The Electrophysiology of Electric Organs of Marine Electric Fishes

III. *The electroplaques of the stargazer, Astroscopus y-graecum*

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ABSTRACT The electroplaques of *Astroscopus y-graecum* were studied *in situ* with microelectrode recordings. Despite the distant taxonomic relations and the different origins of the organs, their properties in the teleost and torpedine marine electric fishes are remarkably similar. Only the innervated membrane (the dorsal) is electrogenically reactive in *Astroscopus*, and it, too, does not respond to electrical stimuli. As in the torpedine fishes, the uninnervated membrane of the electroplaques offers a very low resistance to the discharge of the innervated membrane. Additional direct evidence for electrical inexcitability of the reactive surface was obtained by denervating one of the bilateral organs. The denervated one did not respond to strong electrical stimuli which evoked responses in the opposite, innervated organ. The denervated electroplaques had a normal resting potential and were depolarized by acetylcholine and carbamylcholine similarly to normal cells. Other properties related to electrical inexcitability were also demonstrated. A pharmacological finding of considerable theoretical significance is that desensitization occurred on depolarizing cells with acetylcholine but was absent on depolarizing them with carbamylcholine.

INTRODUCTION

The stargazer, *Astroscopus*, is the only known marine teleost which possesses electric organs. It is found off the Atlantic coast from Delaware in the United States southward to Brazil. Two species (*A. guttatus* and *A. y-graecum*) are recognized in the northern zone of this distribution and one (*A. brasiliensis*) in the southern. Described and studied first by Dahlgren and Silvester (14), and later studied anatomically by White (25), the electric organ of this fish has not hitherto been examined in detail with modern electrophysiological

techniques. Preliminary work[1] showed that the organ responds to electrical stimuli with an irreducible latency, an indication that its electroplaques are electrically inexcitable (16) and like those of elasmobranch electric fishes (17) generate only postsynaptic potentials (p.s.p.'s). This paper, part of a series on electric organs of marine fish (6, 7), confirms that finding and provides other information on the electrophysiological and pharmacological properties of *Astroscopus* electroplaques.

METHODS

Methods similar to those used with the organs and electroplaques of *Torpedo nobiliana* (7) and *Narcine brasiliensis* (6) were employed. A notable difference, however, was the study of single electroplaques *in vivo*, which was made possible by anatomical characteristics of the preparation. The cranial bones in *Astroscopus* are for the most part fused and the head could be gripped in a holder (5) so as to immobilize the electric organs adequately for microelectrode penetrations. A jet of sea water was passed into the mouth for respiration. No anesthesia was necessary, for once fixed in the holder the fish became quiescent and did not discharge their organs.

The location of the organ, immediately below the dorsal skin surface, permitted its exposure merely by lifting a flap of the skin. With the flap replaced, the fish and electric organ survived very well, and the same individual could be studied a number of times.

Anatomical Features of the Electric Organ

The electric organ of *Astroscopus* derives from eye muscles (13), four of the six muscles contributing to its formation (25). The electroplaques lie in the horizontal plane, in a column which extends vertically from the dorsal surface of the head to the roof of the mouth. The cells do not lie uniformly in successive layers, one above the other, as they do in the torpedine electric fishes, but overlap irregularly (Fig. 1 *B*). An electroplaque may also extend out to overlap itself in the same way that successive threads in a screw overlap (14). Approximately eight to ten cells make up a layer of the organ. Those near the center are largest, attaining areas of 0.5 to 1 cm^2.

The dorsal surface of the electroplaque is smooth. The ventral surface has numerous finger-like projections which constitute most of the thickness of the cell (about 30 to 40 μ). There is little free space between cells. The number and length of the projections obviously increase greatly the area of the ventral

[1] That work was carried out in 1957, with E. Amatniek, R. Mathewson, and A. Mauro, at the Marineland Research Laboratory, St. Augustine, Florida. The four fish available for the present study, all *A. y-graecum*, were shipped to Woods Hole from that laboratory through the courtesy of its Director, Mr. F. G. Wood, Jr. We wish to thank him and his staff and Mr. R. Mathewson, Science Curator of the Staten Island Institute of Arts and Sciences, for their help in procuring and maintaining the fish.

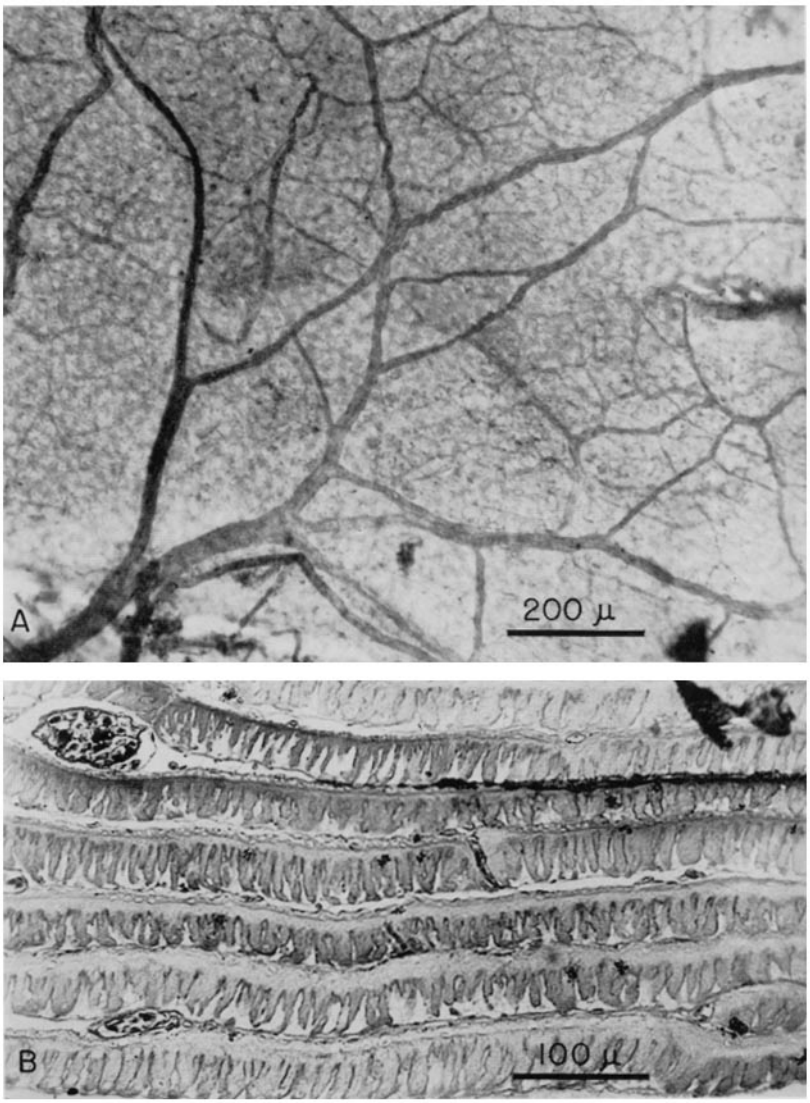

FIGURE 1. Innervation and structure of electroplaques of *Astroscopus*. *A*, region of the dorsal surface of a single electroplaque teased from formalin-fixed material and stained with methylene blue. A nerve bundle enters from the lower left and branches profusely. The branches may run together again after giving off other branches. Small blood vessels are seen in the upper left and below the main bundle. *B*, electroplaques in cross-section, dorsal surfaces uppermost. Preparation fixed and stained in osmic acid. The dorsal surfaces, which receive the innervation, are smooth. The long papillae of the ventral surface form most of the thickness of the cell. Nerve bundles running between cells are seen in the upper and lower left. The edge of a cell lying between two layers (lower right) illustrates the characteristically irregular layering of the electroplaques. Just to the right of center is seen a vertical fissure which probably represents the apposition of two cells in a single layer.

surface as compared with that of the dorsal, but the relative areas have not been determined.

INNERVATION A single, large electric nerve enters the electric organ on its caudal and medial border. The nerve may be sectioned easily at this place, and denervation was performed on one side of one fish. The animal survived the operation and experimental manipulations repeated at various intervals until it was sacrificed after 4 weeks.

The cells are innervated on their dorsal surfaces, by several branches of the electric nerve, each branch being composed of numerous fibers (Fig. 1 *B*). These branches divide extensively and different ones run parallel, diverge, and come together again to form a complicated plexus on the surface. The innervation pattern is somewhat like that found in the accessory organ of *Narcine* (6). Despite the presence of several nerve fibers at many regions of the membrane it was usually possible to obtain an all-or-none response (*cf.* Figs. 3, 6–8). Either the different axons had identical thresholds or one had a much lower threshold.

RESULTS

A. *The Reflex Discharge*

Reflex activity was approximately synchronous in the bilateral organs and was composed of nearly uniform pulses of about 5 msec. duration. Occasional small pulses occurred (Fig. 2 *A*) which could be of somewhat different amplitude on the two sides. The responses were produced at frequencies up to 200/sec. or occasionally somewhat more, so rapidly that one pulse occurred before the end of the previous response. The frequency was variable, a discharge sometimes stopping, then commencing again. The frequency might vary abruptly during the train of discharges. Usually the highest frequency occurred in the early part of the response (*A*, *D*), but the rate of discharge could also accelerate during a train (*B*). A small additional component which sometimes occurred on the falling phases of the pulses, might have arisen from stimulation of the electric nerves by the organ discharge (*cf.* reference 7). Sometimes the organ produced only a short train of pulses (*E*), or even a single pulse (*F*). While both organs were excited during reflex activity, the relative amplitudes of the two discharges occasionally varied (*A*, 3rd response from end) indicating that the electric nerves were unequally activated.

Recording with the dorsal surface of the organ in air, the response amplitude was about 2 to 6 v. The pickup over one organ from discharge of the other was rather small in this recording situation as may be seen from *D–F* in which one organ had been denervated. Recording with the animal im-

mersed in sea water, the amplitude was greatly reduced indicating loading down of the electric organ (*C*).

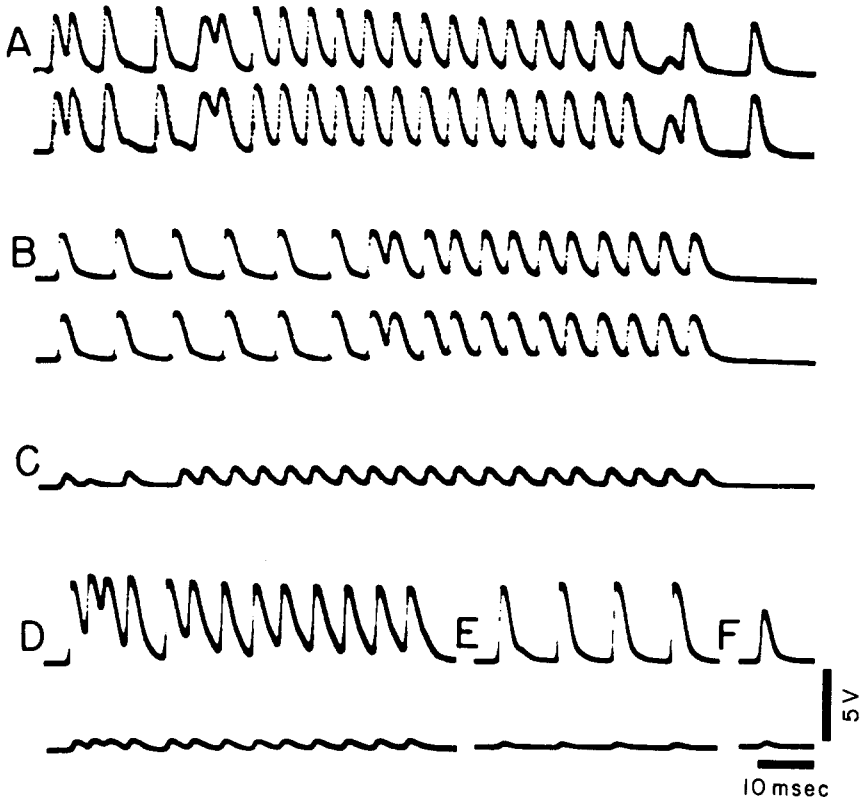

FIGURE 2. Patterns of discharges of *Astroscopus* electric organs. *A*, *B*, simultaneous records from the two organs in each of two different fish. A probe electrode was placed on the skin over each organ, with the dorsal surface of the fish in air. The reference electrode was on the ventral surface and grounded; dorsal negativity up. *C*, same fish as in *B*, but recording with the animal covered by sea water. *D–F*, simultaneous recordings as in *A*, *B* from a fish one of whose electric organs had been denervated. The electrode on the denervated organ (*lower traces*) registered only a small pickup of the activity of the other organ.

B. *Resting and Action Potentials of Single Electroplaques*

Fig. 3 illustrates the records obtained *in situ* when a column of electroplaques was penetrated from the dorsal surface with a microelectrode, while a pair of fine stimulating electrodes excited the nerve on the surface of the uppermost electroplaque. Recording was monopolar against an earth lead on the posterior part of the body. The position of the microelectrode in the prepara-

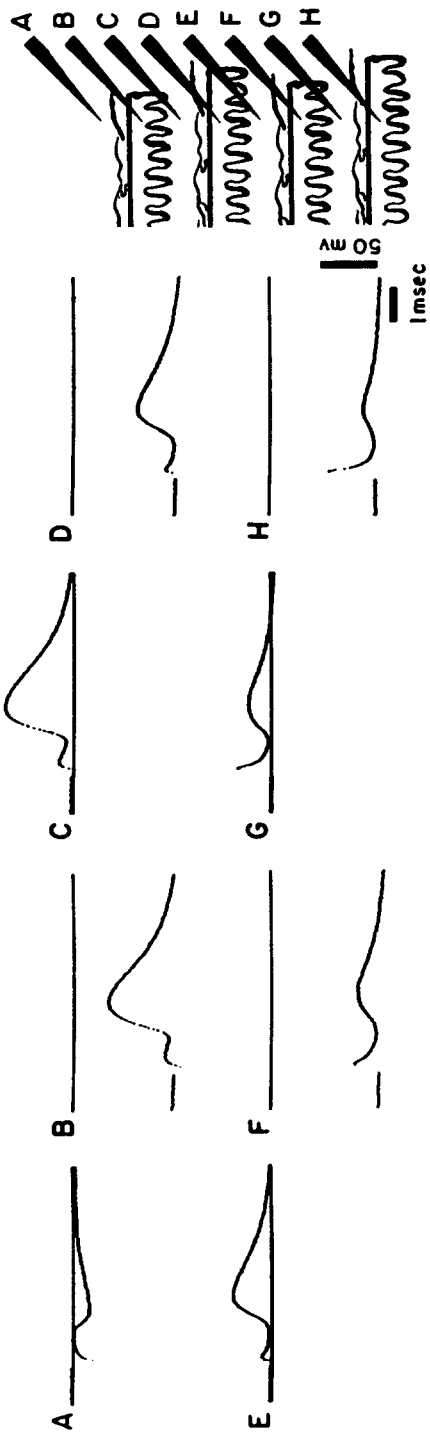

FIGURE 3. Monopolar recording of the activity of the superficial electroplaque from an organ *in situ*. The activity was produced by stimulating with a pair of electrodes on the surface. Sites of the recording microelectrode indicated in diagram are inferred from the successive appearance and disappearance of resting potential as the electrode was advanced. With the stimulus employed activity was restricted to the superficial cell, as is indicated by the sequence of changes in the response sign and amplitude. The changes in the latter indicate that the ventral uninnervated membrane has a negligible resistance compared to that of the dorsal innervated membrane when the latter is inactive. Further description in text.

tion was inferred from the sequential appearance and disappearance of resting potentials.

On the surface there was a small, negative response (Figs. 3 *A*). The appearance of a large resting potential (*ca.* −85 mv.) signalized penetration of the uppermost cell (*B*). The response was a depolarization of about 60 mv. lasting 4 to 5 msec. The change in sign indicates that the innervated membrane of this cell was active. Exit from the cell on its uninnervated surface (*C*) was denoted by disappearance of the resting potential, but the amplitude of the response was little changed. Penetration of the underlying electroplaque (*D*) was indicated by a resting potential that was about the same as in the upper cell. However, the response was decreased to about half. It did not diminish appreciably when the electrode was pushed out of the electroplaque (*E*), but again decreased by half when the next electroplaque was penetrated (*F*). Another sequence of exit (*G*) and entry (*H*) produced the same results.

These data indicate (*a*) that only the innervated surface of the uppermost electroplaque was active, and (*b*) that the uninnervated, ventral surfaces had very low resistance compared to the inactive innervated surfaces of the underlying cells. It is also to be noted that the response of the superficial electroplaque arose with a latency of about 1 msec. despite the fact that the stimulus was applied directly to the innervated surface. The latency could not be reduced with very strong stimulation. The irreducibly long latency indicates that the electroplaques are electrically inexcitable (16, 20). In contrast, the fibers of the eye muscles respond to electrical stimuli.

C. *Evidence for Electrical Inexcitability of the Electroplaques*

EFFECTS OF MEMBRANE POLARIZATION ON THE RESPONSE OF A SINGLE ELECTROPLAQUE Strong depolarization of innervated electroplaques with an intracellularly applied current did not produce a response (Fig. 4). Neither depolarization nor hyperpolarization affected the latency of responses evoked by stimulating the nerve. The amplitudes of these responses were, however, altered in the way expected of depolarizing synaptic activity (16, 20). They were increased by hyperpolarizing the membrane and decreased by depolarizing it. In the experiment of Fig. 4 the relation $\frac{E \text{ response}}{E \text{ polarization}}$ was linear, with a slope of 0.72. The calculated value of the membrane potential beyond which the response would have been inverted was approximately 100 mv. positive from the resting potential. As was discussed with reference to *Torpedo* (7; *cf.* also references 9 and 12), the non-uniformity of depolarization from a point source would lead to overestimation of the reversal potential.

UNRESPONSIVENESS OF DENERVATED ELECTRIC ORGAN TO ELECTRICAL STIMULI Further evidence that *Astroscopus* electroplaques are electrically inexcitable was obtained on the one denervated organ (Fig. 5). Stimuli to

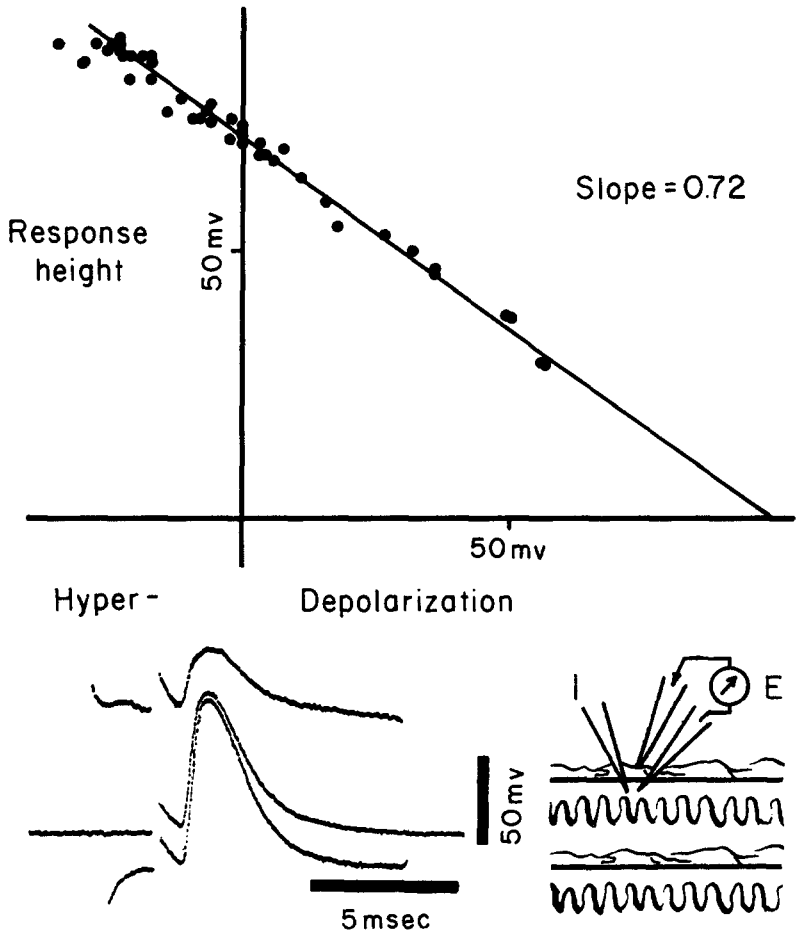

FIGURE 4. Effects of polarizing currents on the response of a single electroplaque. Differential recording across the innervated membrane (E in diagram). Changes of membrane potentials by applying currents with an independent intracellular electrode (I in diagram) produced no response. However, neurally evoked responses increased on hyperpolarizing the membrane, decreased on depolarizing it. Sample records in lower left. Note that the latency of the response did not change with changes in membrane potential. Graph shows complete data for the experiment.

the skin over the innervated organ excited it after a latency of 1 msec. (*A*), but responses could not be obtained from much stronger stimuli applied either directly to the denervated organ (*B*) or through the overlying skin. The denervated electroplaques had resting potentials (*C* and *D*) comparable

in magnitude to those of the innervated electroplaques. As will be shown below (Fig. 13), the denervated electroplaques were depolarized by suitable pharmacological agents in the same way as were innervated cells.

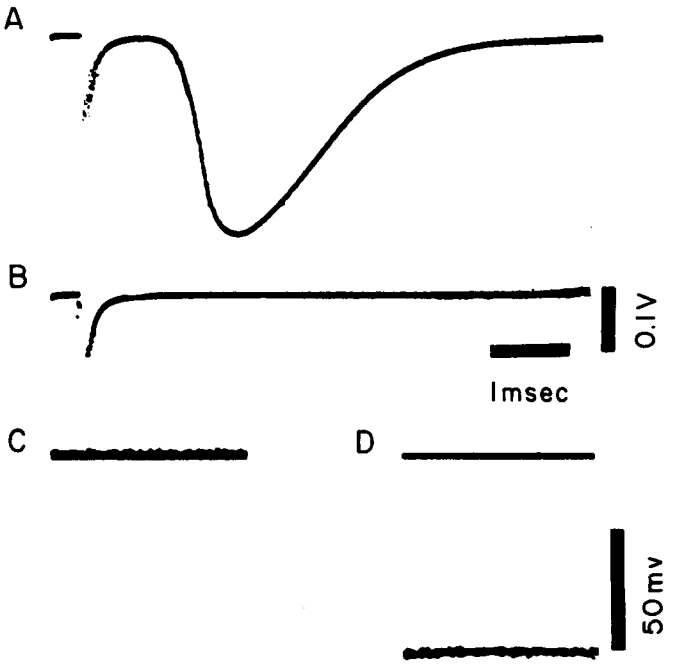

FIGURE 5. Electrical inexcitability of a denervated organ. Strong stimuli were applied to the normal organ through the overlying skin and to the denervated organ both before and after removal of the skin. *A*, the innervated organ produced a response after a latency of about 1 msec. *B*, the denervated organ produced no response. *C*, *D*, microelectrode recording before and after penetrating an electroplaque of the denervated organ. The resting potential of about —90 mv. was like that of innervated electroplaques. The denervated cells responded with depolarization to applications of acetylcholine or carbamylcholine (*cf.* Fig. 13).

D. *Interactions of Responses*

SUMMATION OF TWO RESPONSES EVOKED BY THE SAME NERVE FIBER Repetitive responses of a single electroplaque can summate and fuse (Fig. 6) in a manner that is found in other electrically inexcitable activity (16, 20). The second of a pair of stimuli delivered to the nerve could evoke a second response of the electroplaque while the first was still in progress (*D*, *E*). The potential of the second response then summed with that of the first. The degree of summation indicates that at an interval slightly less than 3 msec. (*E*) the amplitude of the second response was somewhat decreased. At an

interval of about 2 msec. (*F*) no second response occurred, presumably because the nerve was refractory to the second stimulus.

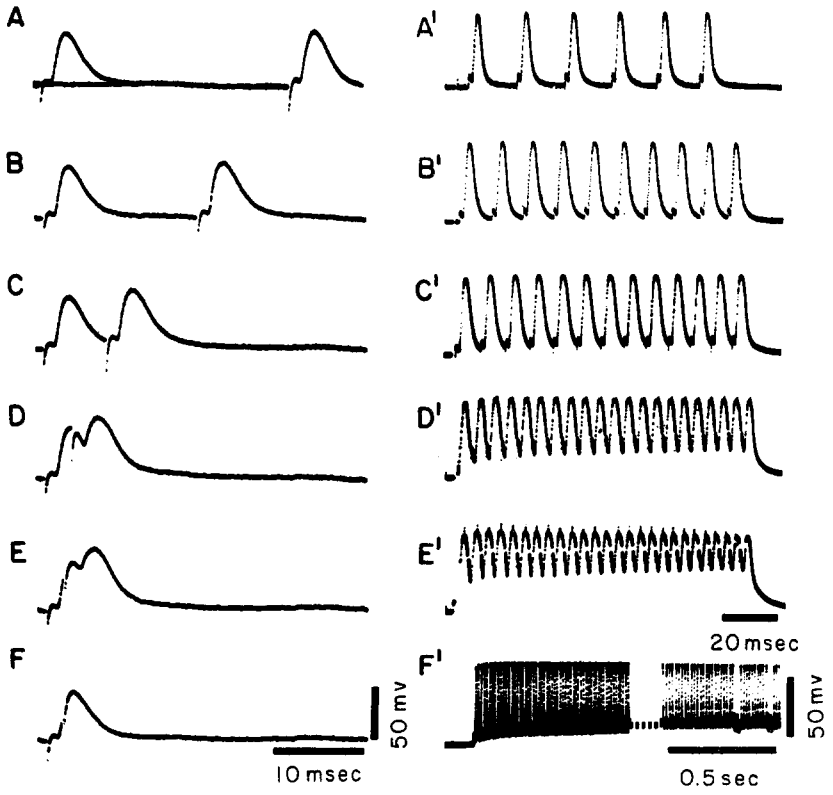

FIGURE 6. Repetitive responses produced by stimulating the same nerve fiber. Intracellular recording from a single electroplaque. *A–F*, responses to paired stimuli. The second response in *E*, occurring about 3 msec. after the start of the first, reached a higher peak value due to summation, but the depolarization produced by the second stimulus was actually slightly smaller than in the testing response in isolation (one of two superimposed traces in *A*). *F*, the second stimulus failed to produce a response when applied 2 msec. after the first. *A'–F'*, another experiment. Summation of responses at different frequencies of stimulation. *F'*, continuous stimulation at about 80/sec., 2.5 sec. intervene at the dotted line. The responses failed to be maintained at full frequency.

However, increase of the total amplitude of the response of a single electroplaque by summation has a limit that is approached asymptotically. This is the characteristic electrochemical potential of the system, or the reversal potential (11, 20). Thus, the maximum height reached by the responses is nearly the same in Fig. 6 *D* and *E*. When repetitive pulses were elicited at various frequencies (*A'–E'*) the responses at progressively higher frequencies occurred on a growing level of summated depolarization, but reached a

nearly constant height. When the stimulus to the nerve was maintained at a high frequency the response sometimes dropped out in an all-or-none fashion (*F'*) indicating that the nerve had stopped producing impulses. During this pause the level of the summated potential decreased, but returned when the response developed again.

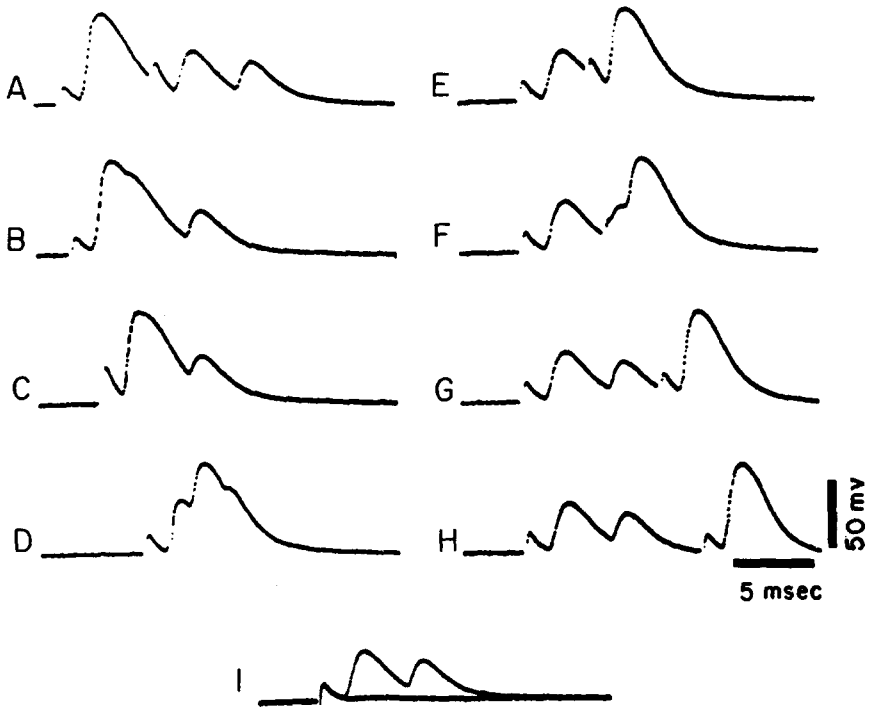

FIGURE 7. Summation of responses produced in one electroplaque by two nerve fibers Intracellular recording. Separate stimulating electrodes were applied to two sites on the superficial electroplaque (about 4 mm. apart). One of the stimuli produced a double peaked but all-or-none response (just threshold stimulus in *I*). The second component was probably caused by delay at some region of the nerve fiber. The response to the other stimulus was a single depolarization of more than 70 mv. amplitude. The responses behaved independently, except that they did not add completely.

SUMMATION OF RESPONSE PRODUCED BY TWO NERVE FIBERS In the experiment of Fig. 7 two nerve fibers to an electroplaque were stimulated by separate electrode pairs. The two responses were different in form. One, perhaps because of a block at a branch of the nerve fiber producing it, had two small elevations separated by almost 4 msec., the second elevation arising on the falling phase of the first. This double response was produced by a threshold stimulus and always appeared in all-or-none fashion (*I*). The other axon evoked a large single component response. Neither of the responses

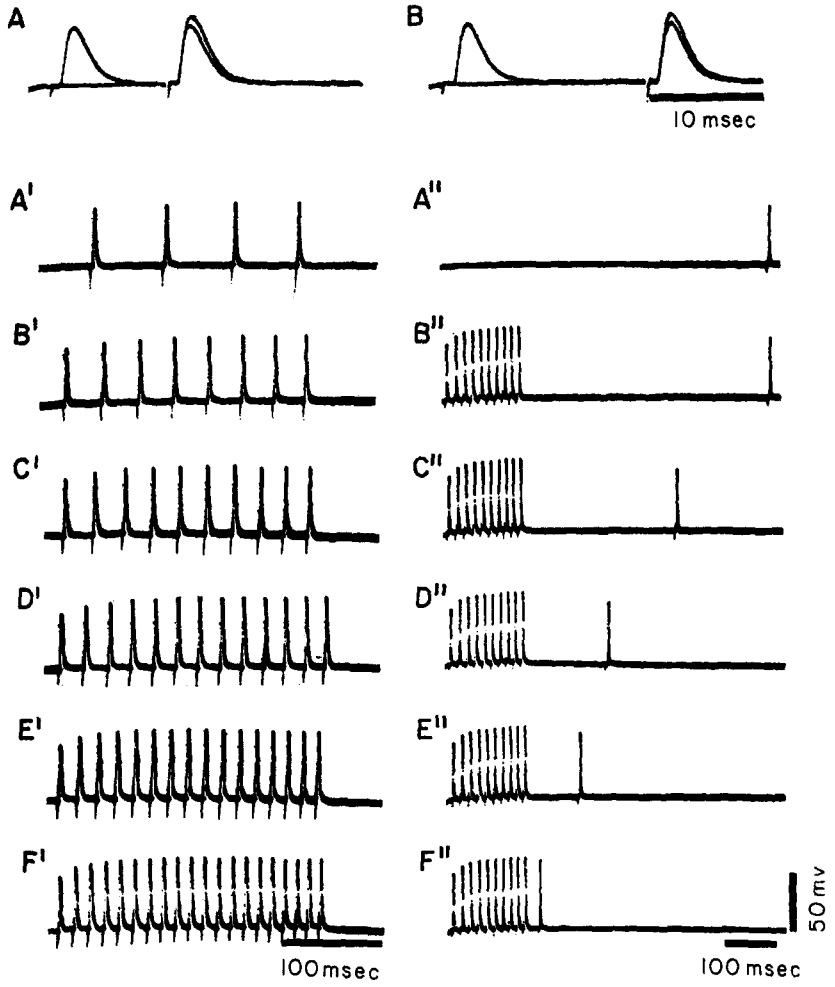

FIGURE 8. Facilitation of responses in a single electroplaque. Intracellular recording. Repetitive responses evoked by stimuli through one pair of electrodes on the surface of the electroplaque. *A*, *B*, two superimposed sweeps, one with the testing response alone, the second with a conditioning response preceding. The amplitude of the testing response increased. *A'–F'*, sequence of responses at increasing frequencies of stimulation. The facilitation was largest in the second response, but the subsequent responses up to the fifth or sixth also increased. *A''–F''*, decay of facilitation after a train of responses at about 60/sec. The testing response 25 msec. after the end of the train (*F''*) was considerably smaller in amplitude than the last response of the train, but about equal to the third response of the conditioning activity.

blocked the other, but summation was limited by the electrochemical characteristics as described above.

When the two responses were elicited concurrently (*C*) the first of the double elevations coincided with the large single elevation. The peak po-

tential generated by the summed activity was only slightly larger than that of the large response, but the second elevation of the other response was still produced. The three peaks signifying the independent activities were evidenced when either nerve was stimulated slightly ahead of the other (*B*, *D*). When the large response coincided with the second of the double elevations (*E*), the peak of the summed potential was again about that of the single response. No matter how the two responses were superimposed (*B–F*), the peak potential was scarcely larger than that of the large single response. This result indicates that the latter was close to the reversal potential of the membrane activity.

HOMOSYNAPTIC FACILITATION During repetitive stimulation of a nerve the amplitudes of later responses increased in some of the cells tested (Fig. 8 *A*, *B*). The facilitation increased during a train of stimuli (*A'–F'*), and somewhat more rapidly when their frequency was higher. Most of the increase occurred early in the train. Once developed, however, it persisted throughout the train, and decayed over a period of about 400 msec. following its cessation (*A"–F"*). The initial decay was most rapid. Thus, the steady facilitation level reached at a stimulation rate of 60/sec. (*i.e.*, an interval of 17 msec.) was markedly diminished when a testing response was evoked after a 20 msec. interval. Heterosynaptic facilitation, increase in the response evoked by one nerve due to prior conditioning by stimulation of another, was not observed, either on paired (Fig. 7) or tetanic stimulations.

E. *Pharmacological Properties*

RESPONSES TO APPLYING ACETYLCHOLINE Three recording microelectrodes were used in the experiments of Fig. 9 (*cf.* diagram). One was intracellular, one just outside the dorsal surface, and one just below the ventral surface of the superficial electroplaque. The upper trace of Fig. 9 (V_3) shows the monopolarly recorded response outside the dorsal surface, a negativity, as in Fig. 3 *A*. The lower (V_1) and middle (V_2) traces show the response as it was recorded differentially across the innervated face and across the electroplaque respectively. A fourth trace, at zero time coincident with V_2, shows the reference zero potential for V_1 and V_2 in all the records of the figure. The resting potential recorded on V_1 was about 85 mv., and the responses recorded on V_1 and V_2 were about equal, indicating absence of appreciable voltage drop across the uninnervated membrane. These characteristics of the differential recordings could be predicted from monopolar records such as those shown in Fig. 3.

On applying 10^{-3} M acetylcholine to the upper surface, the intra- and transcellular records shifted upward about equally, indicating depolarization

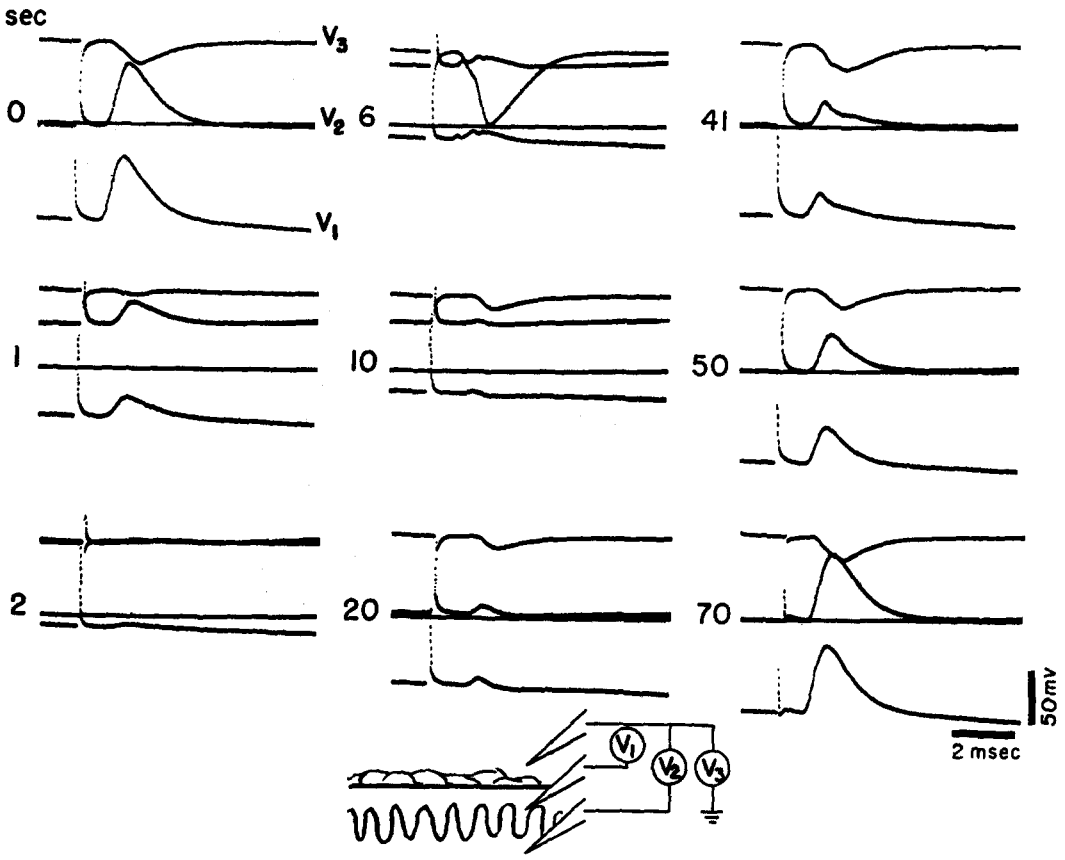

FIGURE 9. Changes in membrane potential and response amplitude on application of acetylcholine to upper surface of an electroplaque, registering with four traces simultaneously. V_1–V_3, recordings with microelectrodes as in diagram. Positivity of the dorsal surface is upward in V_3 and downward in V_1 and V_2. The flat trace is the reference zero for V_1 and V_2. The reference zero for V_3 is given by its position in the first frame. Sweeps were photographed at 1/sec. and the records are samples taken at the times indicated on the left. Acetylcholine was applied between the first two sweeps. It caused upward movement of traces V_1 and V_2 and slight downward movement of V_3, indicating depolarization of the innervated membrane. The response was reduced and could hardly be seen at 2 sec. At 6 sec. the stimulus was increased and excited underlying cells. This response registered as a large deflection on V_3, but had little effect on the differential recordings from the uppermost depolarized cell. The stimulus was again decreased for subsequent records. Note the more rapid recovery of the resting potential as compared with the return of the response amplitude (50 and 70 sec. records).

of the innervated face without a change in potential across the uninnervated face. After 1 sec. the peak of the neurally evoked, intracellularly recorded response reached a slightly greater depolarization than before the drug was applied. However, starting from the depolarized level of the response to the

drug, the neurally evoked response was much smaller in all three modes of recording it.

The maximum depolarization caused by the acetylcholine occurred within 2 sec., to about 15 mv. inside negative. The intracellularly and extracellularly recorded responses were then nearly absent. This level of depolarization probably was at or near the reversal potential for the p.s.p. of *Astroscopus* electroplaques. Thus, the potential initially evoked by stimulating the nerve was not the maximal E.M.F. which the electroplaque membrane was capable of generating. Possibly, the recording electrodes were not in the most active region of the neurally excited membrane, or the amount of the neurally released transmitter was less than required for maximal excitation of the membrane. Also, the neural stimuli probably may not have activated as large an area of the membrane as did the drug, so that resistive loading of the neurally activated membrane by inactive regions would have diminished the response to this stimulus.

At 6 sec., while the drug was still exerting its maximal effect, a stronger stimulus was applied through the external electrodes to ascertain that the added fluid had not reduced the stimulating current. The monopolarly recorded external potential became large, but only small increments appeared in the differential recordings. Thus, the large negativity was due to activity of underlying cells. The small increments contributed to the potential of the depolarized uppermost electroplaque may have been due to the IR drop of the currents generated by the lower cells. The IR drop across the depolarized cell was smaller than that across a resting cell that was not depolarized (Fig. 3), indicating that the drug-activated innervated membrane had a lowered resistance.

The resting potential of the superficial electroplaque returned more rapidly than did the response height (10 and 20 sec. records). The surface was then rinsed with Ringer's solution to wash away the acetylcholine. The resting potential returned rapidly, but the response recovered more slowly (41 to 70 sec.).

The full time course of these effects is shown graphically in Fig. 10. The peaks of the responses in both differential recordings are plotted (crosses), as well as the long lasting potential change produced by the acetylcholine (dots). The upper set of values, which show no resting potential, are for the recordings across the cell. The lower set represents the recordings across the innervated membrane.

It might have been expected that the neurally evoked response and the drug-produced depolarization would have summed, subject to an asymptotic approach to the reversal potential. While summation occurred, the graph of Fig. 10 shows clearly that the response became depressed on depolarization of the cell by acetylcholine. After about 10 sec. the peak of the response was

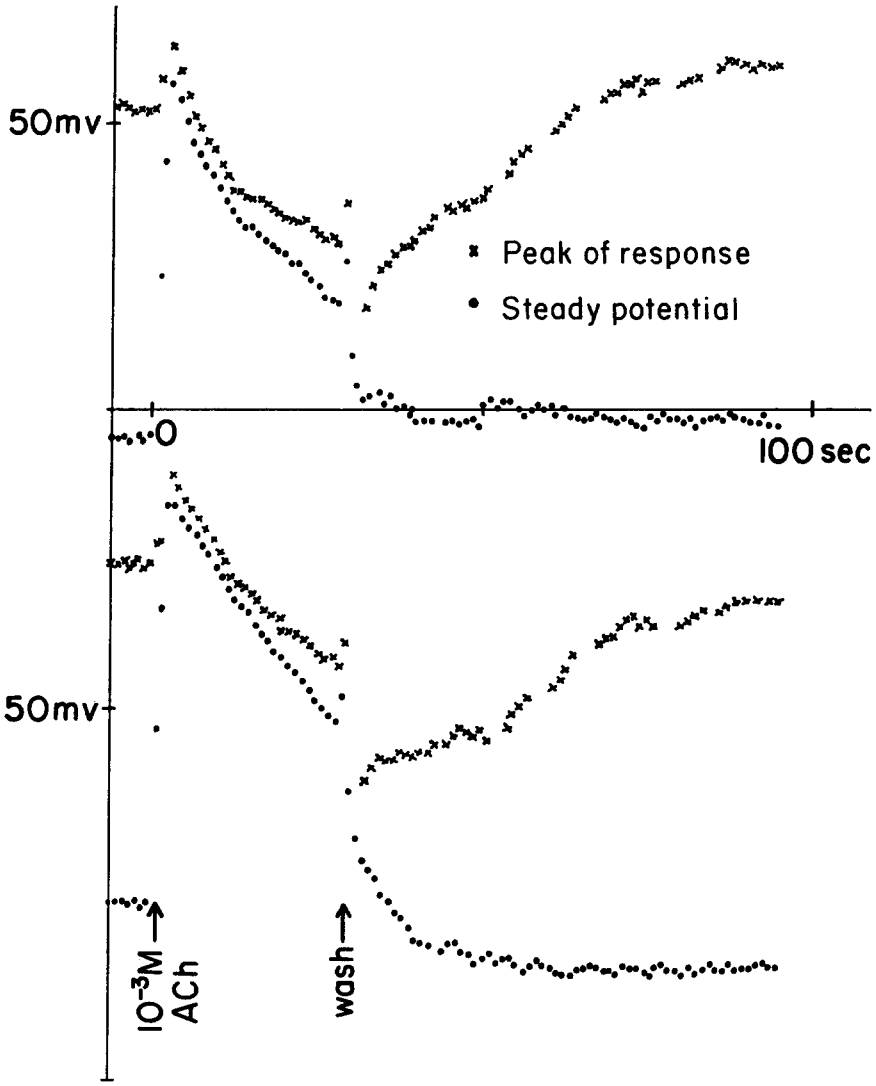

FIGURE 10. Time course of changes in the experiment of Fig. 9. The upper set of values is for V_2 (recording across the cell) and the lower is for V_1 (across the innervated membrane). Note the depression in the response amplitude during application of acetylcholine and long after return of the resting potential.

lower than it had been before application of the drug. On removing the drug the membrane potential returned to its initial value in about 20 sec. The response, however, took much longer to recover. Thus, as in the end-plate preparation (22), acetylcholine has two effects on *Astroscopus* electroplaques. It activates the synaptic membrane (18, 19) causing its depolarization. It also apparently depresses or "desensitizes" the responsiveness of the mem-

brane to the transmitter produced by the neural stimuli, since the possibility that it reduces the amount of neurally released transmitter seems unlikely. The decrease in depolarization that occurred while the acetylcholine was still on the surface presumably was in large measure also due to the desensitization, although some hydrolysis of the drug must also have occurred. The initial increase in depolarization on washing suggests hydrolysis in a layer of

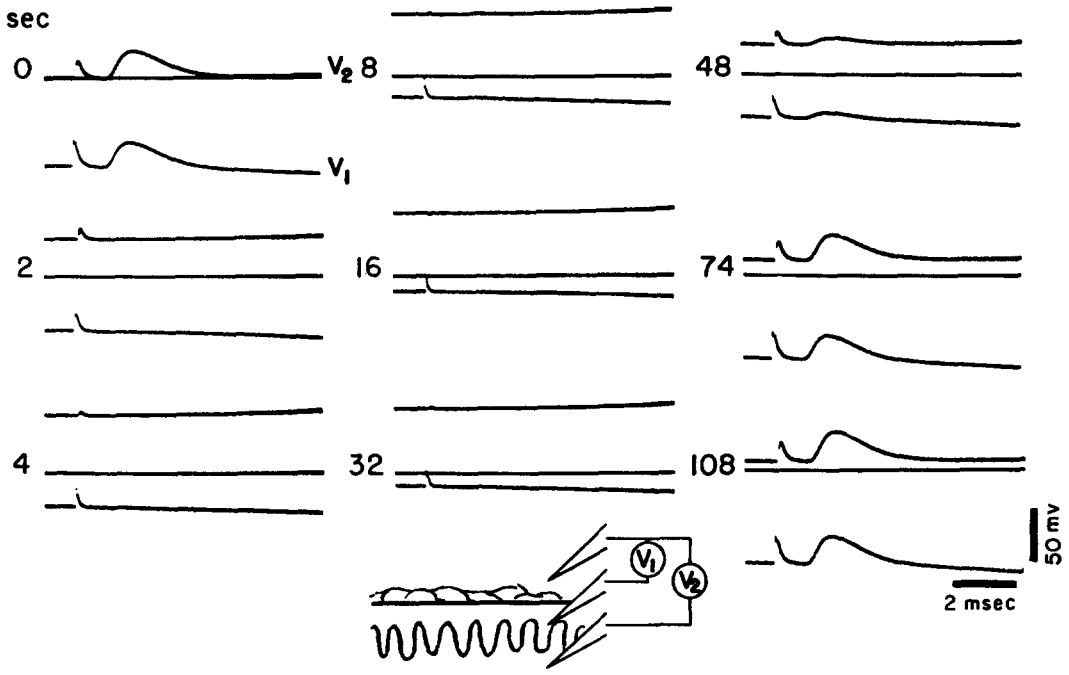

FIGURE 11. Effects of carbamylcholine on resting potential and response amplitude. Conditions as for the experiment of Figs. 9 and 10, except that the monopolar recording (V_3 of Fig. 9) is omitted. The response in this experiment was small and disappeared during application of the drug. The depolarization did not decline until the preparation was flushed with saline solution to remove the drug. Note rapid recovery of response amplitude.

fluid immediately over the cell. The stirring associated with the washing could initially increase the concentration of acetylcholine at the surface.

EFFECTS OF CARBAMYLCHOLINE This compound, which is also a synapse activator drug (18, 19), was effective in lower concentrations than was acetylcholine in depolarizing the electroplaques (Figs. 11 and 12). The experimental conditions for Figs. 11 and 12 were the same as those of the experiment of Figs. 9 and 10. However, the monopolar recording from the dorsal surface has been omitted from Fig. 11. Maximum depolarization was produced by 10^{-4} M carbamylcholine about 8 to 10 sec. after applying

the drug. The responses to nerve stimulation were small in this experiment and disappeared during the application of the drug. The disappearance might have been due to shorting of the external stimulating electrodes, but

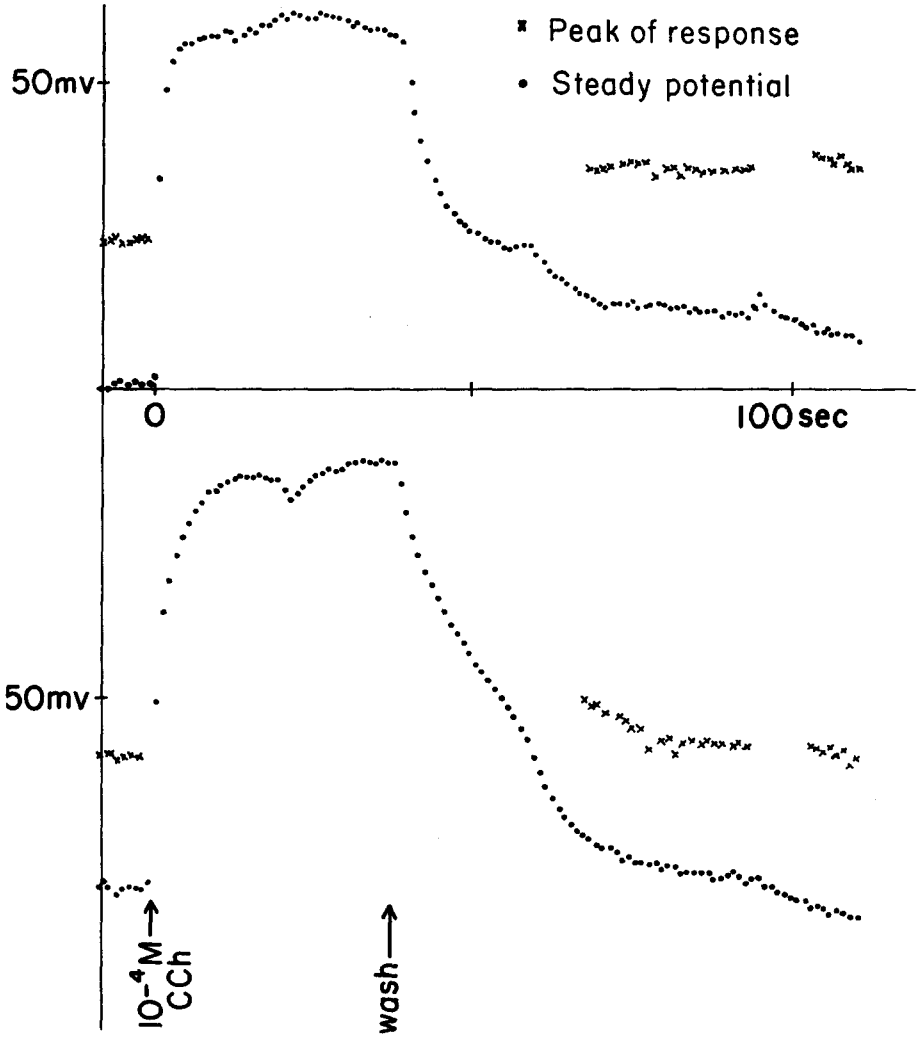

FIGURE 12. Time course of changes in the experiment of Fig. 11, plotted as in Fig. 10. The repolarization on washing was much slower than with acetylcholine but the response amplitude recovered more rapidly.

on subsequent addition of fluid in washing out the drug the response reappeared. It is therefore likely that the depolarization produced by the drug, to −12 mv., brought the membrane potential close to its reversal potential.

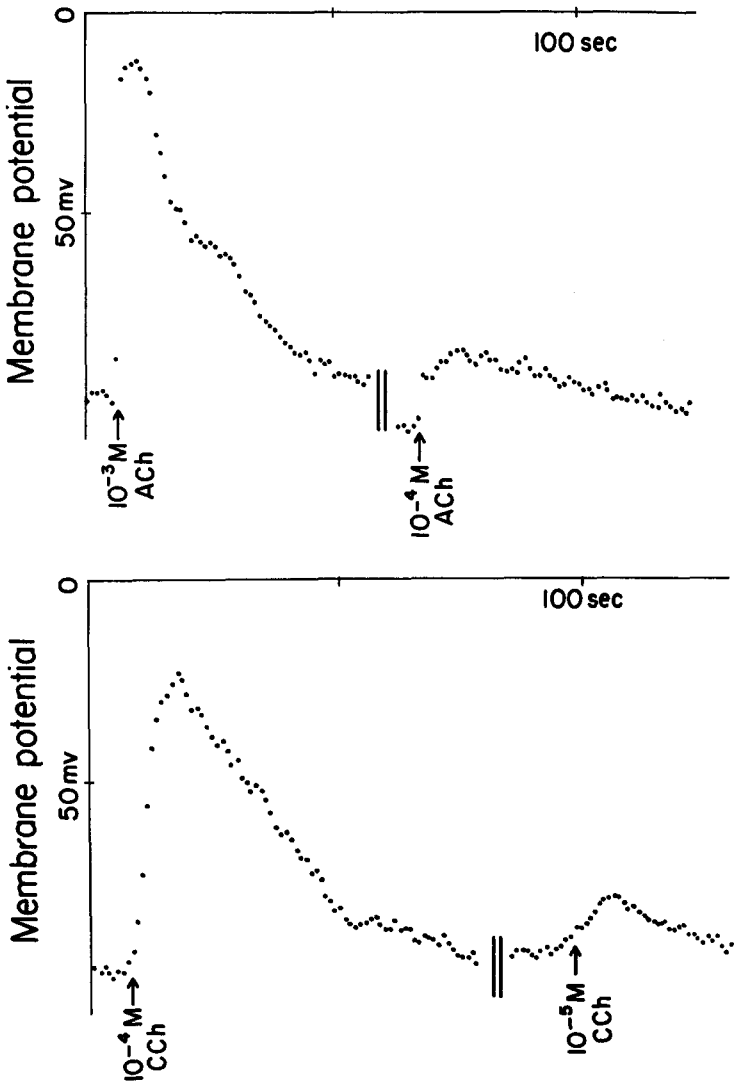

FIGURE 13. Effects of acetylcholine (ACh) and carbamylcholine (CCh) on denervated electroplaques. Same animal as in Fig. 5. CCh was effective at 10^{-5} M, ACh at 10^{-4} M. The depolarizations produced by tenfold higher concentrations of the drugs were probably nearly maximal (*ca.* 85 and 75 mv.). The drugs were washed off soon after producing their maximum effects.

Unlike the case with application of acetylcholine (Figs. 9 and 10) the cells depolarized by carbamylcholine remained depolarized until the drug was washed off (Fig. 11, 32 sec.; Fig. 12). By the time the membrane became repolarized the response to nerve stimulation reached full amplitude. Both the maintained depolarization during application of the drug and the re-

covery of the response indicate that, if it does so at all, carbamylcholine does not desensitize the membrane nearly as much as acetylcholine does. Absence of hydrolysis of the carbamylcholine by esterases must have contributed to the maintained maximal depolarization. This factor was probably also responsible for the slow repolarization on washing. In the case of acetylcholine, hydrolysis would have speeded disappearance of the drug (Fig. 10).

RESPONSES OF DENERVATED ELECTROPLAQUES TO SYNAPSE-ACTIVATING AGENTS. Denervated electroplaques also were depolarized by acetylcholine and carbamylcholine (Fig. 13). After denervation for 4 weeks there seemed

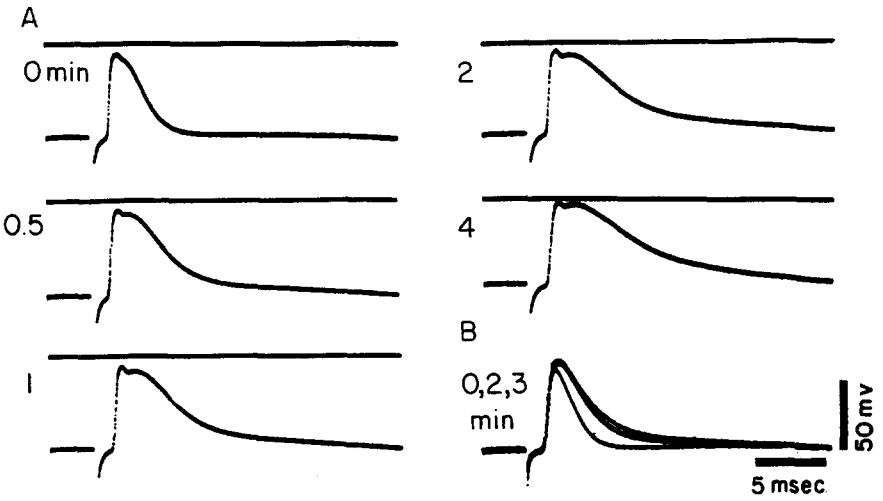

FIGURE 14. Prolongation and increase of responses by 10^{-4} M eserine. Two experiments are shown: *A*, as single traces taken at different times indicated on the left; and *B*, as superimposed traces of records made at 0, 2, and 3 min. after applying the drug.

to be no sensitization of the denervated membrane, such as occurs in muscle end-plate and other synaptic systems (4, 10). Large effects were produced by 10^{-3} M acetylcholine and 10^{-4} M carbamylcholine, as was also the case in the innervated cells. In each case a tenfold lower concentration produced small effects. Therefore, the dose-effect curves must rise steeply in this range of concentrations. However, quantitative determinations of sensitivity and of drug kinetics remain to be carried out.

EFFECTS OF ESERINE Eserine (10^{-4} M) did not affect the resting potential, but it prolonged the response of the innervated electroplaques (Fig. 14). This action was not as pronounced as in *Torpedo* electroplaques (7) and developed rather slowly. The amplitude also increased slightly.

DISCUSSION

ELECTRICAL INEXCITABILITY OF THE ELECTROPLAQUE AND ITS IMPLICATIONS The evidence that electroplaques of *Astroscopus* are electrically inexcitable is direct and conclusive. The electroplaques are not excited by large changes in membrane potential (Fig. 4). Denervated electroplaques which have a resting potential (Fig. 5) and are depolarized by synapse activator drugs (Fig. 13) in the same way as are innervated cells (Figs. 9 to 12) do not respond to stimulation applied to the organ. Elasmobranch electric organs, which are also electrically inexcitable (6–8, 17, 21), likewise become unresponsive to electrical stimuli when denervated or when curarized (15) as do the curarized single electroplaques (7). In contrast, the electrically excitable electroplaques of *Electrophorus* (1) and *Malapterurus* (23) when denervated or curarized continue to produce spikes in response to electrical stimuli. However, the p.s.p.'s which are evoked by indirect stimulation of innervated *Electrophorus* electroplaques by directly applied currents which hyperpolarize the reactive surface but excite the nerves are not produced in the denervated or curarized cells (1, 2).

Direct evidence for electrical inexcitability (16, 20) of the electroplaques is now also available for the other forms of marine electric fishes (6–8, 21). Accordingly, *Astroscopus* electroplaques, like those of all other marine electric fishes, can respond to electrical stimuli only by prior activation of their nerve supply. The responses evoked by the nerve impulses are p.s.p.'s (17), and since these postsynaptic membranes are electrically inexcitable, their responses must be caused by release of a transmitter agent from the presynaptic fibers (16, 20). Presumably, the transmitter is acetylcholine or some cholinomimetic substance, since acetylcholine and carbamylcholine depolarize *Astroscopus* electroplaques (Figs. 9 to 13), and eserine prolongs the neurally evoked responses (Fig. 14).

A constellation of properties that is characteristic of electrically inexcitable activity has been deduced theoretically and the presence of some of the properties may constitute indirect evidence for electrical inexcitability (16, 20). Like the electrically inexcitable electroplaques of the elasmobranch fishes (6, 7, 21) those of *Astroscopus* also exhibit all the features of this constellation for which they could be tested: (*a*) The organ discharge (Fig. 5) and the responses of individual electroplaques (Fig. 3, etc.) were always produced after a latency of at least 1 msec. (*b*) The latency was not affected by depolarizing currents which augment excitability of electrically excitable membrane nor by hyperpolarizing currents which diminish excitability of such membrane (Fig. 4). (*c*) The amplitudes of the responses varied linearly with the membrane polarization (Fig. 4), increasing with internal negativity

and decreasing with depolarization of the membrane. (*d*) The responses showed little refractoriness and could summate as is shown in Figs. 6 and 7 for single electroplaques and in Fig. 2 for the whole organ. The capacity to summate potentials is also shown by the prolonged responses produced by the synapse activator drugs (Figs. 9 to 13). (*e*) The differences in the forms of the responses produced on stimulating two different nerves (Fig. 7) reflect another property of electrically inexcitable membrane, that the responses are local in character since active propagation is absent. As in *Torpedo* (7) the large size of *Astroscopus* electroplaques and their innervation by a number of nerve fibers permit the demonstration of local variations in the amplitude as well as in the form of the non-propagated responses. Variation in the amplitudes of the postsynaptic potentials at different sites was also noted in the electroplaques of *Electrophorus* (1). (*f*) Facilitation a characteristic frequently observed in synaptic responses (20), also occurred, but only occasionally (Fig. 8). The rarity of facilitation in *Astroscopus* electroplaques is probably related to the size of the responses, which were already near the reversal potential. In many cases, therefore, the membrane may already have been maximally activated by the first neural volley. In others, only a small additional potential change would have been evidenced by facilitation, since even a large conductance increase can cause but little change in potential when the membrane is near its reversal potential.

Under the experimental conditions of recording from the intact surviving organ *in situ*, sufficient current could not be applied to an electroplaque to reverse the potential as has been done in the elasmobranch electroplaques (6, 7, 17, 21). The reversal potential calculated from the regression line in Fig. 4 would make the membrane positive intracellularly by some 15 to 20 mv. However, because of the localized source of applied current, the large surface of the cell compared to its space constant, and the distributed nature of the synaptic potential the extrapolation is subject to considerable error (9, 12, 21). More reliable values for the reversal potential are probably given by the maximum depolarization produced by applied drugs (Figs. 9 to 13) or by neural stimuli (Fig. 3). These measures are valid assuming a conductance change of the activated membrane which is large compared to the resting conductance. The value so determined, about -15 mv., is close to that for the neuromuscular junction (11) and for the chemosensitized membrane of denervated muscle fibers (4). Probably, therefore, the ionic mechanisms involved are the same, particularly in view of the pharmacological similarities of the membranes.

PHARMACOLOGICAL PROPERTIES The pharmacological properties of the *Astroscopus* electroplaques also accord with the behavior of electrically inexcitable synaptic activity (18, 19). The dose-effect curves for the cho-

linergic actions were rather steep. The maximum effects required high concentrations and the drugs in tenfold dilution of this value produced very small action (Fig. 13).

The greater effectiveness of carbamylcholine as compared with acetylcholine is referable to two factors. In the first place, acetylcholine is hydrolyzed whereas carbamylcholine is not. In the elasmobranch electroplaques cholinesterase is present in high concentration (3), but it has not been determined in *Astroscopus*. The second factor is desensitization which is produced by acetylcholine, but is caused by carbamylcholine to only a small degree, or perhaps is altogether absent.

Applications of eserine in low concentration to cholinoceptive membrane usually increase the amplitudes and durations of the responses to neural stimuli. Both effects are seen in Fig. 14 but to a smaller degree than in *Torpedo* electroplaques (7). Eserine probably potentiates amplitudes and durations of p.s.p.'s of cholinoceptive synapses by its action as an anticholinesterase (19). The different degrees of action on *Torpedo* and *Astroscopus* electroplaques may indicate that the ratios of esterase to the released transmitter are different or that the transmitter substances and/or the esterase are different in the two forms.

Absence of sensitization of denervated electroplaques to acetylcholine and carbamylcholine (Fig. 13) supports the conclusion (4, 24) that the higher sensitivity of denervated muscle fibers may not be due to sensitization of the denervated end-plate membrane. As a result of denervation some of the muscle fiber membrane which had previously been unresponsive to acetylcholine becomes converted to chemically responsive membrane that appears to have electrochemical properties of the synaptic membrane (*cf.* reference 4, Fig. 4) and to be electrically inexcitable (*cf.* reference 4, Fig. 5). Accordingly, the applications of testing drugs now can act upon a larger area of the fiber membrane and should be capable of producing larger depolarizations. Since the innervation is very dense, denervation could modify only a small area of previously inactive membrane in the electroplaques.

CONCLUSION

It is curious that of the widely dispersed family Uranoscopidae only the genus *Astroscopus* should be electric. In the gymnotid, mormyrid, rajid, and torpedine families of electric fish, all of which are represented by numerous genera, all the species examined have electric organs. Another feature is that *Astroscopus* is the only teleost marine electric fish. Although its electric organ derives from ocular muscles, in its properties it nevertheless resembles markedly the electroplaques of *Torpedo* and other torpedine electric fishes, in which the organs are derived from hypobranchial muscles. The resemblance is

not only with respect to electrical inexcitability but also with respect to the different membrane resistances of the innervated, reactive and uninnervated, unreactive surfaces.

The implications of the electrophysiological findings in marine electroplaques for the theory of comparative bioelectrogenesis have been discussed in detail elsewhere (21). At present it needs only to be pointed out that in cells in which electrically excitable activity complicates synaptic responses, indirect evidence for electrical inexcitability is more readily attainable than is direct evidence. Thus, the numerous findings regarding the properties of postsynaptic potentials in many systems (16, 20) may be taken as evidence that these electrogenic activities occur in electrically inexcitable membrane.

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