situation (Fig. 6, lower left), did not evoke responses of the electroplaque, but the neurally evoked potentials were diminished. Hyperpolarizing the innervated surface increased the neurally evoked responses. The latency was not affected even by the largest changes in membrane potential.

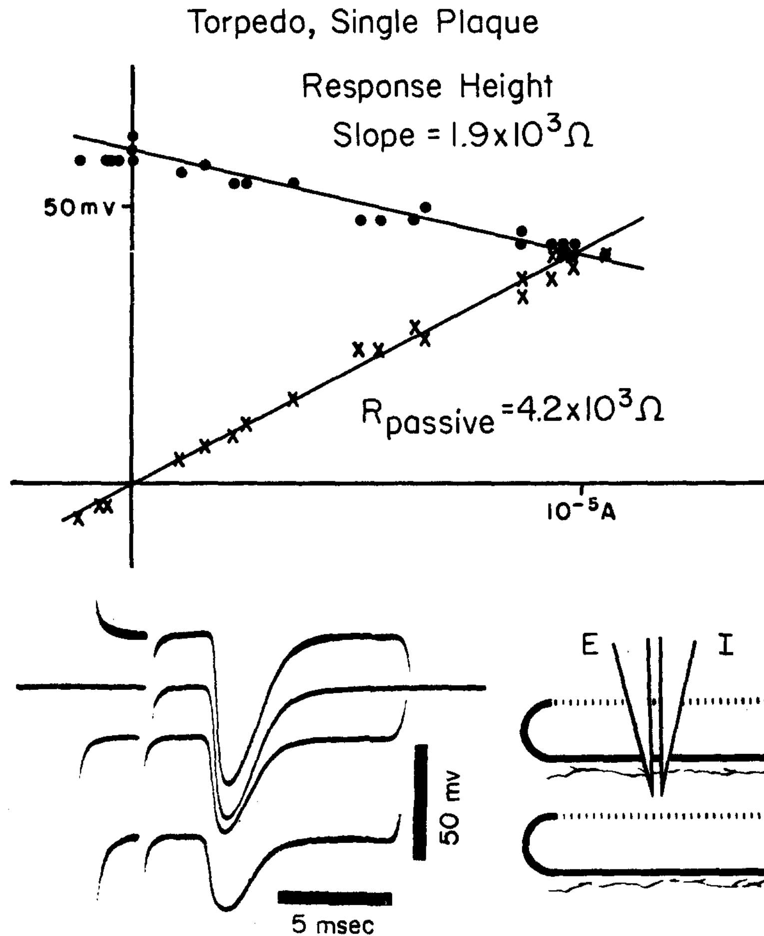

FIGURE 6. Effect of polarizing current on amplitude of responses of a single electroplaque. Arrangement of microelectrodes for recording (E) and for applying currents (I) shown in diagram. At left are sample records, but for a different experiment than that from which the graph was constructed. The base line trace shows the response (downward deflection) of the upper cell to a brief stimulus applied through a pair of electrodes on the surface (not shown in the diagram). The other traces show the responses during application of current pulses lasting about 15 msec. and marked by deviations of the traces from the base line. *Upward deflection* was produced by a current hyperpolarizing the innervated membrane (I electrode positive). The neurally evoked response increased in amplitude. The two downward deflections represent depolarization with two strengths of current (I electrode negative). The responses decreased in amplitude. Note that the latency of the responses was not changed. The crosses on the graph represent the changes in the base line potential (ordinates) with applied currents (abscissa), depolarizing current on the right of the origin. The E-I relation was linear for all values of current. The responses declined in amplitude with increasing depolarizing currents. Only small hyperpolarizing currents were applied in this experiment and did not affect the response amplitudes. Further description in text.

Further details of the properties of the excitable membrane are shown in the graph of Fig. 6 taken from another experiment, that with the maximum rate of change of response height with polarization. In other experiments, including ones in which larger hyperpolarizations were applied, the membrane potential changed linearly and with the same slope for currents applied in both directions, indicating that the active membrane does not rectify. The changes in the response amplitude produced by changes in membrane polarization indicate that the membrane conductance increased during activity.

Experiments of the type shown in Fig. 6 are subject to limitations by two factors. The amount of current which can be delivered through a microelectrode is too restricted to polarize the large electroplaques as much as desired. Also, the "space constant" (Fig. 8) is small compared with the cell diameter. Therefore the polarization is non-uniform over the surface, decrementing electrotonically away from the polarizing electrode. This leads to overestimation of the reversal potential for the postsynaptic potential which is generated at numerous sites distributed on the surface of the cell (*cf.* reference 29). In the experiment of Fig. 6, the reversal potential calculated by extrapolation was about 140 mv., a value much higher than is likely (*cf.* Discussion).

EFFECT OF POLARIZATION ON A COLUMN OF ELECTROPLAQUES To explore the current-voltage properties of the membrane more extensively, preparations of a single column of electroplaques were used (Fig. 7), to which current could be applied through large electrodes. A portion of a column, several centimeters in length, was dissected free of neighboring columns, blotted dry, and placed in an oil bath. The polarizing currents (I) were applied with large electrodes at the ends of the column as shown in the diagram. The potential across part of the column was recorded with another pair of electrodes (V). On the basis of the findings of Figs. 4 and 5, it may be assumed that little voltage drop occurred across the uninnervated face of the cells.

A brief stimulus to the column, maximal for the response, was immediately followed by a longer polarizing pulse. The response latency could not be reduced by increasing the strength of the stimulus nor did it change on application of a large range of polarizing currents in either direction as seen in the sample records. With currents depolarizing the innervated faces, however, the responses diminished in amplitude, disappeared, and with still stronger polarizations reappeared inverted in sign. With opposite currents they increased in amplitude continuously. The slope of the current-voltage curve decreased during activity, indicating lowered membrane resistance. The percentage change during activity (about 35 per cent) was

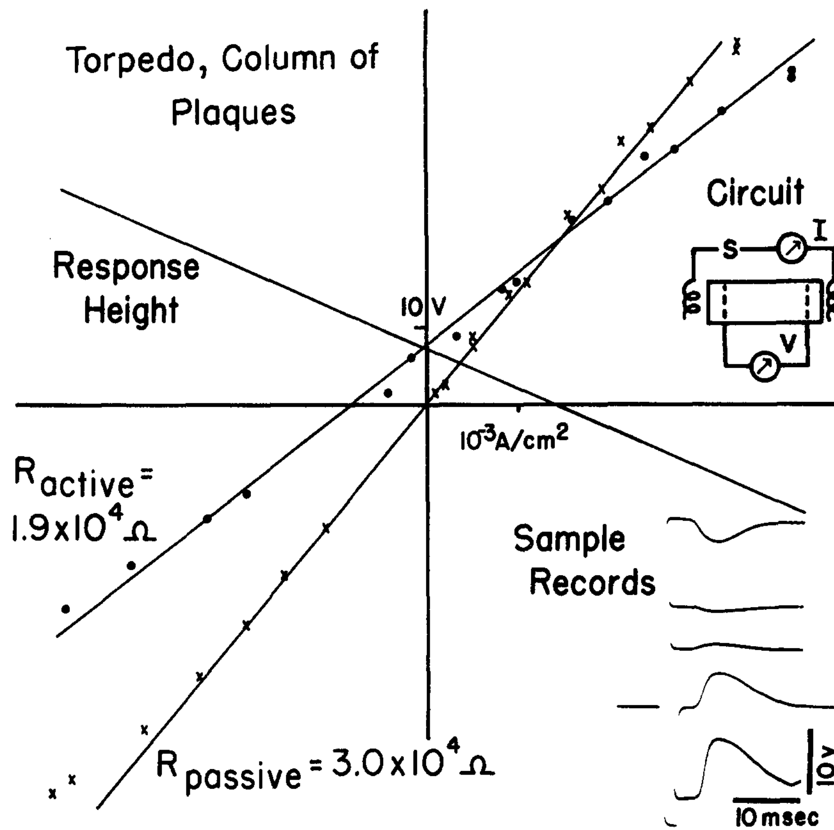

FIGURE 7. Effect of polarizing currents on a column of electroplaques. Experimental arrangement is shown in inset diagram. Two stimulators (S) were used in series to give a brief stimulating pulse and a longer lasting polarizing pulse, the current being monitored (I). The current electrodes had a large surface and were applied to the ends of a column of electroplaques mounted in oil, as described in the text. A separate pair of electrodes recorded the voltages (V) produced by the applied currents in the resting state (crosses) and during the peaks of the neurally evoked responses (dots). The difference between these two values is the response height which is also shown on the graph. The responses increased in amplitude when the current direction was such as to hyperpolarize the innervated membranes of the electroplaques (to the left of the origin). Currents outward through the innervated membranes caused diminution and then reversal of the sign of the response. These changes are also seen in the sample records which show, furthermore, that the latency of the evoked responses was not affected by what must have been very large changes in membrane potential. The slopes of the two voltage-current curves show that the effective resistance of the membrane was not changed by applying currents, but was decreased during activity. Further description in text.

probably diminished due to shunting by fluid and connective tissues. However, as will be noted below, it is likely that some of the nerve fibers to the preparation were injured and did not respond. Therefore only part of the excitable membrane may have been active during the response.

Counts were made, in sectioned material, of the number of electroplaques in the columns used in two of the experiments. This permitted reduction of the various electrical data to values for single cells (Table I). The large differences in all the values involving the responses and the small average voltage per cell reflect the likelihood that in the course of dissection the nerves suffered damage. However, these experiments achieved their primary purpose in demonstrating three features: (*a*) constancy of response latency in the face of large changes in membrane polarization; (*b*) reversal of the response on sufficiently strong depolarization; (*c*) proportionality of the response height to the difference between the reversal potential and the polarized value of membrane potential.

TABLE I
ELECTROPHYSIOLOGICAL PROPERTIES OF TORPEDO ELECTROPLAQUES CALCULATED FROM MEASUREMENTS ON COLUMNS OF CELLS; TWO EXPERIMENTS

No. of cells in column	Area of column	Resting membrane resistance	Decrease during activity	Open circuit voltage	Short-circuit current	Reversal potential
	cm.²	*ohm-cm.²*	*per cent*	*mv./cell*	*ma./cm.²*	*mv./cell*
450	0.47	32	36	18	0.90	51
300	0.28	44	44	46	0.53	104

LATENCY OF RESPONSES Responses to electrical stimuli in all cases known are evoked by critical depolarization of the electrically excitable membrane. As the stimulus is made stronger, the firing level is reached earlier and the response therefore arises earlier. In *Torpedo* electroplaques no response was observed even on very strong depolarization of the innervated membrane (Fig. 6), and the responses that were produced either in single electroplaques or in columns of electroplaques never occurred with a latency shorter than about 3 msec. (Figs. 4 to 7, etc.). The response latency was not appreciably changed by polarization either of single cells (Fig. 6) or of a column of cells (Fig. 7). In the latter experiments the calculated changes in membrane potential were very large.

D. *Interaction of Responses*

The foregoing experiments demonstrated in the most direct manner possible (54, 56) that the innervated membrane of *Torpedo* electroplaques is electrically inexcitable. The responses of the cells therefore must be due to stimulation of the nerve supply. Electrically inexcitable membrane cannot actively propagate locally generated depolarization (54). Since a single nerve fiber

innervates a restricted region of the *Torpedo* electroplaque and several fibers innervate the surface (Fig. 1), the response of the electroplaque due to a single fiber may be expected to be subject to electrotonic decrement and to differ in different regions. Also, the responses to different nerve fibers recorded in the same region may be expected to differ for the same reasons. These differences will be appreciable if the electrotonic spread has a rapid decay; that is, if the space constant is small in comparison with the diameter of the electroplaque. The electrotonic spread is small, as described in the experiments of the next section.

ELECTROTONIC SPREAD ALONG THE ELECTROPLAQUE MEMBRANE The passive spread of current from a point between two parallel membranes is a problem in non-uniform transmission lines (30). Intuitively it may be seen that decrement of an applied depolarization will be faster than exponential. For the present purposes it was only necessary to obtain the approximate amount of spread and a detailed investigation was not attempted. The term "space constant" will be used to indicate distance from the polarizing site at which the applied potential decayed to e^{-1} times its initial value.

In the experiment of Fig. 8, the space constant was determined with three microelectrodes placed outside the innervated membrane of the superficial electroplaque as in the diagram. One electrode (I) delivered a hyperpolarizing pulse. One of the two recording microelectrodes (E_1) was fixed close to the stimulating one. The other (E_2) was moved to varying distances on the surface. The graph of Fig. 8 shows the decline of the potential at E_2 in terms of the ratio $\log \frac{E_2}{E_1}$ for the different spacings of the two recording electrodes. The space constant was about 0.8 mm. The decay did not differ markedly from an exponential function, as is indicated by the degree of fit of the points to a straight line.

In the hypothetical case of an isolated electroplaque, the electrotonic spread would be limited by the low resistance of its uninnervated membrane. In the conditions of the present experiments the superficial cell had many cells lying beneath it. Polarizing current applied beneath the innervated membrane of the uppermost cell (as in the diagram of Fig. 8) therefore was confined by the resistance of the underlying innervated membranes. The electrotonic spread thus must have been greater than with an isolated electroplaque.

RELATION OF RESPONSES EVOKED IN A SINGLE ELECTROPLAQUE BY STIMULATION OF DIFFERENT NERVE FIBERS Two recording microelectrodes were placed 2.5 mm. apart beneath the innervated surface in two adjacent fields, each innervated by a different nerve fiber (Fig. 9 *A*, *B*). Subsequently, one of these electrodes (C) was placed in the region between, 1.5 mm. from

electrode B. Two pairs of stimulating electrodes excited independently the two nerve fibers, respectively those supplying the "A" and "B" regions. Traces A_1, B_1 represent the potentials at these two sites resulting from stimulating first the A axon and then the B axon. Traces A_5, B_5 record the potentials evoked in the reverse order. The two responses recorded within the

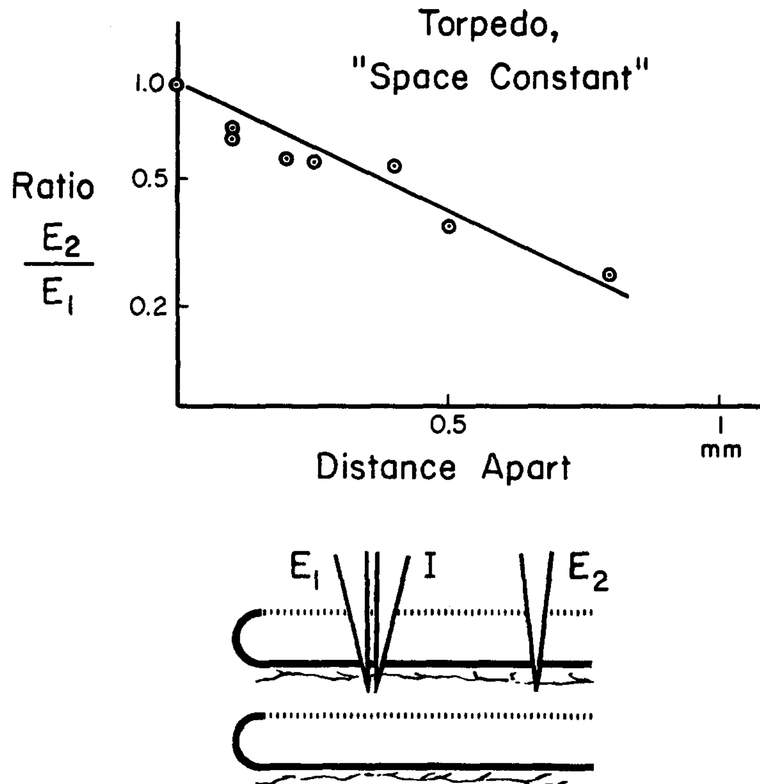

FIGURE 8. Electrotonic spread of current beneath the superficial electroplaque. Current was applied through microelectrode I (diagram) situated in the space between two cells. The resulting potential was measured at a site close to that of application of the current with a fixed microelectrode (E_1) and at various distances with a moving microelectrode (E_2). The ratio $\frac{E_2}{E_1}$ is plotted on a logarithmic scale against the distance.

A and B regions were different in form. At recording site C, however, the responses evoked by both the A axon and the B axon were similar (C_1, C_5).

The responses to stimulating the two axons were additive, no matter in what order they were produced (A_2, B_2–A_4, B_4; C_2–C_4). However, the summation was not algebraic. This behavior reflects the existence of a reversal potential for the p.s.p.'s (*cf.* reference 56) and the lowering of the space constant during activity. It should be noted that the degree of summation was greater in C_2–C_4 than in A_3, B_3.

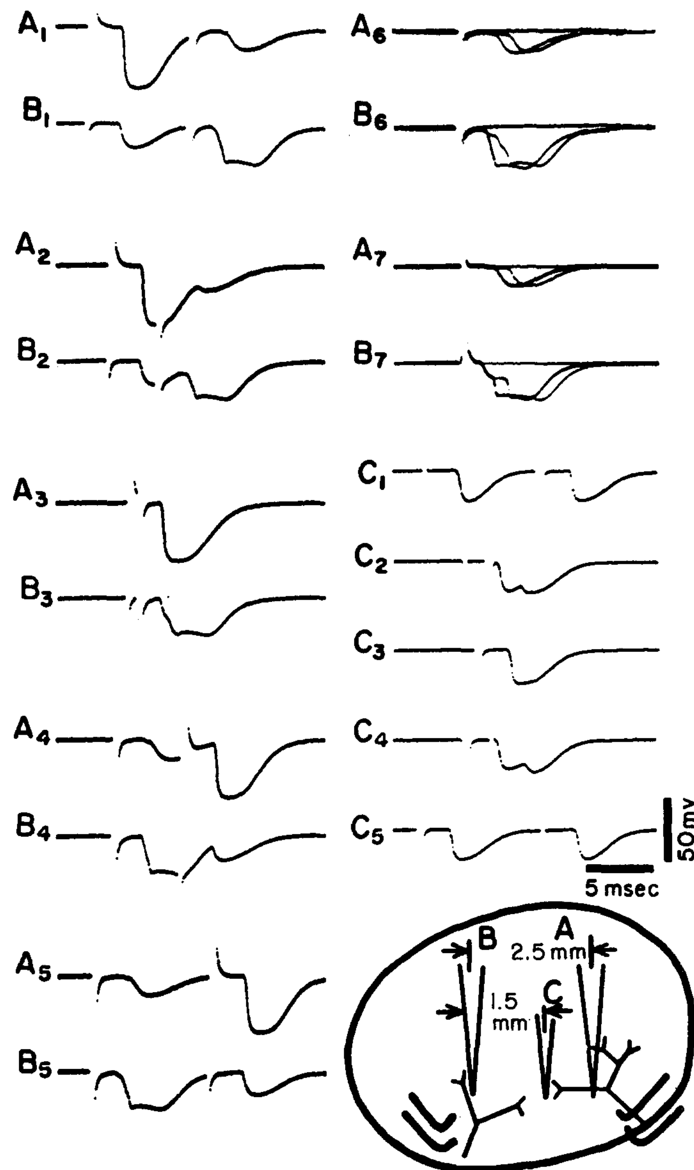

FIGURE 9. Absence of propagated activity in the electroplaque. Experimental arrangement shown in inset diagram which indicates an outline of the innervated surface and two nerve fibers. One pair of stimulating electrodes (right, near A) evoked a response that was recorded as a larger potential by the microelectrode at A than by that at B, 2.5 mm. away. The second pair of stimulating electrodes (near B) produced a larger response at B than at A. These responses are seen in succession on each of two traces recorded simultaneously at the two electrode sites (A_1, B_1 to A_5, B_5). The intervals between the two stimuli were varied in these series of records, the "A" stimulus leading in A_1, B_1 to A_3, B_3 and lagging in A_4, B_4 and A_5, B_5. Note the different forms of the responses to the "B"

At recording site B, stimulation of its innervating axon produced a response in which there appeared a number of irregularities. These were most marked with just threshold stimuli (B_6, B_7). The smaller, early components could not be produced separately, indicating that the complex was due to excitation of a single axon. The irregularities were absent in the responses evoked at recording sites A and C by stimulating the same axon. Presumably, these irregularities indicate delays that were caused by partial blocks in conduction along the branched axon. These may have resulted from damage in some cases, but the irregularities were most often seen in preparations in which the stimulating and recording electrodes were close together. Stimulation of one of the branches of the nerve fiber would cause "antidromic" propagation of the impulse and invasion of the larger trunks of the axon would tend to be associated with delays arising from propagational hindrance. The irregularities thus probably represent delays in initiating the local p.s.p.'s as the invasion of the axon branches was delayed. Stronger stimuli would tend to excite the axon in many regions and would overcome these delays. This result is evidenced by an earlier onset of several components of the responses in B_6 and B_7. These components did not, however, develop any earlier than did a small component obtained with threshold stimuli. The latter presumably represents activity initiated in branches which conducted for only a short distance and "orthdromically," and which therefore were not subjected to propagation delays. The stimuli were of opposite polarity in B_6 and B_7. The shape of the responses was different in the two cases, which is consistent with the explanation offered above. With the stronger stimulations the potentials recorded at the same time at A (A_6, A_7) also arose correspondingly earlier. These potentials, and those recorded at C, were smooth in form. Being distant from the stimulating site they might have arisen by electrotonic spread from site B. However, the decrement was not as great as would have been expected in that case (Fig. 8) nor was it great enough to produce the observed smoothing. Therefore, the responses probably arose by excitation of regions of the membrane near the recording electrodes through orthdromically conducted impulses in the axon.

FACILITATION Heterosynaptic facilitation, augmentation of the response to one nerve fiber by activity in another was never seen. However,

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stimulus as recorded at A and at B. A_6, B_6 and A_7, B_7, three superimposed sweeps in each trace. One was for a just subthreshold B stimulus; a second just threshold, and a third well above threshold. The direction of the stimuli was reversed in the two sets of records. Further description in text. C_1 to C_5, single traces of records made at site C, 1.5 mm. away from B. The responses to both the A and B stimuli were similar in form and magnitude, and they summed. C_1 and C_2, the A stimulus leading; C_4 and C_5, the B stimulus leading. C_3, both stimuli were delivered simultaneously. Further discussion in text.

homosynaptic facilitation was frequently observed when a testing stimulus was preceded by even a single stimulus to the same nerve fiber. The amount of facilitation was variable, being particularly large in the electroplaque of the experiments of Fig. 10. Facilitation was maximal when the testing stimulus followed the conditioning by about 30 msec. (*B*, *F′*). It declined slowly

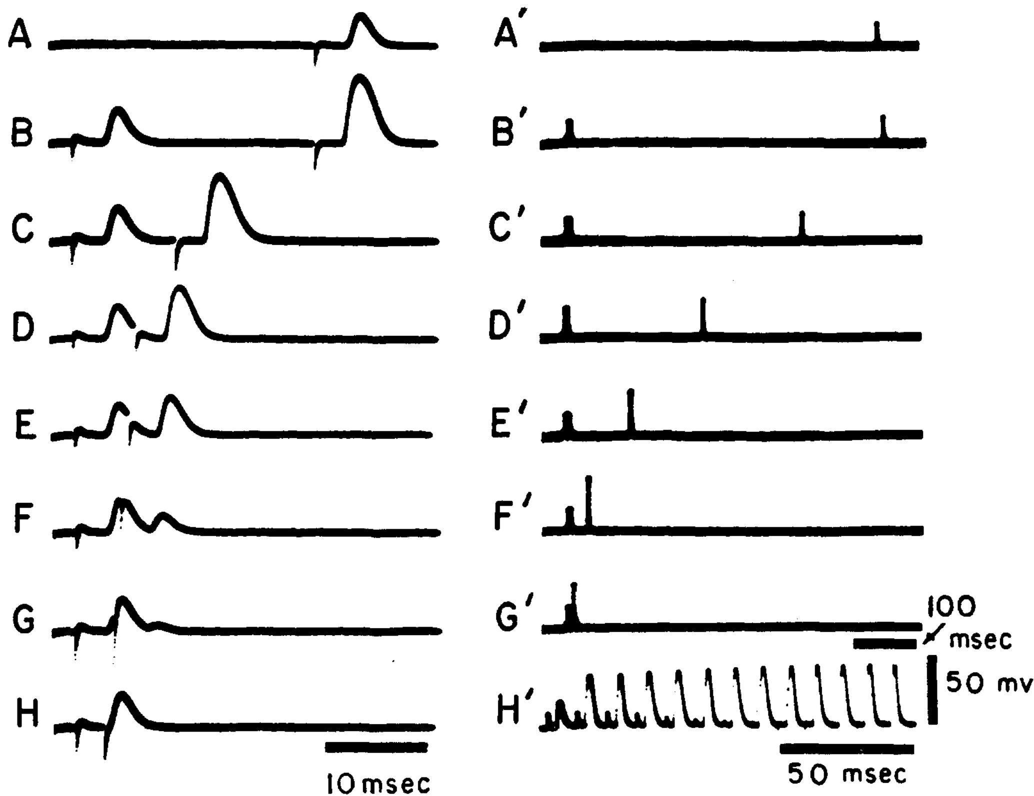

FIGURE 10. Homosynaptic facilitation in an electroplaque. *A*, *A′*, testing response in isolation; *B–H* and *B′–G′*, preceded by a conditioning response. Both evoked by stimulating the same nerve fiber through one pair of electrodes. At brief intervals (*F–H*) the testing response was small or absent, but at longer intervals, up to more than 400 msec. (*B′*), the testing response was facilitated. *H′*, responses to a train of stimuli at 100/sec. The facilitation was greatest for the 2nd response, but continued to increase also during subsequent responses.

at longer intervals, persisting for more than 400 msec. (*B′*, *C′*). When the axon was stimulated repetitively at 100/sec. (*H′*), the second response in the train showed the greatest increase, but the subsequent responses continued to grow in size, which reached a plateau at about the 10th impulse.

At short intervals (*F–G*) the testing response decreased markedly and was absent at *H*, at an interval of 3 msec. between stimuli. Since the innervated

membrane of the electroplaques is capable of sustained activity (Figs. 15 and 16) as are other electrically inexcitable membranes (54), the effects in records *F–H* are to be ascribed to presynaptic processes. Absence of response at an interval of 3 msec. presumably is related to failure of a second impulse to be produced in the axon (*cf.* Fig. 12), or to propagate into the presynaptic terminations. The reduction in the response at slightly longer intervals (*F, G*) suggests that some of the axonal terminals may not have been invaded (*cf.* reference 66 *a*) or that the smaller impulse which occurs during relative refractoriness may have resulted in less release of transmitter in all the presynaptic terminals. Depression at short stimulus intervals was a regular finding, but in many experiments facilitation did not occur at any interval between two stimuli.

PRESYNAPTIC CHANGES DURING HOMOSYNAPTIC FACILITATION Facilitation was usually accompanied by reduction in the latency of the response of the electroplaques (Fig. 10 *A*, *B* and Fig. 11 *A*, *B*) and in the threshold of the nerve (Fig. 11). These changes were also seen when there was little or no increase in the postsynaptic response.

In the experiment of Fig. 11, the strength of consecutive stimuli (measured as the input voltage to the stimulus isolation unit) was gradually increased until the electroplaque responded and was then decreased again until the response failed. Measured in this way the threshold of the nerve was nearly 15 per cent greater for increasing than for decreasing stimuli. The difference was somewhat greater for stimuli delivered at 2/sec. than at 1/sec. The return to higher threshold during inactivity of the nerve required some seconds as may be seen in the 3rd sequence of responses in Fig. 11. After the 6th subliminal stimulation (at 2/sec.) the stimulus evoked a response, and this diminished the threshold for subsequent responses.

The reduced latency of facilitated responses could not be ascribed to the lowering of the threshold. The latency to the first supraliminal stimulus was always longer than the latency of a facilitated response to the final supraliminal stimulus. Also, the latency sometimes increased again to its initial value, before the progressively weaker stimuli had reached their new threshold level.

A complete description of the time course of these changes was not attempted, since the responses were not stable for periods long enough to collect the necessary data. However, the threshold decrease was observed to occur with paired stimuli at intervals as short as 3 msec. when the second response of the electroplaque was actually reduced (as in Fig. 10). When a single axon in a small nerve trunk running to a column of electroplaques was stimulated repetitively a reduction in threshold usually was not observed, even when the response of the electroplaque itself exhibited marked facilita-

tion. Rather, there tended to be refractoriness lasting more than 15 msec. This result suggests that the parts of the nerve fibers in the trunks had properties different from those of portions nearer the terminals on the electroplaque surface, either normally or as a result of injury.

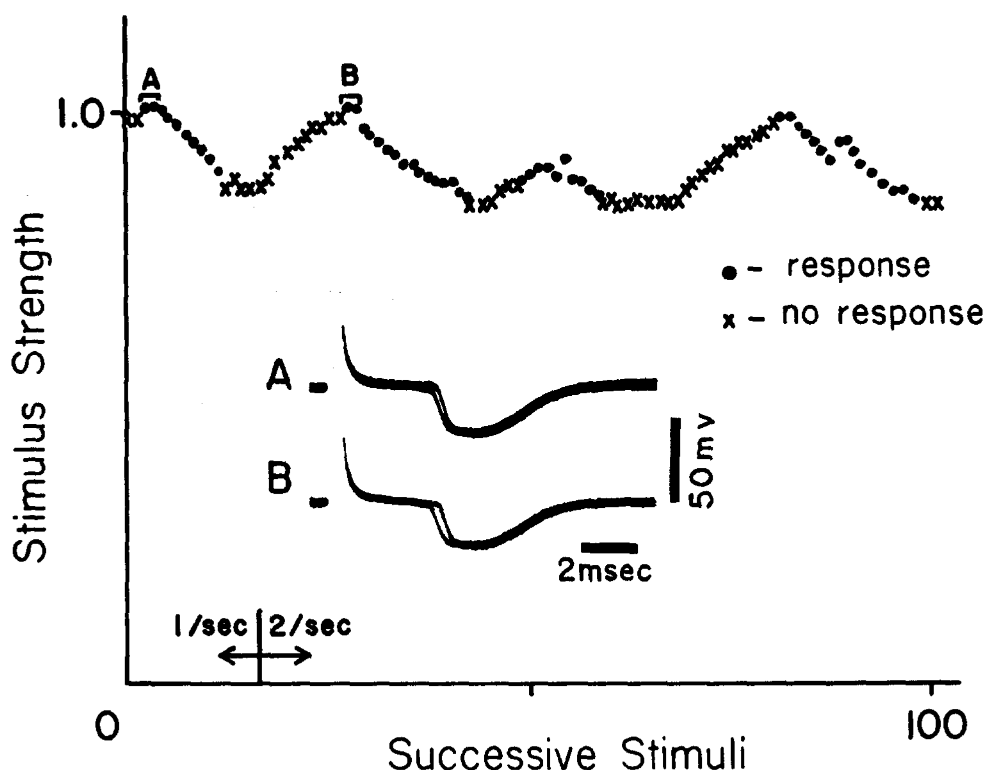

FIGURE 11. Reduction in threshold and latency during repetitive excitation of an electroplaque at 1/sec. and 2/sec. *Ordinate,* relative stimulus strength; *abscissa,* successive stimuli. The stimulus strength was gradually increased until a response was evoked. It was then gradually decreased until responses failed. Effective stimuli are indicated by dots, the ineffective by crosses. The first effective stimulus of an ascending series was much larger than the last such stimulus of a descending series. *Inset,* superimposed records of the first and second responses in the initial (A) and following (B) series of effective stimuli. Reductions in latency and threshold were slightly greater on stimulating at 2/sec. than at 1/sec.

The decrease in threshold suggests a long lasting depolarization of the nerve fibers following activation. The reduced latency could arise from reduced presynaptic conduction time which would also result from depolarization. Attempts to record the action potential of the fibers on the electroplaque surface were not successful. Intracellular recordings from axons in nerve trunks 1 cm. or more from their terminations were made, however,

although these data are of less significance with respect to the points in question.

In axons yielding spikes more than 80 mv. in amplitude (Fig. 12) there was an after-depolarization lasting only about 5 msec., and long lasting

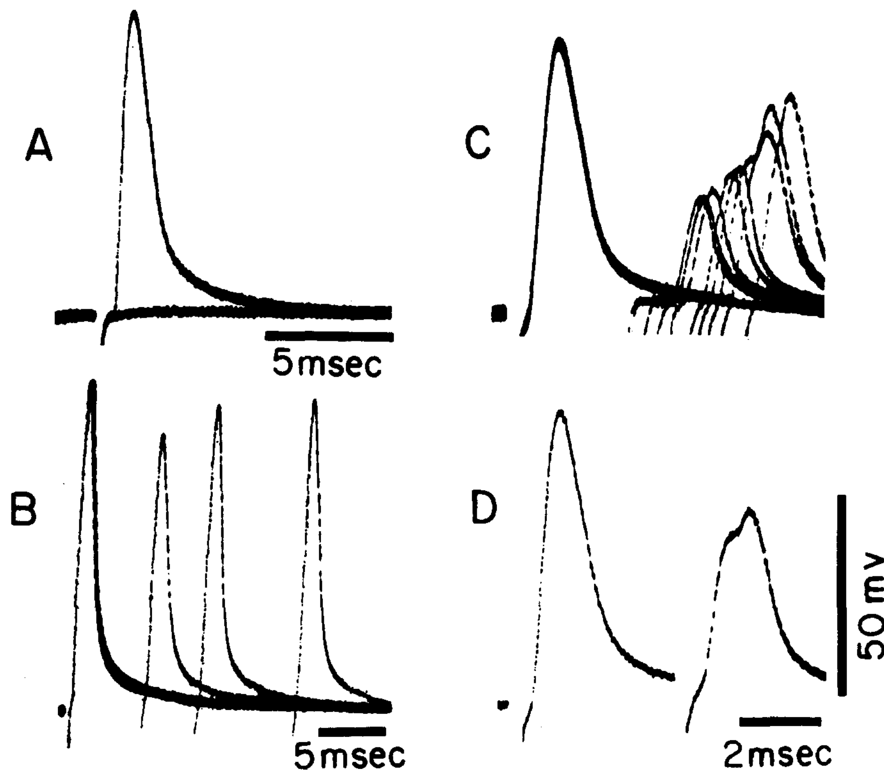

FIGURE 12. Intracellular recordings from an axon of an electric nerve, about 1 cm. from its terminations on the electroplaques. *A*, response in isolation on the base line of another sweep with a just subthreshold stimulus. Note brief after-depolarization, or negative after-potential. *B*, four superimposed traces show a conditioning response, in one sweep in isolation and, in the others, followed by a testing response at different intervals. The testing response was reduced at all the intervals. *C*, *D*, testing stimuli applied at shorter intervals show break-up of the response during refractoriness. The asynchronous activation of different regions, probably of different nodes, is shown by the notch in the testing response of *D*. Similar notches are also seen in some of the responses of *C*, as well as disappearance of the later component at the briefest intervals when the axon was still responsive.

depolarization was never seen. Refractoriness outlasted the after-depolarization, as denoted by decrease in response height (*B*) and failure of activation by strong stimuli (*C*). At short stimulus intervals (*C*, *D*) the response fractionated into components which probably represent activity evoked at different nodes (39, 77).

SPATIAL AND TEMPORAL INDEPENDENCE OF LOCAL FACILITATORY PROCESSES The response due to a single nerve fiber might be differently facilitated in different parts of an electroplaque, as in the three regions tested in the experiment of Fig. 13. Two stimuli were applied to the surface near recording site A. The testing response alone, recorded simultaneously at A and B (1.5 mm. apart) is seen in set *II*. The testing response recorded simultaneously at A and C (1 mm. apart) is shown in *V*. Conditioned by previous

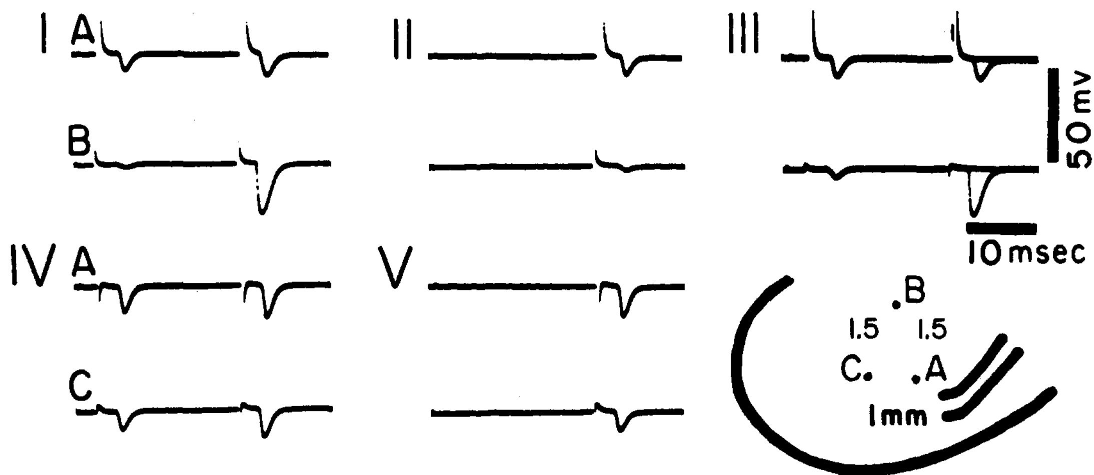

FIGURE 13. Independence of facilitatory processes at different sites of the electroplaque surface. External recording with microelectrodes at sites A, B, and C (diagram) of the responses evoked by stimuli delivered through a pair of electrodes on the surface. The responses to single stimuli were largest at A, only slightly smaller at C, and much smaller at B. Facilitation, however, was very large at B, marked at C, and slight at A. The testing response was due to a single nerve fiber, as is indicated by the all-or-none response to a threshold stimulus (*III*). The testing responses in isolation (*II*, *V*) were of the same amplitude as were those to the conditioning stimuli (*I*, *IV*). Note the decrease in latency of the greatly facilitated response at B (*I*, *III*).

activity, the testing response was slightly augmented at A (*I*, *IV*), markedly increased at B (*I*), and moderately at C (*IV*). The response was produced by a single nerve fiber, as is seen in *III*, in which superimposed traces show the effect of varying the strength of the testing stimulus slightly. The latency of the facilitated response at B decreased markedly, while the small degree of facilitation at A and C was accompanied by only a slight change in latency.

In the course of repetitive activation the responses to a conditioning stimulus and those to a closely following testing stimulus could undergo marked changes, varying from facilitation to depression. These effects could be somewhat independent in different regions innervated by a single nerve fiber. In the experiment of Fig. 14, one axon was excited by a series of pairs

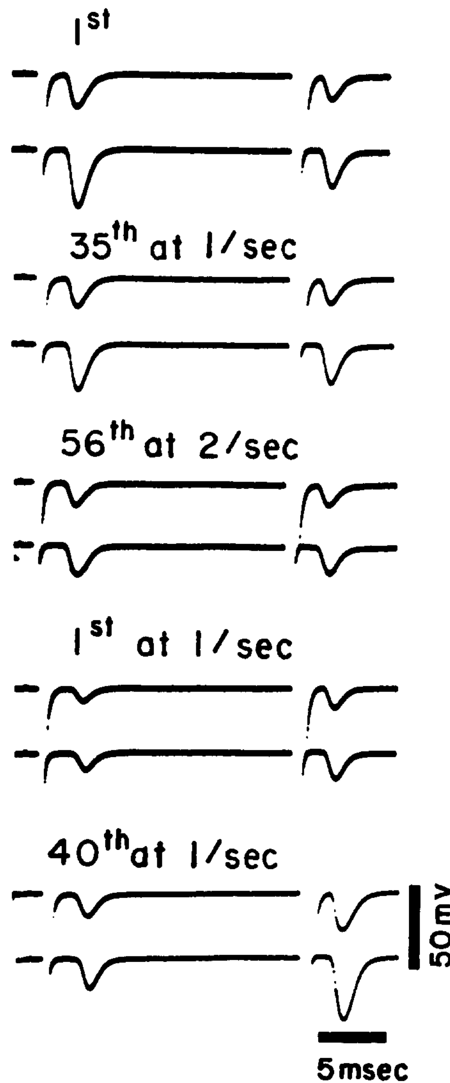

FIGURE 14. Locally independent temporal changes in responses. Each pair of traces records activity evoked at two sites in response to two stimuli about 25 msec. apart. They represent 5 sets of responses out of a train of about 131 evoked first at 1/sec., then at 2/sec., and again at 1/sec. Description in text.

of stimuli (*ca.* 25 msec. apart) presented at 1 or 2/sec. Simultaneous recordings were made at two sites of the innervated membrane. Initially at both sites the second (testing) response was smaller than the first (conditioning) response. This was one of the relatively rare instances of depression of a second response at a long interval. By the 35th paired stimulation at 1/sec. the conditioning response of the lower trace was diminished, whereas the

response of the upper trace was changed little. The diminution seen in the lower trace was even more marked in the immediately subsequent series at 2/sec. Both conditioning responses were now smaller. The testing responses had become the same size as the respective conditioning responses. The frequency of stimulation was then changed to 1/sec. (4th sequence of traces). The first pair of conditioning responses became smaller than the conditioning responses in the last stimulation at 2/sec. The corresponding testing responses were unchanged, however. Thus, in spite of the depressed responses at 2/sec., there had been some facilitation lasting between the pairs of stimuli at that frequency. The maintained amplitude of the testing responses in the first pair of stimulations at the lower frequency confirms the presence of facilitation. During the course of stimulation at 1/sec. the amplitudes of the conditioning responses gradually increased, but at the 40th response (lowest sequence of traces), they were still smaller than in the 35th pair of responses at the same frequency earlier in the experiment (2nd sequence of traces). However, the testing responses now showed marked facilitation and had become as large as had been the conditioning responses at the beginning.

E. *Response of Torpedo Electroplaques to Synaptically Active Drugs*

EFFECTS OF ACTIVATORS OF CHOLINOCEPTIVE SYNAPSES In this series of experiments advantage was taken of the special recording conditions which prevail in a column of electroplaques because of the low resistance of the uninnervated membrane. The electroplaques were placed with their innervated surfaces up and a recording microelectrode was inserted under the superficial electroplaque. In this position, movement of the electrode into the superficial or the underlying cell would have caused appearance of a resting potential and a downward shift of the recording trace. Thus, a positive shift, which would result from depolarization of the upper surface, could not result from electrode movement. In the experiment of Fig. 15, acetylcholine (10^{-2} M) was applied with a hypodermic needle to the surface of the electroplaque in the interval between two sweeps photographed at 1/sec. The electroplaque was depolarized only by about 15 mv. despite the high concentration of the drug. It continued to be depolarized maximally for about 5 sec. The depolarization gradually subsided, ending in about 14 sec. The response to a neural stimulus was somewhat decreased during the depolarization and the amplitude did not recover fully when the depolarization induced by the drug ended. Complete recovery of response height did not occur.

A high concentration of acetylcholine (10^{-2} to 10^{-1} M) was always required. It is likely that these high concentrations were necessary because the drug was destroyed rapidly by cholinesterase at the electroplaque surface. This conjecture was confirmed by the effects of applying a cholinesterase inhibitor

(Fig. 16, inset records). The experimental technique was the same as that described in connection with Fig. 15. Eserine (10^{-5} M), which had been applied several minutes before the records were made, prolonged the response to neural stimuli as did prostigmine in other experiments. The cells also became more sensitive to acetylcholine and the depolarizations shown in Fig. 16 were produced by applying that drug in 10^{-4} M concentration. Return

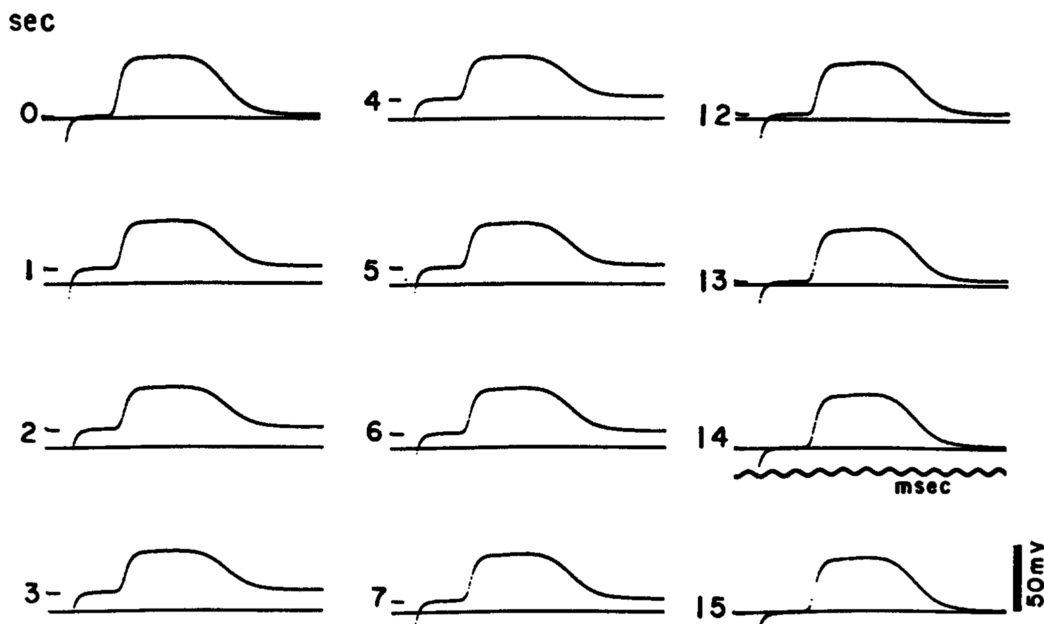

FIGURE 15. Effect of acetylcholine on resting potential and response of an electroplaque. External recording outside the uninnervated surface of the superficial cell of a preparation with the innervated surfaces uppermost. The responses were evoked and recorded at a frequency of 1/sec. Between the first two sweeps, acetylcholine (10^{-2} M) was applied to the surface with a hypodermic syringe. The depolarization which developed was relatively small, but lasted about 13 sec. The response amplitude decreased and was still depressed at the end of the series.

to the resting potential was earlier than recovery of the height of the response, which had decreased markedly during the maximum depolarization produced by the drug. Also, the response became shortened (6 to 30 sec. records), and the recovery of duration occurred in part after the resting potential returned to normal. Depolarization by acetylcholine did not affect the response latency.

Temporary depression of the response to a testing stimulus after depolarization by acetylcholine had disappeared was characteristic, and is shown graphically in Fig. 16 for this (upper graph) and for another experiment (lower graph) also after applying eserine. In the experiment shown in the

upper graph and inset records the depolarization subsided in about 15 sec., but the testing response was depressed maximally for about 25 sec. Traces of depression persisted another 10 to 20 sec.

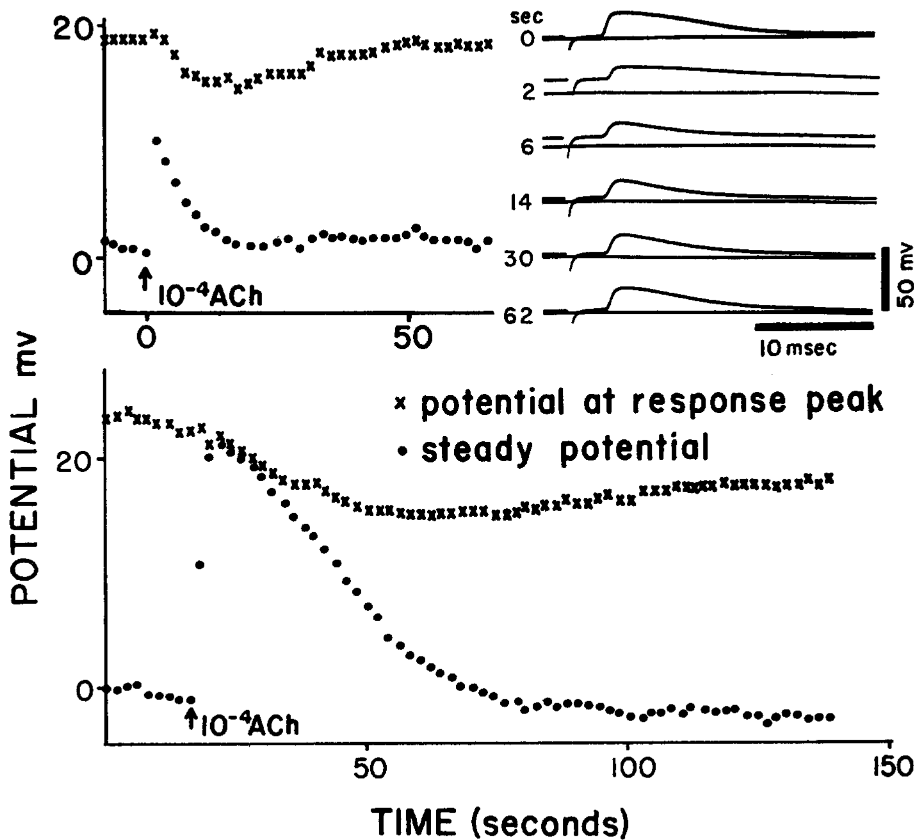

FIGURE 16. Effects of eserine and acetylcholine on electroplaques. Two experiments. *Upper right,* neurally evoked responses of a single electroplaque were prolonged after applying eserine (10^{-5} M). The cell was depolarized by a lower concentration of acetylcholine (10^{-4} M) than were uneserinized preparations (time of application between the zero and 2 sec. records). The changes in membrane potential are also shown in the graph on the left. Note that the depression of the response outlasted the depolarization produced by the acetylcholine. *Below,* the changes in the potentials produced by acetylcholine in another preparation were larger. The response amplitude remained depressed long after the depolarization by the acetylcholine had subsided.

A larger amount of depolarization was produced in the experiment of the lower graph. At the peak of the depolarization the testing response almost disappeared. While the depolarizing effect of the drug was terminated after about 60 sec., the depression of the testing response continued for much longer. A similar effect ("desensitization") has been described at the muscle

end-plate (64). In uneserinized preparations recovery from acetylcholine depolarization was much less nearly complete, and there was no indication of reversible desensitization. It seems likely that the high concentration of

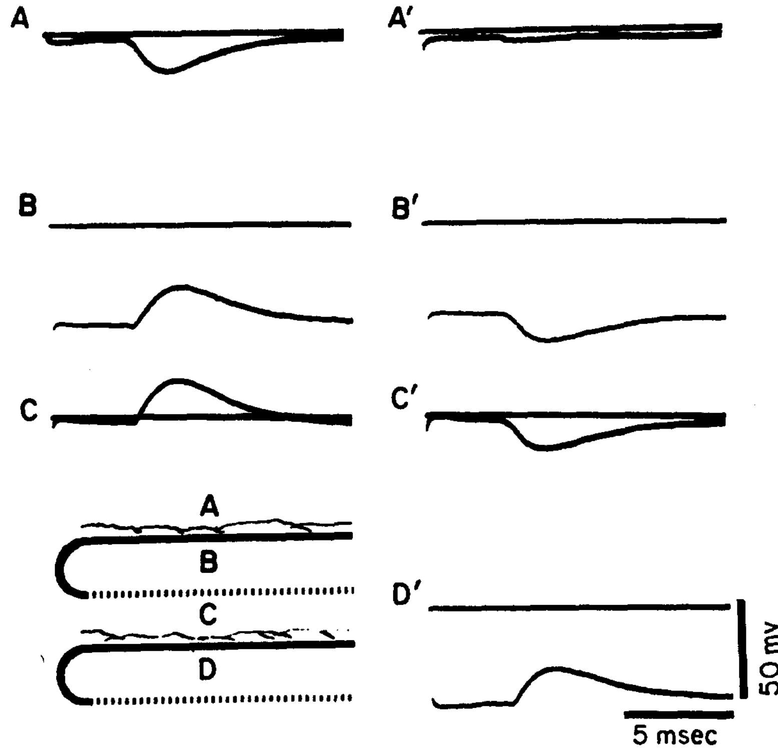

FIGURE 17. Block of response in superficial electroplaque by *d*-tubocurarine. Recording conditions shown in diagram. *Left,* potential before, and *right,* after applying *d*-tubocurarine. A weak stimulus which excited only the uppermost cell was used in the control recordings. The activity is identified by the size and sign of the potentials recorded above, inside, and below the cell (*A–C*). The marked surface negativity in *A* was due to removal of most of the fluid over the surface. *A′–D′*, the uppermost electroplaque became unresponsive after *d*-tubocurarine, even to a stronger stimulus which excited the underlying electroplaque. The response of the latter appeared as negativity within (*B′*) and just below (*C′*) the uppermost cell. It reversed to positivity when the microelectrode penetrated into the lower cell *D′*.

acetylcholine required in the absence of the anticholinesterase produced some secondary effect.

Only a few experiments were done with some other synapse activator agents. Carbamylcholine was as active in eserine-free preparations as acetylcholine was in the eserinized, and rapidly caused depolarization at 10^{-5} M to 10^{-4} M. Succinylcholine was effective in eserine-free preparations at 10^{-4} M. Whether or not these drugs caused desensitization was not determined.

Eserine itself, at concentrations of 10^{-3} M to 10^{-2} M, depolarized the electroplaques, but more slowly than did acetylcholine or its analogues.

EFFECT OF SYNAPSE INACTIVATORS Only the actions of *d*-tubocurarine and dihydro-β-erythroidine were tested in the present series of experiments. Both drugs eliminated the responses, without affecting the resting potential of the cell (Fig. 17).

SITE OF ACTION OF THE DRUGS In the above experiments, drugs were always applied to the innervated surface of the superficial cell. Exploration with microelectrodes of underlying cells showed that these were not affected by the highest concentrations of the depolarizing drugs applied to the superficial cell. Similarly (Fig. 17) the response of the next lower cell was not affected by *d*-tubocurarine which had eliminated the response of the superficial electroplaque. On applying eserine, however, the response to nerve stimulation of the underlying electroplaques was prolonged about as much as was that of the uppermost cell.

When the electroplaque preparations were made with the uninnervated surfaces uppermost, the superficial cell was also insensitive to the quaternary ammonium compounds, both synapse activators and inactivators. Eserine, however, prolonged the response of the uppermost cell about as quickly as when applied to its innervated surface. It may be concluded, therefore, that the quaternary ammonium compounds did not penetrate the uppermost cell nor did they diffuse through the connective tissue around the column, while the tertiary ammonium compound (eserine) was capable of reaching the underlying innervated membranes.

DISCUSSION

Electrophysiological Properties of Torpedo Electroplaques

A. DIRECT EVIDENCE FOR ELECTRICAL INEXCITABILITY

A notable feature of the electroplaques of *Torpedo* is their electrical inexcitability. The membrane potential changes linearly with applied current over a wide range (Figs. 6 and 7). The strongest electrical stimuli, whether depolarizing or hyperpolarizing (Fig. 6), fail to elicit a response, or to affect the latency of responses evoked by neural stimuli. These data are evidence of the most direct type possible for electrical inexcitability (54, 56).

Since the suggestion that *Torpedo* discharges are a form of responses in electrically inexcitable membrane (9) a number of other systems have been discovered which respond electrogenically to neural stimulation or to chemical agents, but not to electrical stimuli. Among these are the slow muscle fibers in frog (29), some muscle fibers of crayfish (40) and of grasshopper (32),

gland cells (52, 70), and electroplaques of all marine fishes that have been studied thus far (17–19, 21, 27, 57). Portions of certain other cells do not respond directly to electrical stimuli but do respond to other types of excitation. Among these are the end-plate membrane of frog skeletal muscle fibers (80), the dendrite and cell body of the *Limulus* eccentric cell (49, 79), the soma of lobster cardiac ganglion cells (62), the apical dendrites of mammalian cortical neurons (58), and the dendrites of the olfactory epithelium of frog (52, 73). In every case in which this matter has been tested, postsynaptic potentials are not evoked directly by electrical stimulation (54, 56).

B. INDIRECT EVIDENCE FOR ELECTRICAL INEXCITABILITY

The occurrence of certain other properties that have been theoretically correlated with electrical inexcitability provides indirect evidence for this condition in numerous electrogenic sites in which direct evidence has not been obtained as yet (54, 56). These properties are also found in *Torpedo* electroplaques. Special anatomical features such as the large size of the cells and their pattern of innervation (Fig. 1) as well as the absence of complicating effects produced by an electrically excitable response have made possible particularly clear demonstrations of these correlated properties. Similar data have also been obtained on electroplaques of *Astroscopus*, *Narcine*, and *Raia* (17 to 19, 57). The possibility of testing electrophysiological and pharmacological properties of responses in systems that are directly shown to be electrically inexcitable enhances the theoretical importance of the study of marine electroplaques (*cf.* reference 57).

Synaptic Latency Conduction time in the terminals should be minimal for stimulation applied to the surface of directly innervated electroplaques (53). Nevertheless, an appreciable latency, usually larger than 1 msec. and up to 3 msec. in duration was always found for p.s.p.'s evoked in gymnotid electroplaques (7, 15). However, spike responses of these electrically excitable cells also could be produced, developing with virtually no latency for responses to strong stimuli. Stimulation of *Torpedo* electroplaques in the same experimental conditions never evoked responses except after an appreciable latency. The synaptic latencies are particularly long in *Torpedo* and in other marine fishes (17–19, 57). As noted above, the synaptic latency is not appreciably altered by large hyperpolarizing or depolarizing changes in membrane potential.

Even though direct evidence for chemical transmission is unavailable, it may be reasonably assumed that this is the mode of transfer of excitation from the presynaptic nerve terminals to the electrically inexcitable postsynaptic membrane (*cf.* references 53 and 57). Accordingly, synaptic latency may be composed of three distinct factors: the time for mobilization and

emission of the transmitter agent by the presynaptic terminal; the time for the passage of the transmitter agent from the surface of the terminal across the synaptic cleft onto the surface of the subsynaptic membrane; and the latency of activation of the depolarizing processes of this membrane by the transmitter (*cf.* reference 56). The magnitude of the synaptic latency, its occurrence under conditions well suited to its elimination if it were due to conduction delay, and its independence from the membrane potential are particularly striking characteristics which should prove useful in the future for analyzing the different aspects of these processes. Noteworthy also is the fact that the latency was not affected by drugs (Figs. 15 and 16). Intervals between the arrival of the presynaptic impulse and the postsynaptic response that are ascribable to synaptic latency (54) have also been found in the squid giant axon synapse (28, 61), cat motoneurons (*cf.* reference 38), and frog skeletal muscle fibers (23, 24, 41, 67).

The occurrence of an appreciable and irreducible latency in responses that are as rapidly rising as those of *Torpedo* electroplaques precludes an appreciable electrotonic (or "ephaptic," *cf.* reference 56) contribution from the axons. The electrotonic current, due to activity in presynaptic terminals, is also negligible in other synaptic systems: cat motoneurons (*cf.* reference 38), squid giant axons (28, 61), frog skeletal muscle fibers (41), muscle fibers of *Romalea* (32) and lobster (60), and various electroplaques (7, 15, 57).

Electrochemical Gradation and Reversal of the Response The amplitude of electrically inexcitable activity is electrochemically graded (54, 56). The absence in *Torpedo* electroplaques of complications introduced by electrically excitable processes such as spikes permitted the demonstration of this property over a wide range of membrane potentials. However, the large size of the electroplaques was to some extent a hindrance for the experiments, since a single microelectrode could not polarize a cell uniformly nor deliver sufficient current to change the membrane potential over a large range. However, when currents were applied with large electrodes to a column of electroplaques, sufficient current could be passed so that the response could be reversed.

Reversal of responses by applying strong currents was first described by Albe-Fessard in *T. marmorata* (2), and the interpretation of the effect as a manifestation of activity in electrically inexcitable membrane was provided subsequently (53).

Electrochemical gradation and/or reversal of depolarizing postsynaptic potentials has been demonstrated in a large number of cells (*cf.* references 54 and 56). Among recent data are studies on the synaptic currents of the frog muscle end-plate (76) and the synaptic potentials of cardiac ganglion cells of lobster (62) and squid giant axons (61). Gradation has also been shown

in the "fast" and "slow" p.s.p.'s of *Romalea* (32) and in lobster (60, 75) muscle fibers. Reversal has been produced in the slow p.s.p. of the grasshopper muscle fibers (32). The inversion of i.p.s.p.'s has been demonstrated in cat motoneurons (*cf.* reference 38) and crustacean synaptic systems (26, 42, 60, 68).

The reversal or equilibrium potential of the p.s.p. has not been determined satisfactorily in the present experiments for a number of technical reasons. The amplitudes of the responses produced by individual electroplaques are frequently somewhat larger than the resting potential; that is, there is some overshoot. However, the thinness of the electroplaque of *Torpedo* predisposes toward recording a reduced resting potential. Also, the canalicular network which penetrates most of the cell volume as inpocketings of the uninnervated surface (72) may affect the determination of the resting potential. The intracellular electrode would probably rupture many of these canaliculi, tending to diminish the recorded resting potential. This effect would not, however, diminish the response amplitude since little or no voltage drop is developed across the low resistance, uninnervated membrane. Calculations of the reversal potential from the responses of columns (Table I) gave divergent results, but experiments of this type are not quantitatively reliable.

C. CONSEQUENCES OF ELECTRICAL INEXCITABILITY

Absence of Rectification It has already been noted that rectification is absent from *Torpedo* electroplaques (Figs. 5 and 6). Rectification is absent in the electroplaques of *Narcine* (17) and *Astroscopus* (18). It is also absent in several other systems in which there are p.s.p.'s but no spikes (*e.g.*, lobster cardiac ganglion somata; *cf.* reference 62) whereas in most spike-generating cells (though not all; *cf.* reference 38) "delayed rectification" (33, 63) is present. In the frog slow muscle fibers (29) and in *Raia* electroplaques (19, 57) delayed rectification is very marked, although in both systems the membrane producing the p.s.p.'s appears by other criteria to be electrically inexcitable. In *Raia* electroplaques the delayed rectification occurs primarily in the uninnervated surface.

Absence of Propagation by Active Processes Since propagation through stimulation by local circuit currents cannot occur in electrically inexcitable membrane, propagation is passive, by electrotonic spread only (54, 56). The large active surface of *Torpedo* electroplaques which is divided into distinct zones innervated by different nerve fibers (Fig. 1) provides advantageous anatomical conditions for demonstrating the limited spread of potentials that are generated at local synaptic sites. This spread was small because of the small "space constant" of *Torpedo* electroplaques (Fig. 8), and this emphasized regional differences in the electrically inexcitable, neurally evoked activity.

Local Variations in the Response The amplitude changes at one site produced during repetitive activity differed markedly from those at another site (Figs. 13 and 14). The forms of the responses could also differ (Fig. 9) from one recording situation to another, in contrast to the general fixity of the spike in geometrically uniform, electrically excitable membrane. However, successive individual responses under given conditions were also rather constant in amplitude and form. The relative constancy of these responses is due to the stimulation of a single axon in the course of the present experiments. The all-or-none appearance of the response therefore indicates that the all-or-none pattern of activation of the nerve terminals was relatively invariant. Thus, even when different nerve terminals probably fired asynchronously, giving rise to responses of irregular form, the pattern of the responses was the same in successive records (Figs. 4, 5, and 9). Both amplitude and form could be varied by facilitation or by the action of drugs.

D. FACILITATION

Marked facilitation occurred in some electroplaques, but it was absent in others. It was not observed in the organ discharges, suggesting that most, if not all, electroplaques were active during a discharge and were activated maximally, or nearly so. Heterosynaptic facilitation was never observed. The local character of the facilitatory processes is shown by the different degrees of facilitation at different sites on the same electroplaque (Figs. 13 and 14).

The present data do not, however, provide information to separate presynaptic and postsynaptic factors. The reduction in threshold of the nerve due to activity (Fig. 11) suggests that the presynaptic terminals may become depolarized during successive responses. Depolarization of the nerve could also account for the shorter synaptic latency during facilitation (Figs. 10, 11, 13, and 14) since it might cause earlier invasion of the presynaptic terminals. However, the total conduction time in the present experiments must have been short. The decreased latency of the facilitated response might be due to shortened latency of interaction between transmitter and postsynaptic membrane, which might be due to more rapid release or release of a larger quantity of the transmitter. These various presynaptic effects would involve special properties in the terminal portions of the fiber that were not seen in the axon about 1 cm. from the terminal (Fig. 12). However, differences in the properties of the terminals and axons have been observed in lobster (59, 74, 75).

The occurrence of depolarization in the presynaptic terminals, which is indicated by the present data, is not consistent with hypotheses explaining facilitation as resulting from increased spike height in the positive afterpotential (31, 38, 69). However, it is possible that facilitation is produced by several mechanisms. In the present case, the innervating terminals are densely

applied to the electroplaque surface as a profusely branching system (Fig. 1). Facilitation by aiding invasion of the branches might therefore play an important role.

The amount of homosynaptic facilitation varies in the electroplaques of other electric fishes. In the accessory organ of *Narcine*, facilitation is very large (17). In *Gymnotus carapo* (15), in the main organ of *Narcine* (17), and in *Astroscopus* (18) facilitation is small, or non-existent. The electroplaques in the two latter cases produce responses which appear to be nearly maximal, since little increase in height is produced by summation or by depolarization by drugs. Thus, facilitatory processes, if they occurred, would probably not have been detected. However, in the experiment of Fig. 14, "fatigue" occurred in the course of repetitive stimulation. The conditioning responses became reduced and facilitation of the testing responses became marked.

Pharmacological Properties

The Transmitter System The electroplaques of *Torpedo* have cholinoceptive membranes. Acetylcholine and other cholinomimetic synapse activators (52, 55) cause depolarization if the drugs are applied to the innervated surface. The response lasts a long time (Figs. 15 and 16). Close arterial injection of acetylcholine in *Torpedo* also produced prolonged depolarization (43). The neurally evoked responses were prolonged by anticholinesterases, and presumably as a related phenomenon, these drugs potentiated the action of acetylcholine. Carbamylcholine, which is not destroyed by esterase, was as active in normal tissue as acetylcholine was in the eserinized. Inactivators of cholinoceptive synapses also blocked the neurally evoked response.

Transmission is also cholinergic in all other electric organs that have been tested. However, the cholinesterase of *T. marmorata* differs from "true" acetylcholinesterase (22). Thus, even if the transmitter were acetylcholine in all cases, the presence of different amounts of it, of different varieties and amounts of cholinesterases, or of pharmacological differences in the pre- and postsynaptic membrane might result in a variety of electrophysiological properties in different electric fish.

Transmitter Action The time constant of the electroplaques is shorter than about 0.2 msec. (Fig. 6) and probably much more so, since it is about 30 μsec. in *T. marmorata* (1). Thus, the duration of passive decay of the p.s.p., after transmitter action has disappeared cannot be very long. The duration of the response therefore represents the time during which the transmitter is available after its release by the presynaptic terminals. The duration of membrane activity is also nearly that of the response duration in *T. marmorata*, since the conductance change during a discharge lasts about the same

time as does the discharge (1). The durations of p.s.p.'s are also short in electroplaques of other electric fish (7, 15, 17–19) except the rajids (19, 27, 57). Since the time constants of the membranes are small in all, the durations of the p.s.p.'s reflect almost exactly the durations of transmitter action, in contrast to the distortion produced by membranes of large time constant (*cf.* reference 57).

If the transmitter is acetylcholine itself the quantity released during a nerve impulse must be quite large, since *Torpedo* electroplaques are rather insensitive to it. Even in the presence of eserine or prostigmine, about 10^{-4} M acetylcholine had to be applied for depolarizing the cells. Carbamylcholine, which is not destroyed by esterase, also effected depolarization, but in the range of 10^{-5} to 10^{-4} M. Electroplaques of *Astroscopus* likewise have low sensitivity (18).

The sites of transmitter action are probably the regions of the innervated membrane that can be localized histochemically by their esterase activity (34). These are sharply limited to the sites of synaptic contact (72). The uninnervated dorsal surfaces of *Torpedo* electroplaques do not react to the drugs, and quite possibly, the regions of the ventral surface which are not synaptic sites are similarly inert.

A finding that needs more detailed study is the desensitization of the reactive membrane by acetylcholine (Fig. 16). Desensitization was also produced in *Astroscopus*, but only by acetylcholine, not by carbamylcholine (18). While the response height did not recover completely after acetylcholine in *Torpedo* electroplaques it did so in *Astroscopus.* In both, there was a return to the resting potential, but the synapse activator drugs cause irreversible depolarization of eel electroplaques (8).

Functional Adaptation of Electric Organs

Functional Differences in Innervated and Uninnervated Membranes The occurrence of only one reactive surface is characteristic of electroplaques of the torpedine fish and *Astroscopus* (17, 18, 57). In this respect there is a resemblance to the electroplaques of *Electrophorus* (7, 66). Bernstein (23 *a*) had suggested this type of electrophysiological system to account for the high voltages produced by electric organs. However, other electric fish are now known in which both major surfaces of the electroplaques become active but are nevertheless capable of series summation of their voltages. This applies not only to weakly electric fishes which emit diphasic or triphasic pulses (15, 16, 19) but also to the strongly electric *Malapterurus* (20, 65). Despite the fact that both surfaces are active in the electroplaques of *Malapterurus* and in those of the weakly electric *Mormyrus rume* (16), the electroplaques also emit monophasic or nearly monophasic pulses.

The difference in the membrane resistance of the innervated and unin-

nervated major surfaces is another similarity between the electroplaques of *T. nobiliana* and those of *Electrophorus*. In *Astroscopus* and in both main and accessory organs of *Narcine* the uninnervated membrane of the electroplaques also has a low resistance relative to the innervated surface (17, 18). This characteristic leads to a kind of impedance matching, since the resistance of the innervated surfaces decreases during activity. Other modifications which are probably adaptive for impedance matching occur in electroplaques of various electric fishes in which both surfaces have about the same resistance (19).

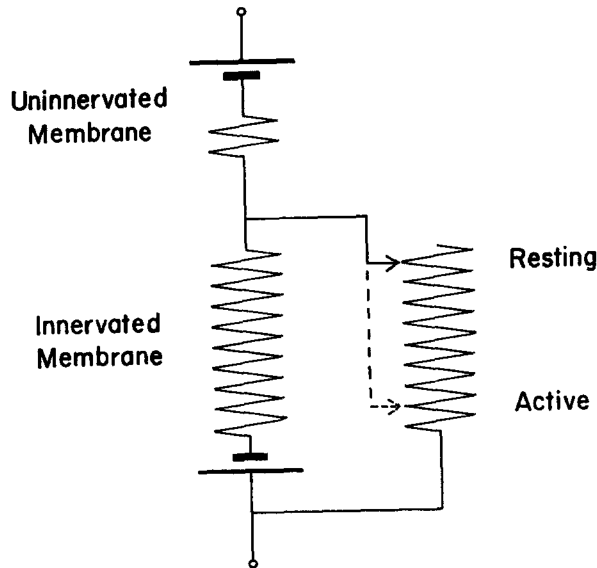

FIGURE 18. Equivalent circuit of *Torpedo* electroplaques. The membrane capacity is omitted, since the time constant of the membrane is very brief. Each membrane has a resting potential generator, the two being equal but oppositely oriented with that of innervated membrane having a much higher internal resistance. The reactive component of the innervated membrane is shown as a resistance shunting the resting potential generator. This resistance is high at rest and low during activity.

Equivalent Circuit While the electrogenesis of *Torpedo* electroplaques is similar in nature to that of the muscle end-plate (53), the cell resembles the eel electroplaque in its anatomical and functional polarization (7, 66). Accordingly, the equivalent circuit of the bioelectric generator in *Torpedo* (Fig. 18) resembles in some respects the equivalent circuits constructed for each of the other structures (41, 66). Since the time constant is small the capacity of the membrane has been neglected. The membranes of the two major surfaces form equal and opposed batteries, but of different internal resistance. Assuming ionic mechanisms similar to those at the end-plate, the high resistance battery of the innervated membrane has in parallel during

activity of the synaptic sites a low resistance tending to short-circuit it. Current is thus permitted to flow under the drive of the potential across the uninnervated and unreactive low resistance membrane.

The same equivalent circuit also applies to the electroplaques of *Narcine* and *Astroscopus* (17, 18). It is not valid for *Raia*, however, since in these cells there is delayed rectification (19, 57).

The conductance change during activity is independent of changes in membrane potential as is indicated by the linear relation between response height and membrane potential (Figs. 6 and 7). The time course of the responses is also unaltered, indicating that the degree of action and the rates of destruction and diffusion of the transmitter are unaffected by applied polarization.

As in eel (66) and in *Narcine* (17) and *Astroscopus* (18), the membrane resistance of the uninnervated surface is so low that it develops no appreciable IR drop during activity (Figs. 4 and 5). The specific resistance of the innervated surface, determined from columns of tissue, was of the order of 30 ohm-cm^2. Calculations from the data on *T. marmorata* (1, 2) indicate a lower range of values (5 to 25 ohm-cm.^{2}). The largest observed current density developed by *T. nobiliana* was about 75 ma./cm.^{2} and the maximum with zero external load is probably larger. The value of 240 ma./cm.^{2} obtained by Cox and Coates (*cf.* reference 36) is probably in error since the measured area appears small for the given weight of organ and the recorded voltage was up to 200 v., much larger than has been obtained in all the fish of the present experiments. Cox and Breder (35) obtained 70 ma./cm.^{2} in *Narcine* which extrapolates to 100 ma./cm.^{2} for zero external load resistance, and 55 ma./cm.^{2} has been obtained in *T. marmorata* (2). The calculated current density for dissected columns of tissue was only about 1 per cent of the above values, and the discrepancy is probably to be accounted for by damage to the nerve fibers in the course of dissection. Similarly, Albe-Fessard (2) obtained maximum resistance changes only in very fresh preparations. The values were about twice those of Table I, and are also low compared to the changes during the responses in the organ.

Series-Parallel Summation The electroplaques in a single column of the *Torpedo* organ are all oriented in the same way and all produce monophasic pulses. Summation of the electrical contributions of individual electroplaques is therefore simple, and results in a monophasic pulse of the whole organ or of its parts. The very large number of parallel columns in combination with the low internal resistance of the individual generators in membrane makes possible the high currents which are produced in the discharge.

Torpedo is a strongly electric fish, despite the fact that the individual electroplaques produce responses of about half the voltage of those of *Electro-*

phorus (7, 66) and *Malapterurus* (20, 65) which are also strongly electric fishes. The individual cells of *Eigenmannia* or *Sternopygus* (14) and *Mormyrus rume* (16) generate similarly large monophasic spikes, but are weakly electric fishes. It is the far smaller number of series-parallel elements in weakly electric fishes that is responsible for their low voltage and current outputs. The distinction between strongly and weakly electric fishes therefore is one of anatomical characteristics and not of fundamental differences in electrophysiological properties (53, 57).

An adaptation of the shape of the electric organs of torpedine fishes to their marine habitat was noted by du Bois-Reymond (24). The flattening of the organ along its axis allows the emission of a high current at relatively low voltage, thus developing maximum power in the high conductivity sea water. The two fresh water, strongly electric fishes, *Electrophorus* and *Malapterurus*, generate high voltages at lower currents and in the long axis of the fish, adaptations to the high resistance of fresh water.

Synchronization The responses of single electroplaques to excitation of single nerve fibers varied from about 3 msec. (Fig. 5) to nearly 10 msec. (Figs. 9 and 15), while the organ discharges were pulses of some 5 to 6 msec. (Figs. 2 and 3). As has already been described, however, the form and duration of the responses in single electroplaques are subject to variation by experimental interference with conduction in the nerve terminals. A more accurate comparison is between reflexly evoked discharges (Figs. 2 and 3) and those produced by stimulating the electric nerve (Fig. 3). The electrically evoked response was never longer than the reflex discharge. Presumably, therefore, *Torpedo* does not have any of the various central synchronizing mechanisms which are present in *Electrophorus* (4), *G. carapo* (15), other gymnotids, and possibly the mormyrids (16). It also appears that the reflex responses may be asynchronous to a considerable degree. Some equalization of conduction times probably occurs in the periphery since the nerves innervating the medial cells first pass more laterally to innervate peripheral columns and then return.

Synchronization of discharges in two groups of electroplaques that are brought into contact has been observed in preparations of *T. marmorata* (3). However, these effects cannot be ascribed to enhancement of the excitability of as yet inactive cells by the current of those that are already active, since the electroplaques themselves are electrically inexcitable. Furthermore, applied currents do not change the latency of responses evoked by stimulation of the electroplaque surface (Fig. 6). Probably, therefore, the synchronizing actions were caused by effects on the excitability of the nerves through the current generated by the discharging electroplaques. Stimulation of the innervating nerve by the evoked discharge caused by a brief stimulus was

occasionally observed in preparations of columns of the organ and presumably also occurs in stimulating branches of the electric nerve (Fig. 3 *C*). This effect was not observed in the activity of single electroplaques (Figs. 4 and 5) and seems to be absent in organ discharges (Figs. 2 and 3).

Skin Resistance The immunity of electric fish to their own discharges is an intriguing problem (25, 53). At least one factor appears to be the markedly lower resistance of the skin above and below the organs in torpedine fish, in comparison with skin elsewhere on the body (Fig. 19). This adaptation tends to channel the flow of current through the organ and reduces flow through other parts of the fish. The skin resistance in several nonelectric marine fishes both teleost (*Spheroides* and *Ctenolabrus*) and elasmobranch (*Squalus*) lies between the highest and lowest values found in *Torpedo.*

Electrogenic Membranes as Current Generators The current density in *Torpedo* organ is high compared to that in most other known electrogenic tissue. The peak current density in eel electric organ is about 70 ma./cm.^{2} (57). High current density probably indicates that a large proportion of the surface becomes active, which in *Torpedo* electroplaques is undoubtedly related to the high density of innervation. However, the capacity to generate high current is not specific to synaptic membrane. Although the peak inward current in squid axon is only about 10 ma./cm^2 (78) that of frog node is about 70 ma./cm.^{2} (37). Presumably the higher value in the node represents an adaptation to provide a high safety factor for saltatory conduction.

That all electrogenesis of marine electric fish is of the synaptic variety without the spike activity that is present in responses of all fresh water electric fish suggests some adaptive value of the former type of activity to the habitat of the marine fish. It is possible, for example, that the high current may be produced at the expense of less metabolic work in synaptic membrane than in electrically excitable membrane.

Evolution of Electrogenic Membrane The occurrence of electrically inexcitable electroplaques might be due to persistence of a primitive evolutionary condition, or it might be a regressive feature, the loss of an electrically excitable component. The hypobranchial tissue from which *Torpedo* electroplaques derive is capable of developing into electrically excitable muscle fibers (Fig. 20). Studies on embryological material may be required to decide whether electroplaques pass through an electrically excitable stage. A discussion of evolutionary and phylogenetic relations among electric fishes will be found elsewhere (57).

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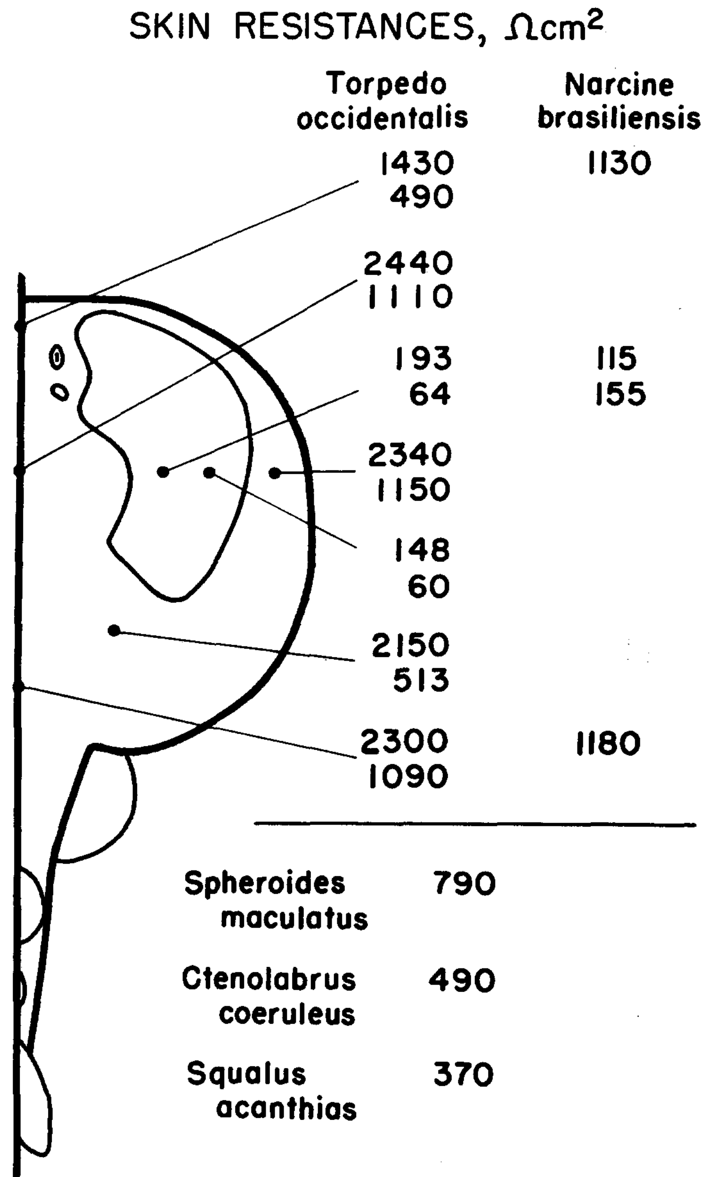

FIGURE 19. Adaptive modification of skin resistance in *T. nobiliana* and *N. brasiliensis*. Resistance measurements were made with an A.C. bridge or with a 4 electrode D.C. bridge. Skin was taken from the dorsal and ventral surfaces at the indicated sites, the values of resistance of the skin from the dorsal surface being given on the top row in each set. The electric organ is indicated in outline. Below are values of resistance of flank skin in three non-electric fish.

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Dr. Bennett is a Fellow of the National Neurological Research Foundation. He held a Grass Foundation Fellowship at the Marine Biological Laboratory during the summer of 1958.
This series of three papers reports work on the electric organs of three marine electric fishes, *T. nobiliana*, *Narcine brasiliensis*, and *Astroscopus y-graecum*. Other data on several species of *Raia* will be reported later. A preliminary note on this work appeared in 1958 (21). The relation of the data to

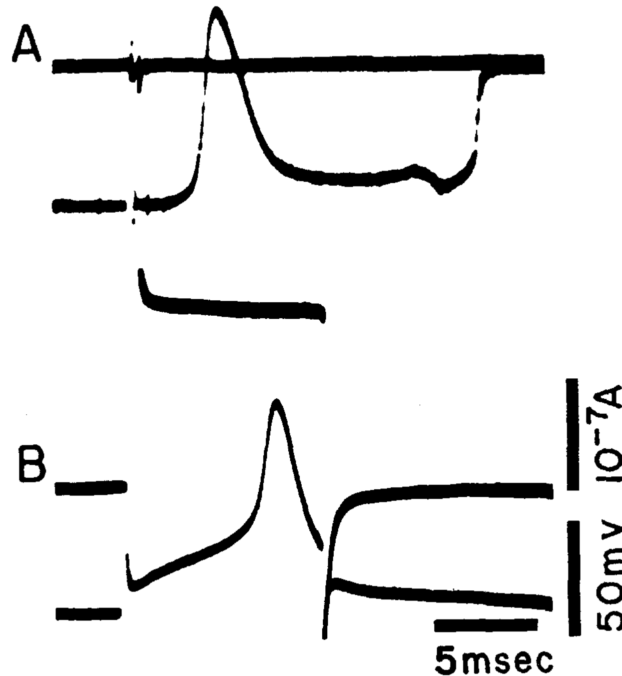

FIGURE 20. Responses in electrically excitable hypobranchial muscle fibers of *Torpedo*. Lower trace, intracellular recording, upper trace, zero resting potential and (in *B*) the intracellular stimulating current. *A*, propagated spike evoked by external stimulus about 1 cm. distant from the recording site. Owing to movement the electrode came out of the cell following the spike. *B*, spike evoked by a 10 msec. depolarizing pulse applied with a second intracellular electrode close to the recording site.

the general theory of bioelectric activity was described at the International Symposium on Comparative Bioelectrogenesis that was held in Rio de Janeiro in 1959 (57).
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