The Electrophysiology of Electric Organs of Marine Electric Fishes

II. *The electroplaques of main and acccessory organs of Narcine brasiliensis*

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ABSTRACT Studies on the electric organs of *Narcine brasiliensis* and particularly of the responses of the electroplaques of the accessory organ confirm and amplify data obtained on the electroplaques of *Torpedo nobiliana*. Only the innervated surface is electrogenically reactive and the uninnervated surface has a low resistance, as in *Torpedo* electroplaques. However, in the accessory organ of *Narcine* the innervated surface is the dorsal, rather than the ventral, and it has a different pattern of innervation. The responses of single cells of the accessory organ exhibit marked facilitation on repetitive stimulation. The facilitated responses, like the individual responses of *Torpedo* and of the main organ of *Narcine*, are electrochemically graded on changing the membrane potential with applied currents, and are inverted in sign when outward currents through the innervated face are very strong.

INTRODUCTION

Two distinctly different electric organs, the main and the accessory, are found in *Narcine brasiliensis* (16). The accessory organ is absent even in closely related species (15). It is also absent in *T. ocellata* (16) and *T. nobiliana* (4), and has not been described in the commonly studied European torpedine species (*cf.* reference 8).

Our interest in the accessory organ stemmed from the finding (*cf.* reference 16) that the two organs differ markedly in physiological as well as in anatomical features. The study of some of these differences and the correlation of functional with anatomical findings are not yet completed. The present paper reports in detail, however, evidence that the different activities which are evoked by neural or chemical stimuli in the two types of electroplaques

of *Narcine* do not arise in electrically excitable membrane. In both kinds of electroplaques the membranes that become active are electrically inexcitable, as in all other marine electric fishes that have thus far been studied (2–5, 12, 16).

METHODS

Specimens of *Narcine* were obtained from the Marineland Research Laboratory[1] and delivered by air to Woods Hole. All microelectrode recordings were done on animals which had had operations on the kidneys or ureters and the electrical responses may have been diminished as a result of operative trauma. However, the grossly recorded responses were the same, except for amplitude, in the operated and normal animals in this study and in earlier, preliminary work done at Marineland Research Laboratory (16, and unpublished data; *cf.* also reference 7). The techniques of study were described in the previous paper of this series (4).

General Anatomical Features

The main organ of *Narcine* is similar in structure to the organ of *Torpedo* (1, 8), but the accessory organ differs in a number of characteristics (16). Each accessory organ is composed of about 10 columns of electroplaques that are innervated solely by the 4th electric nerve, the last electric branch of the vagus. The columns of the organ twist in their course from the scapular process, ventrally, slightly forward and toward the midline to the ventrocaudal border of the main electric organ. Unlike the ventrally innervated electroplaques of the main organ, those of the accessory organ are innervated on their dorsal or dorsocaudal surfaces. There are about 200 electroplaques in each column of the accessory organ. They have about the same diameter as do the electroplaques of the main organ (4 to 6 mm.), but are somewhat thicker (about 20 μ, rather than 7 to 10 μ) and lie farther apart (about 60 to 100 μ).

INNERVATION OF THE ELECTROPLAQUES Each electroplaque of the main organ is supplied by four or five axons which run over the ventral surface (Fig. 1 *A*) from different points on the periphery (16). Each axon branches profusely (*A*, *B*). As in *Torpedo*, there is little if any overlap, each axon supplying a specific region. In the accessory organ, however, each electroplaque is innervated on its dorsal surface by two and sometimes three nerve trunks,

[1] We wish to thank Dr. Rudolph Kempton of Vassar College for furnishing us this material which was supplied through the cooperation of Mr. F. G. Wood, Jr., Director of the Marineland Research Laboratory.

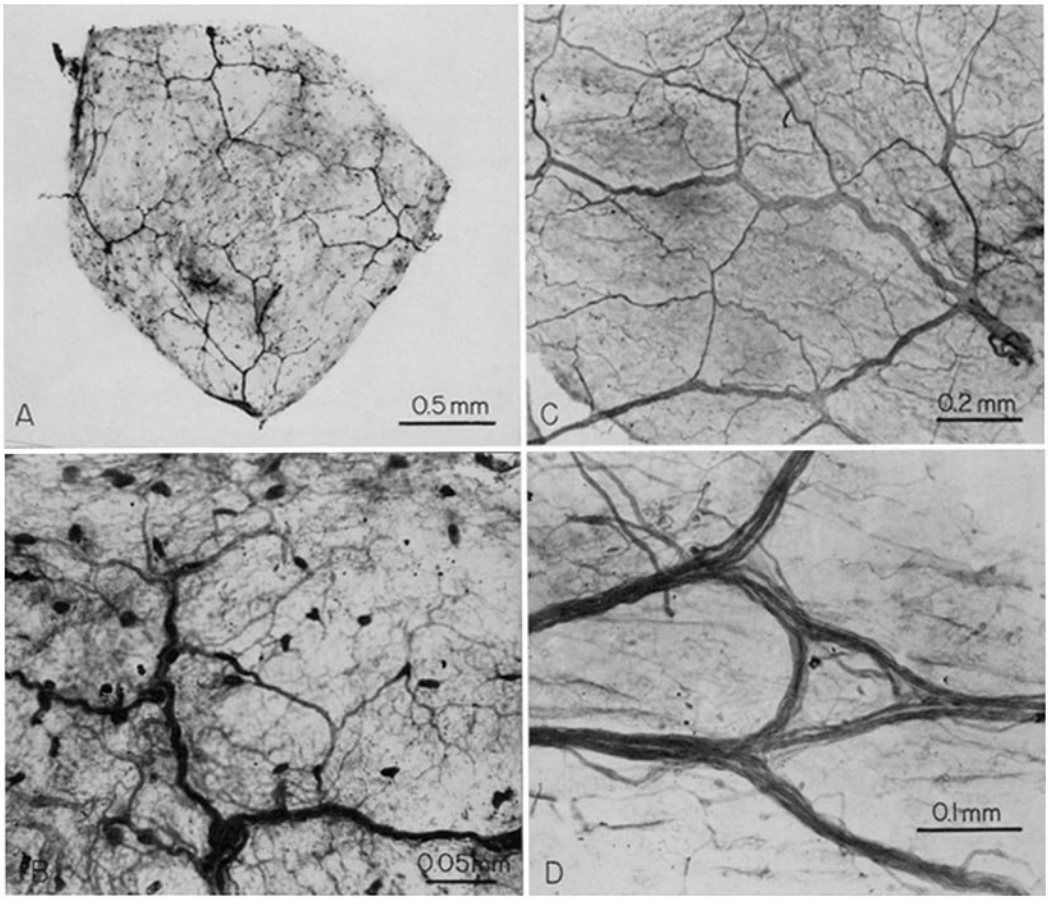

FIGURE 1. Innervation of electroplaques of the main and accessory organs of *Narcine*. Formalin-fixed material. Single cells teased out and stained with methylene blue. *A*, ventral surface of an entire electroplaque of the main organ. Four nerve fibers enter the surface from different points on the periphery. They branch profusely, but each innervates a separate segment of the surface. *B*, the same cell, detail from the upper left corner. Nuclei of the electroplaque were stained as well as many fine nerve branches. *C*, electroplaque of the accessory organ. A nerve bundle enters from the lower right and gives off branches which may divide, the fibers joining other branches. *D*, another cell, detail of a region of branching and recombination of several small bundles of nerve fibers.

each of which contains several axons (*C*). The different axons divide to form numerous branches which run together in some places, and separate to find new partners in others (*D*). However, no anastomoses have been observed. A single axon innervates a number of separate areas of the electroplaque rather than a single segment as in the main organ.

RESULTS

A. *Responses with the Electric Organ in Situ*

THE REFLEX DISCHARGE The organs of both sides respond synchronously and repetitively on stimulating the fish by vigorous prodding (Fig. 2). The individual discharges are brief, lasting less than 5 msec. The pulses in the train attain frequencies up to about 150/sec. and are fairly uniform in amplitude. In these characteristics the organ discharges resemble those of

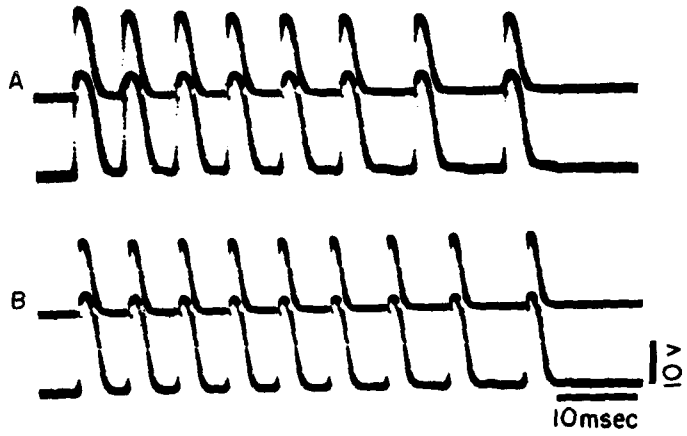

FIGURE 2. Reflexly evoked discharges. In each sequence the two traces show the activity of both organs, recorded simultaneously with separate probe electrodes on the dorsal surface, one over each organ. The reference electrode was a common earth lead in the sea water in which the ventral surface of the fish rested. Positivity of the dorsal surface upward.

T. nobiliana (4). However, the amplitudes are lower, reflecting the smaller number of series elements in each column and also the smaller number of columns in parallel array (16). The accessory organ, with its few columns of electroplaques, produces relatively little externally recorded potential (Fig. 3). Therefore, the reflex activity of Fig. 2 was due predominantly to that of the main organ.

RESPONSES EVOKED BY STIMULATION OF NERVES TO THE MAIN AND ACCESSORY ORGANS In the experiments of Fig. 3 the fish was placed in a shallow pan of sea water. The bottom of the pan was covered with wax, but the entire rim was in contact with the water. The responses were recorded monopolarly against the pan as the indifferent lead and thereby approximated in form what would have been recorded in a much larger volume of sea water. Part of the main organ activity was evoked by stimulating the

3rd electric nerve repetitively. The responses (*F*) were recorded on the dorsal surface, at their site of maximum amplitude (*B*). The discharges were monophasic and positive, reflecting the ventral innervation of the electroplaques. The largest response in the train was the first. Some decrease in amplitude occurred throughout the sequence, but chiefly in the earliest responses.

A similar train of maximal stimuli to the nerve of the accessory organ produced responses which were monophasic and positive on the ventral

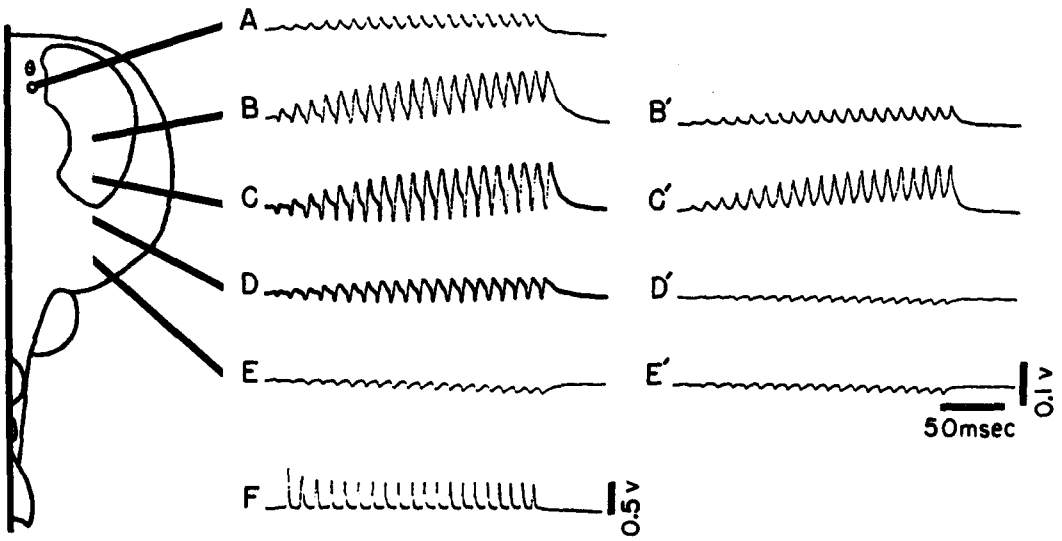

FIGURE 3. Potentials recorded at different sites during activity of the accessory organ evoked by repetitive stimulation of the 4th electric nerve. Differential recording as described in the text, positivity of the probe electrode upward. A train of stimuli was delivered to the nerve during each record. *A–E*, potentials obtained with the probe electrode on the dorsal surface at the sites indicated in the diagram. *B'–E'*, recording with the probe electrode on corresponding points of the ventral surface. *F*, records during a similar train of stimuli delivered to the 3rd electric nerve. The probe electrode was at site B where the responses were maximal. The responses are those of the main organ.

surface of the main organ (*B'*, *C'*). Posteriorly on the ventral surface (*D'*, *E'*) the response became negative, showing the longitudinal vector of the potential produced by the obliquely oriented electroplaques. The responses were positive also anteriorly, on the dorsal surface of the fish (*A*), and were negative posteriorly (*E*). In the regions nearest the accessory organ (*B–D*) the responses were diphasic. Since the responses of single electroplaques are monophasic (*cf.* Fig. 5), the diphasicity indicates asynchrony in the firing of the cells. The negativity shows the vertical component in the output. The

most striking aspect of the responses of the accessory organ is the great increase in amplitude during a train of stimuli.

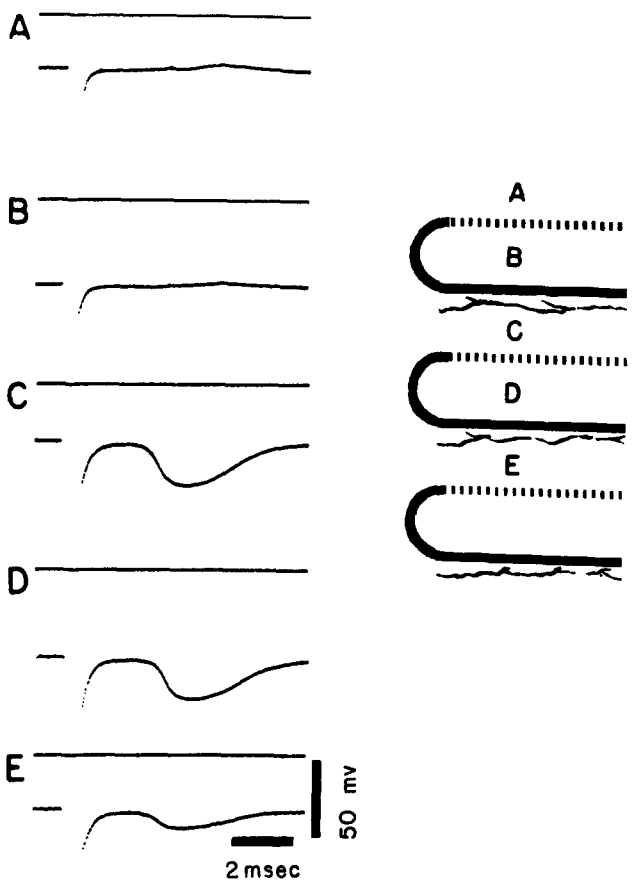

FIGURE 4. Responses of a single electroplaque of the main organ. The column of cells was in the normal position, with the dorsal, unreactive surfaces uppermost, as in the diagram. Letters on the diagram refer to the electrode positions, inferred from the appearance and disappearance of the resting potentials as the electrode was advanced. *A–E*, successive changes in the potentials. Further description in text.

B. *Electrical Inexcitability of the Main Organ*

The responses of single electroplaques and columns of the main organ closely resembled the responses in *Torpedo* electric organ (4).

RESPONSES OF SINGLE ELECTROPLAQUES In the experiment illustrated in Fig. 4, silver wire stimulating electrodes were applied to the uninnervated surface of the uppermost cell in a column. The position of the microelectrode as it was advanced through the preparation was inferred from the successive

appearance and disappearance of a resting potential. On penetrating the superficial cell with the microelectrode the resting potential was observed (*B*) with only a small positive going response, seen also external to the uppermost cell (*A*). As explained in the previous paper (4), this indicates that the uninnervated membrane is unreactive and that it has a low resistance. When the microelectrode penetrated the innervated membrane, leaving the cell as denoted by the disappearance of the resting potential (*C*), the response became negative and large. This negativity persisted almost unchanged when the electrode entered the next underlying cell (*D*) where a resting potential was again observed. Exit of the electrode from the cell (*E*), again marked by loss of the resting potential, was also accompanied by considerable diminution of the response. Therefore, the second cell was not excited by the stimulus and its innervated membrane had a high resting resistance. These changes in the response on moving the recording electrode are similar to those observed in *Torpedo* (4).

The maximum response height was about the same as the resting potential, or somewhat more (Fig. 4). The resting potential attained a maximum of about 60 mv., but often was as low as 20 to 30 mv. The low values probably reflect the extreme thinness of the individual cells. The responses were about 3 msec. in duration. Although the stimuli were delivered close to the nerve terminals of the uppermost electroplaque, the response occurred only after a latency of nearly 3 msec. The long latency suggests that the responses are of electrically inexcitable membrane (9, 10). Further evidence was provided by the electrochemical gradation and inversion of the responses, under the influence of applied polarizing currents.

GRADATION AND INVERSION OF THE RESPONSES BY APPLIED POLARIZATION An isolated column from the main organ was used for the experiment of Fig. 5 to permit application of large polarizing currents (*cf.* reference 4). The latency of the responses did not change with changes in polarization of either sign. When the current was applied inward through the innervated membranes, tending to hyperpolarize them (lowest trace of Fig. 5), the amplitude of the responses increased. On depolarizing the cells by a current in the opposite direction the response diminished, disappeared, and reappeared in reversed sign, growing with a stronger applied current (top trace).

PHARMACOLOGICAL PROPERTIES Only preliminary studies have been made thus far on the pharmacological properties of *Narcine* electroplaques and only in the main organ. Like those of *Torpedo* (4) the cells are depolarized by acetylcholine and cholinomimetic agents, indicating that the electroplaques have cholinoceptive membrane. The innervated membrane, and only this, appears to be the site of considerable esterasic activity (17).

C. *Electrical Inexcitability of the Accessory Organ*

As shown in Fig. 3, the responses of the accessory organ exhibit marked facilitation, differing in this respect from those of the main organ. The facilitation occurs in the responses of single electroplaques. Other response characteristics are similar to those of the main organ.

RESPONSES OF SINGLE ELECTROPLAQUES A column of electroplaques from the accessory organ, oriented with the innervated surfaces upper-

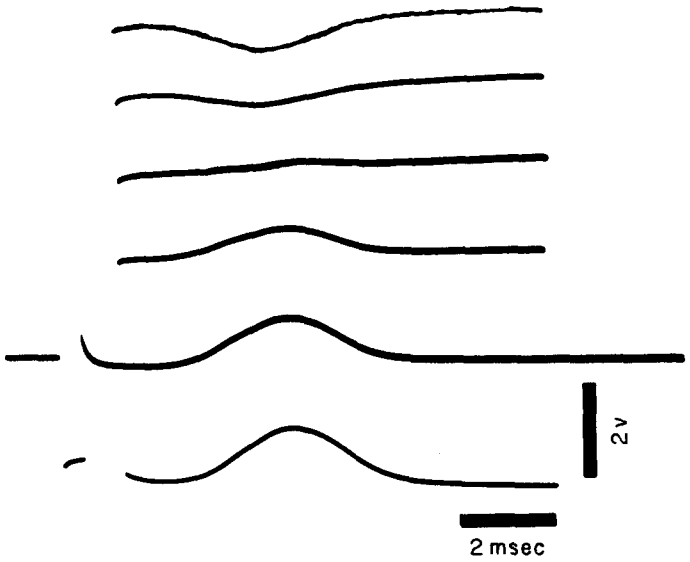

FIGURE 5. Changes in responses of a column of electroplaques from the main organ on applying polarizing currents. The long trace (second from bottom) shows the response to a brief pulse in the absence of polarizing current. Current which hyperpolarized the innervated membranes increased the response (lower record). In the sequence of four records above the base line the current flowed in the opposite direction, outward through the innervated membrane. The responses were diminished in amplitude by the weaker currents. With stronger currents they were reversed in sign. Note that the latency (about 2 msec.) was not affected by the applied currents.

most, was used in the experiment of Fig. 6. A train of stimuli at about 80/sec. was applied to the surface of the uppermost cell through a pair of fine wire electrodes. On the surface, a microelectrode recorded small negative potentials visible in the records only as responses to the later stimuli (*A*). On penetrating the cell (*B*), indicated by the appearance of a resting potential of about 30 mv., the responses became positive. Even at the time scale of the recording an appreciable latency is evident between the first stimulus and a small response. The amplitude increased in the course of the next few responses, as did the duration, so that successive responses developed on a

larger residue of potential from the previous activity. As the summated potential increased, the excursions of the individual responses decreased, but their peaks attained a maximum value which was approximately that of zero membrane potential.

When the microelectrode passed through the uninnervated membrane, leaving the cell as indicated by the loss of the resting potential (*C*), the responses were not diminished in amplitude and remained of the same sign. Penetration of the next cell (*D*) registered its resting potential which was somewhat larger than that of the superficial cell. The second electroplaque

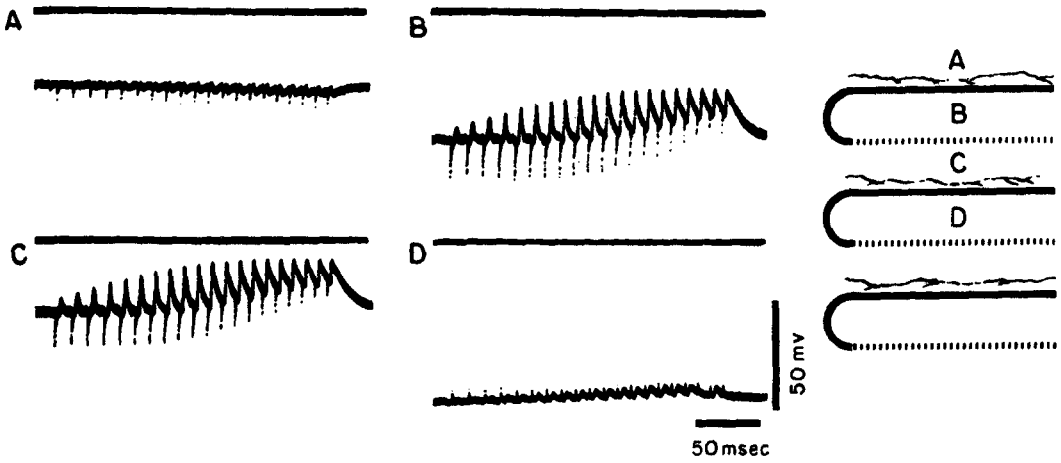

FIGURE 6. Responses of a single electroplaque of the accessory organ recorded as in the diagram. Positions of the advancing microelectrode (*A–D*) inferred from the changes in resting potential. The responses were evoked by trains of stimuli of constant strength applied to the innervated (dorsal) surface. Marked facilitation, which is characteristic of the responses of the accessory organ (*cf.* Fig. 3), occurs in the individual cells. Note the summation of the potentials in the terminal responses of the sequence. Further description in text.

was not excited by the weak stimulus and the responses of the upper cell were recorded markedly diminished in amplitude, but still positive.

The durations of the responses were considerably longer than in the electroplaques of the main organ. As in the cells of the main organ only one membrane was active, that which was innervated, the dorsocaudal surface. The uninnervated membrane was inactive and had a low resistance compared with the resting resistance of the innervated membrane.

The marked facilitation seen in the responses of single electroplaques was essentially duplicated in discharges of the whole electric organ (Fig. 3). Summation was particularly evident in the large monophasic or predominantly surface-positive responses of Fig. 3 *B* and *C'*, but also appeared in the

smaller monophasic surface-positive (*A*, *B'*) and surface-negative (*E*, *E'*) records.

FREQUENCY DEPENDENCE OF FACILITATION The initial responses at any frequency of stimulation, and the responses even to very strong stimuli delivered at intervals more than 100 msec. apart were always small. Thus the facilitation could not have been due to recruitment of nerve fibers. The amplitude grew with repetitive stimulation, even when the stimuli were as much as 50 msec. apart (Fig. 7 *D*). A given amount of facilitation occurred

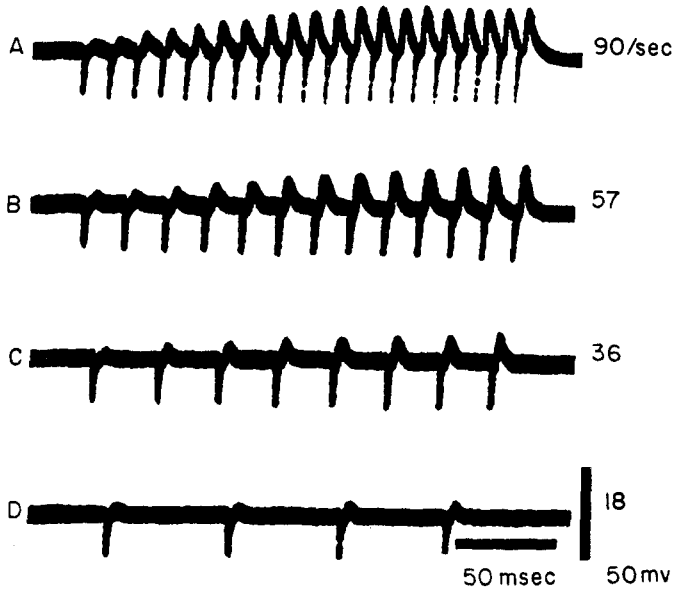

FIGURE 7. Facilitation of responses in a single electroplaque of the accessory organ as a function of the frequency of stimulation. *A–D*, frequencies of stimuli to the surface shown on the right.

with fewer stimuli at higher frequencies (*A–D*). The individual responses became maximal by about the 12th stimulus at frequencies of 60 to 100/sec. (*A*, *B*). The durations of the responses increased to different degrees in different cells. The prolongation was more marked in the experiments of Fig. 6, and accordingly summation was greater than in the experiment of Fig. 7. The fully facilitated responses were about 10 msec. in duration.

GRADATION AND INVERSION OF RESPONSES BY APPLIED POLARIZATION An experiment similar to that of Fig. 5, but recording from several parallel columns of the accessory organ, is illustrated in Fig. 8 *A*. A train of stimuli at about 80/sec. was given to the tissue, and the later responses were facilitated (lowest trace). On depolarizing the innervated membranes a level was reached at which all the responses disappeared (middle trace). They re-

appeared in opposite sign on further depolarization (upper trace). The uniform effects of the membrane polarization upon the responses of different amplitudes show that they were all produced by electrogenic processes having the same reversal potential, and that the facilitation was unaffected by the polarization.

The records of Fig. 8 *B* show the last two of a similar train of stimuli, and the single responses may be seen in more detail. The responses were augmented when currents of different strengths were applied in the direction of hyperpolarizing the innervated, reactive membrane (lower two traces). When the currents were in the opposite, depolarizing direction (traces above the base line) the responses were diminished in amplitude. With further depolarization the potentials disappeared. Stronger currents caused a return of the potential in reversed sign. This behavior and the fact that the latency of the responses was not affected by the extreme membrane polarization signify that the reactive membrane is electrically inexcitable. Thus, the responses of *Narcine* electroplaques, whether those of the main or of the accessory organ, have the properties of postsynaptic potentials (p.s.p.'s) (9, 10).

The data of the voltage-current relation of the same experiment are plotted in the graph of Fig. 8. The "passive" and "active" resistances are described by the two lines with positive slopes. The active resistance was about 40 per cent less than the passive. However, shunting of the electroplaques by inactive tissue would have reduced the measured change. If the equilibrium potential of the p.s.p. is assumed to be close to zero membrane potential, or about 50 mv. away from the resting potential (*cf.* Fig. 6 *B*), an estimate can be made of the membrane resistance of the cells from the data on the change of response amplitude with applied current, the line with a negative slope in Fig. 8. The current required to produce inversion was 1.8×10^{-3} amp./cm^2. If the resting potential were 50 mv. the resistance would be about 30 ohm-cm^2. This is to be ascribed chiefly to the innervated membranes, for the resistance of the uninnervated membrane appears to be negligible (Fig. 4). This value is similar to that obtained for the innervated membrane of *T. nobiliana* (4).

DISCUSSION

Like the electroplaques of other marine electric fish (2, 4, 5, 12) those of both organs of *Narcine* have only a single surface that becomes active, the innervated membrane. As in the other forms, this membrane does not respond to electrical stimuli and possesses other characteristic properties of synaptic membrane (10, 11). Electrical stimuli excite the cell only by stimulating the nerve fibers. The resulting response, a p.s.p., occurs only after a

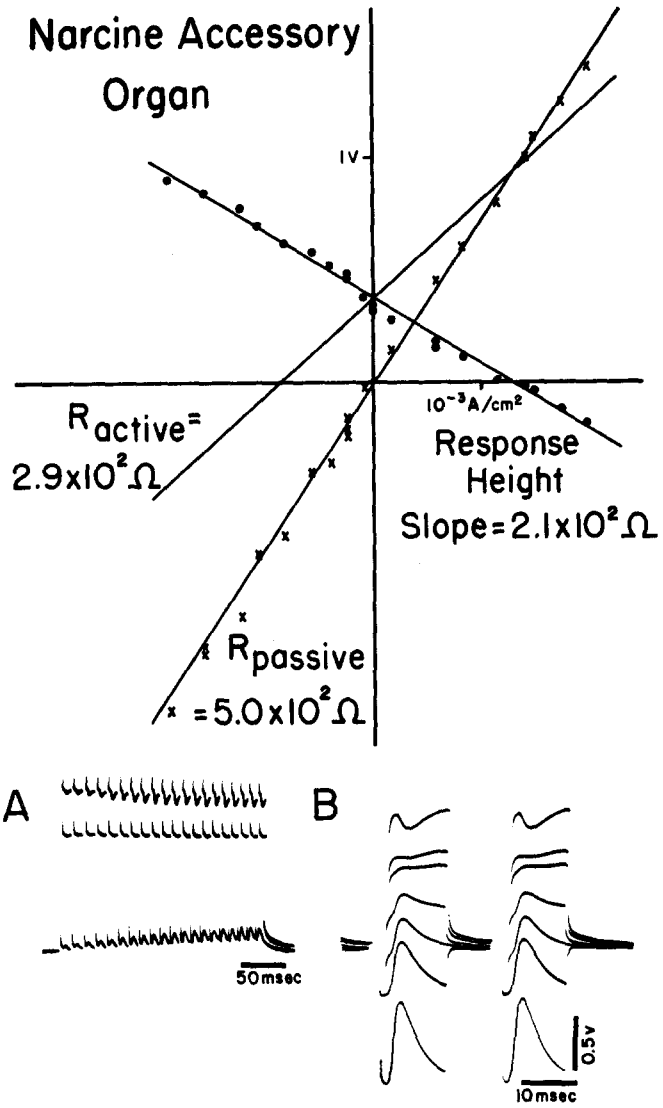

FIGURE 8. Effects of applying currents to a column of electroplaques of the accessory organ. *A*, sample records of responses to a train of stimuli at 90/sec.: without applied current (below); when the current flowing outward through the innervated membrane was sufficient to make the responses very small (middle trace) and to reverse their sign (upper trace). *B*, sample records of the last two responses in a train at 50/sec., registered on a fast time base. Each of the responses was evoked during a polarizing pulse. Those evoked during hyperpolarization of the innervated membranes (lower two traces) were larger than the responses at the resting potential (shown on the base line). When the current pulses were in the opposite direction the response diminished and then reversed in sign. *Above*, graph from the complete experiment of *B*, showing (dots) the changes in amplitudes of the last responses in each train (ordinate) as a function of the applied current (abscissa; hyperpolarizing current to the left of the origin). The polarization of

considerable latency, about 3 msec., even when, as in the present experiments, the electrical stimuli were applied at the surface of the electroplaque and conduction time must have been minimal (*cf.* reference 9). The latency was not affected by strong hyperpolarizing or depolarizing currents. The maximal responses were about equal to the resting potential, or were slightly larger (Figs. 4 and 6). However, the recorded resting potentials were probably too small, since the cells are thin (*cf.* reference 4).

The responses of the electroplaques of both organs were augmented by hyperpolarizing the reactive membrane and were diminished by depolarization. They appeared as responses of reversed sign when a critical value of applied current was exceeded (Figs. 5 and 8). The amplitudes, and to some extent the durations, of the responses of single electroplaques of the accessory organ could be increased by facilitation (Figs. 6 and 7). The process or processes underlying the facilitation were not affected by large changes in the membrane potential (Fig. 8).

Since the cells are electrically inexcitable, the neurally evoked responses of the membrane must be effected by release of a transmitter by the presynaptic terminals. The pharmacological evidence as well as the histochemical (17) indicates that the agent is acetylcholine or is cholinomimetic. The latency of the responses, the variable amplitudes and durations, and the summation of successive potentials in the electroplaques of the accessory organ are all explicable as the effects of chemical excitation of an electrically inexcitable membrane. The electrochemical gradation and inversion of the responses are also consequences of their electrical inexcitability (10, 11).

The amount of facilitation which occurs in the electroplaques of the accessory organ is striking, and particularly so since it is not seen in the cells of the main organ, or in those of *Torpedo* (4) or *Astroscopus* (2) electric organs.

The kinds of changes that might produce augmented responses of electroplaques are limited by the electrical inexcitability of the cells. While the time constant of the membrane was not determined accurately, it is certainly very short compared to the responses (Fig. 8 *B*). Prolongation of the responses therefore indicates that the transmitter action was prolonged as well as augmented. These effects might arise through changes either in the individual nerve fibers or in the subsynaptic membrane. Successive presynaptic impulses might be more effective in releasing larger quantities of the transmitter, as has been suggested for the end-plate (6, 13, 14) and for a longer time. Alternatively, postsynaptic membrane might have become sensitized

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the membrane by the applied current preceding the responses is shown by the crosses. Note absence of rectification. The slope of this line gives the resistance of the resting membrane (R passive). The difference between the two lines joining the experimental points is a line the slope of which is the resistance during the peak of the response (R active); this resistance is lower than the resting value.

to the transmitter, or an inactivating agent present at the synaptic sites and competing with the transmitter may have been neutralized by the initially emitted transmitter.

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