

MECHANISMS OF DIRECT AND NEURAL EXCITABILITY
IN ELECTROPLAQUES OF ELECTRIC EEL*

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INTRODUCTION

Knowledge of the mechanism of the electric discharge of electric fishes has been considerably advanced recently by studies of the activity of single cells of the electric eel (*Electrophorus electricus*), recorded either with intracellular (33) or external microelectrodes (4). Both demonstrated that the voltage change of the spike is located only at the posterior, innervated face of the cell. The finding confirms the theory which Bernstein (7) proposed to account for the unique ability of electric fishes to develop very high voltages, this special adaptation of electric cells permitting series summation of the low electromotive forces produced by the individual units. The earlier work of this laboratory (4) using single or few-layered preparations of plaques with intact innervation, analyzed in some detail the various components of the electrical response when the cell was excited either directly or through one of the several nerves which multiply innervate the cell, as well as some of the properties of the excitatory phenomena associated with these different modes of activation. Stimulation of a nerve causes excitation of the innervated cells, evidenced by the production of a prepotential lasting about 2 msec. In those cells in which the excitation is sufficiently large to cause their discharge, a spike is superimposed on the prepotential. The excited state produced by a nerve volley persists, though with no accompanying observable potential change, for as long as 1 second and has a characteristic time course with 2 components. The

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long period of enhanced excitability is manifested, however, only when the second, testing neural volley is delivered through the same nerve (homosynaptic excitation (31)). When the conditioning and testing volleys derive from different nerves (heterosynaptic excitation) the time course of enhanced excitability is brief, approximately 2 msec. The response to direct electrical excitation of the normal or denervated cell is also prefaced by a small, graded potential. After subthreshold direct stimulation, enhanced excitability persists for about 300 msec., but is small in magnitude.

This paper extends the earlier work, reporting primarily the results obtained with microelectrodes recording in single cells of the few-layered preparation. Again, observations were directed to the responses obtained by neural as well as by direct stimulation of the plaques. In a number of the experiments simultaneous recordings with 2 microelectrodes inserted into different regions of the same cell gave information not otherwise obtainable. The emphasis of this paper, which concerns the nature of direct and neural excitation of the electroplaque, is somewhat different from that of Keynes and Martins-Ferreira (33), but our results in parallel experiments are in agreement with theirs. A preliminary note by Brock, Eccles, and Keynes (11) reports data on the innervated preparation of electroplaques from *Raia clavata* which are related to the results detailed here.

Methods

(a) More than 50 preparations of electroplaques made as described in the previous paper (4) were used for these experiments. Two or more cells were studied in most of the preparations. The experiments were carried out at room temperatures ranging from 22–24°C.

(b) Microelectrodes were drawn prefilled with 3.0 M KCl (32) from 0.7 mm. pyrex capillary tubing. Tips were 1 to 2 μ in diameter and the resistance 2 to 5 megohms. In experiments with dual recording 2 microelectrodes were inserted at different sites of the cell as shown in the diagram of Fig. 1. For reasons discussed by Keynes and Martins-Ferreira (33) each internal electrode was situated close to the caudal membrane and was paired with an external reference microelectrode of the same dimensions.

(c) The direct coupled amplifiers were highly differential. With the relatively low resistance microelectrodes used in these experiments the frequency response was sufficiently high to reproduce a 0.1 msec. square pulse with less than 2 to 5 per cent loss. The input stages were cathode followers with grid currents about 10^{-11} amp., mounted as probes close to the recording electrodes.¹

(d) The 2 traces of a cathode ray oscillograph were each further split into two by an electronic switch operating at 100 kc. Thus 2, 3, or 4 traces were available for inde-

¹ The amplifiers were designed in this laboratory by Mr. E. Amatniek and constructed by Messrs. A. Cross and O. N. Dagavarian. We wish to thank them for the excellent performance of the equipment.

pendent simultaneous recording. Two cathode ray tubes in parallel, one for monitoring and the other for photographic recording, increased the convenience of operation.²

(e) The large working distance and great depth of focus of the Zeiss Opton binocular microscope, together with improved illumination enhance visibility to such an extent that the protoplasmic structures of the electroplaques of living, unstained preparations become easily distinguishable. Exploration of the relatively large surfaces of the unit electroplaque requires the use of manipulators affording the possibility for moving the electrodes over distances of 1 cm. or more. The improved type III manipulators manufactured by the Brinkman Instrument Co., proved convenient for this purpose.

TABLE I
Characteristics of Bioelectric Potentials of Single Electroplaques

	Con- duction velocity	Resting potential* across		Spike†			
		Caudal face	Rostral face	Neural excitation		Direct excitation	
				Amplitude	Duration	Amplitude	Duration
M.P.S.	mv.	mv.	mv.	msec.	mv.	msec.	
Innervated cells	1.05‡	73.1	72	126	2.16	127	2.20
Dener- vated range	1.68	±4.76 74 66-78	±4.45	±15.55	±0.55	±13.55 132 120-136	±0.69 2.5 1.7-4.5

* 75 cells.

† 50 cells.

‡ Average of 6 experiments. Range 0.71 to 1.59 M.P.S.

|| Average of 6 experiments except conduction velocity which was determined in only one cell.

RESULTS

I. Resting Potentials

Table I includes a summary of the measurements of the resting potentials observed in our experiments. The average (73 mv.) is smaller than the 84 mv. average obtained by Keynes and Martins-Ferreira (33), although values of that magnitude were also obtained in the present work. Keynes and Martins-Ferreira used animals relatively fresh from their natural habitat whereas ours, as noted previously (4), had been kept for a long time in the New York Aquarium. The resting potential had the same value, within the experimental error, when measured across either the rostral or caudal membrane (33, and Table I). The resting potentials of denervated cells are in the same range as those of the normal electroplaque, although the physical appearance of the cells alters when neural excitability is lost within about a month after denervation. The

² The equipment was designed by Messrs. E. Amatniek and L. Strauss and constructed by Messrs. A. Cross and O. N. Dagavarian.

cells then appear opaque, but at the longest denervation interval of the experiments ($8\frac{1}{2}$ months) they remained excitable. On denervation the resting potential of muscle fibers of mice decreases from about 100 mv. to about 75 mv., the major change taking place in the first 10 days (46).

The cell of the Sachs organ occupies only a small portion of the compartment and of this, the long digitations at the anterior face comprise a major part. A microelectrode entering the cell from the anterior face and somewhat obliquely after penetrating one of the digitations will often pass through and back into the exterior if forward movement is continued. Sometimes a succession of such penetrations and exits occurs as the electrode is continuously moved toward the caudal face of the cell. With each penetration there is a change of potential at the electrode corresponding to the resting potential and with each exit a return to the base line potential. The continuous protoplasmic portion of the cell is thin. This type of structure was first inferred from the electrophysiological findings and later observed in the living cell with improved optical arrangements. It has since been shown histologically (8, 12).

II. Site of the Bioelectric Action Generator

That the innervated caudal face of the electroplaque is the site of the bioelectric generator of the spike had been earlier demonstrated with different methods by Keynes and Martins-Ferreira and ourselves (4, 33). Our own measurements with intracellular electrodes, carried out independently of the work by Keynes and Martins-Ferreira, are in agreement with their finding that the spike is recorded only across the caudal face of the cell and that the small potential which appears at the rostral face is due to current flow across the resistance of the membrane.

III. Response to Direct Stimulation

1. *Denervated Cells.*—The denervated cell is capable of being directly excited (3, 4, 38) and its response is therefore unequivocally that electrically initiated in the electroplaque.³ Weak direct stimulation, applied across the cell with external metal electrodes insulated except at their tips and with the current directed so as to flow outward through the caudal surface (4) provides a cathodal stimulus to the excitable membrane (20). This evokes first a small potential (4) which grows (Fig. 1 A-B) with increasing stimulus strength and eventually an all-or-nothing spike is elicited (Fig. 1 C). Since direct stimulation, subthreshold for the spike, produces a small remnant excitability persisting for about 300 msec. it is also possible to duplicate the same events by using repetitive weak stimulation at rates of 5/sec. or higher (5). Oppositely directed stimuli of maximal strength cause no response, but when they are

³ Although other authors seem to have had difficulty in obtaining denervated preparations (38), histological examination showed that in ours, nerve fibers were absent. We wish to thank Mr. Paul Bernstein of this laboratory for the histological material.

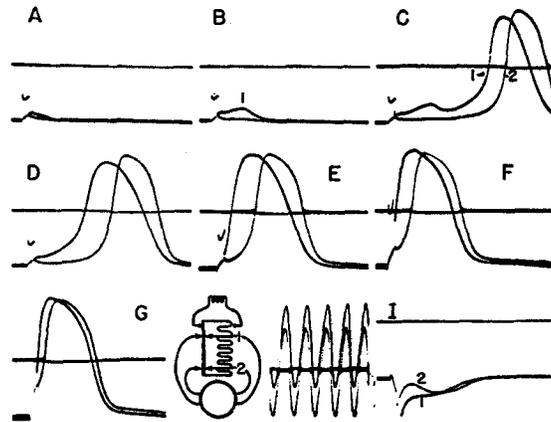


FIG. 1. Responses of an electroplaque denervated $8\frac{1}{2}$ mos. to cathodal stimulation of the caudal membrane. Two microelectrodes were inserted in the cell close to its caudal membrane as shown in the diagram. In this and subsequent figures, direct stimulation was applied through external electrodes near the medial end of the cell. Activity therefore was initiated first at site 1 and later at 2, nearer the outer end. Distances in millimeters between the stimulating electrodes and microelectrode 1, and between 1 and 2 are given in that order at the end of this and subsequent legends. Calibration 100 mv. and 1000 c.p.s. in all figures except Fig. 13. Where 2 time and amplitude calibrations are given, as in this figure, they refer to traces 1 and 2 of the three simultaneously recorded. The upper trace is the reference (zero) potential before insertion of the microelectrodes. The distance of the two lower traces (1, 2) from the zero line represents the resting potential of 70 mv. with the microelectrodes in the cell. Weak stimulation (A) produced a small local response on trace 1, which grew with a stronger stimulus (B, trace 1 identified by the numeral), and evoked a propagated response recorded on both traces (C, spikes at the two recording sites identified by the appropriate numerals). The latency of onset was shortened with stronger stimulation (D to G), but at first spike 2 developed by propagation (D, E). With stronger stimuli (F, G) there was a tendency toward simultaneous activation of the cell. A very strong anodal stimulus (current flow into the caudal membrane) (I) evoked no response in the cell but excited nearby cells. This activity caused small potentials of reverse sign. These nearby cells were activated first near the stimulating electrodes, as seen by the earlier onset of their potentials in trace 1. Distances 0.83 and 2.56 mm., XI-17-0.*

* XI-17-0, etc., signify the respective experiments.

made strongly supramaximal a potential of opposite sign occurs, which is due to pick-up of activity in other cells excited by spread of the stimulus (Fig. 1 I).⁴

⁴ This artifact appears to account for the positive phase recorded in Fig. 3 of the earlier paper (4).

The outwardly directed threshold stimulus applied across the cell is relatively weak and only rarely able to excite nerve fibers (Fig. 4) terminating at the caudal membrane. In Fig. 1 *C* the depolarization of the membrane by the stimulus amounted to 30 mv. Transverse stimulation of nerve or muscle fibers under similar conditions requires current densities some 300 times greater than does longitudinal excitation (45). The difference may derive from the specialized structure of the electroplaque, in which excitable changes occur only at the caudal face of the cell membrane.

The graded prefatory potential is absent at a recording electrode 3.39 mm. distant from the site of the stimulation (Fig. 1 *A* to *C*). The potential produced in the vicinity of the stimulating electrode is therefore a local response analogous to those observed in other excitable tissues. Both the prefatory response and the spike maintain the same sign when recorded with internal microelectrodes as with external recording. Both are therefore generated at the excitable membrane of the electroplaque. The prefatory, graded, localized potential is therefore a local response of the cell, and will be so designated henceforth. Occasionally, even when recording close to the site of stimulation, the spike arose without preliminary growth of a local response. It appears likely that the excitability of the cells was then very high, the rising local response initiating the spike with so little delay as to cause the two potentials to appear fused into one, as in the responses of Fig. 1 *E*, *F*. Decrease of the excitability by action of various drugs brings out the existence of the local response (5).

As a consequence of the local nature of the prefatory response the directly evoked spike always arises at the stimulated region and is propagated from this site (Fig. 1) with a velocity of about 1 m.p.s. (Table I). This is lower than the value found by Keynes and Martins-Ferreira (33). Propagation block sometimes occurs.

The spike elicited by threshold direct stimulation may have long latency (Fig. 1 *C*). In Fig. 1 (*A* to *C*) the local response developed by spread of excitation from the stimulating locus, 0.83 mm. from site 1. The local response in *C* was large, but not enough so to initiate the spike which arose near the stimulating electrodes and developed at site 1 by propagating at about 2 m.p.s. The conduction time was therefore approximately 0.4 msec. and latency at threshold at the site of stimulation about 3 msec. When the stimulus strength is further increased, the latency is shortened (*C* to *G*). The response at the distal electrode (spike 2) at first arises at a constant time interval (*C* to *E*) after the proximal (spike 1). The former therefore arises by propagation, but earlier because of shortening of latency at the site of stimulation. On increasing the stimulus strength further, however, spike 2 tends to arise still earlier (Fig. 1 *F*). With very strong stimuli the two may be nearly simultaneous and the latency, particularly of the first, extremely short (*G*). The spread of current from strong stimuli therefore can activate the entire cell with short latency (20, 33). Measurements of apparent conduction velocity may thereby give rates which are too high.

The spike amplitude and the form vary in different sites of the cell (Fig. 1) but a considerable part of this variation is ascribable to asymmetric recording conditions (section X). Spike amplitudes and durations are given in Table I.

2. *Innervated Cells.*—The responses of innervated cells to direct stimulation follow the pattern described for the denervated cells. The potentials have the same characteristics (Table I) and examples are to be seen in most of the figures.

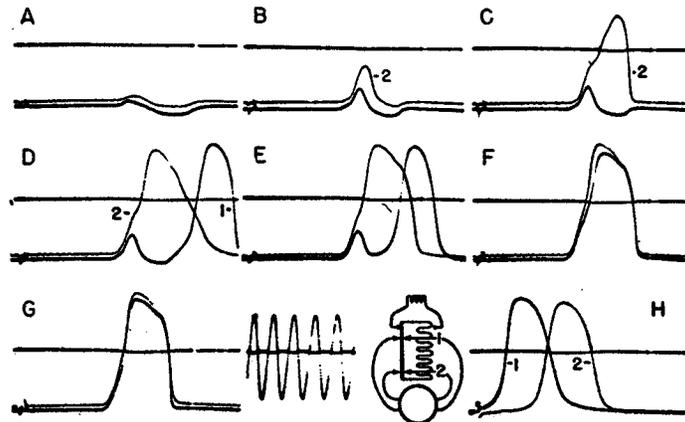


FIG. 2. Responses in the electroplaque to increasing stimulation of a nerve trunk. Trace 2 was displaced upward for clearer identification of the activity at the two recording loci. A weak stimulus evoked only a small postsynaptic response simultaneously at both sites (A). The discharge of a nearby cell was responsible for the downward (negative) deflections. Stronger stimulation increased the postsynaptic activity (B), but more so at 2. The response at 1 was not markedly increased with a stronger stimulus (C) but that at 2 developed an overshoot. With increasing stimulation propagation occurred (D), spike 2 arising first. A local response prefaces spike 1. As the neural stimulation was increased spike 1 arose earlier (E), coincided with (F), or slightly preceded spike 2 (G). The responses, in reverse order, to a moderate direct stimulus are shown in H. Distances 1.02 and 1.86 mm., XI-4-13.

IV. Response to Stimulation of the Nerve Trunk

In experiments with external recording electrodes (4) increasing the intensity of the stimulus to the nerve trunk first caused a small, graded potential of the same electrical sign as the spike. This is also true when recording intracellularly (Fig. 2). The sign and the magnitude of this potential remain unchanged when it is recorded with a variety of electrode arrangements (Fig. 17). The response arises simultaneously at 2 recording loci (Figs. 2, 10, 12, 15 to 17) and therefore presumably simultaneously in all or most regions of the excitable surface of the cell. Everywhere in the cell, therefore, it is a reflection of internal change toward positivity. Since the direction of electrical change of this

response is the same as that of the all-or-nothing spike, in whatever way the potentials are recorded, the small prefatory potential elicited by a neural volley also occurs at the excitable surface which is the generator of the previously described local response and of the spike. Hence, the potential appears to be similar to those recorded in nerve cells (10) or at end plates (17). It will be demonstrated in section VI that the prefatory potential produced by a neural volley has properties which are different from those of the local response. Identification of the neurally evoked potential with an end plate response is tempting, because the electroplaque is a structure derived from striated muscle. However, since this potential appears to be generated everywhere in the cell within its excitable surface the term "postsynaptic potential" is more suitable. Therefore that prefatory response which is generated only by a neural action will be henceforth so designated.

The neural volley to the outer (skin) end of the plaque traverses a path 5 to 12 mm. greater than the path to the medial end of the cell. The nearly simultaneous development of the postsynaptic potential over the entire caudal face of the cell therefore raises 2 possibilities: (i) The conduction velocity in the terminal portions of the nerve fibers supplying the outer end of the electroplaque may be higher than of those innervating the medial part so as to ensure nearly simultaneous neural activation of the whole cell membrane. If this be the case, the nerve fibers innervating the outer end therefore should have larger diameters (23), but this matter has not yet been examined. (ii) The innervation at the outer end of the cell may have greater synaptic effectiveness leading to an earlier development of the postsynaptic potential. The neural volley does have greater effectiveness at the skin end of the cell since the postsynaptic potential developed there is larger than the potential at membrane sites a few millimeters away (Fig. 2 *B*), and the neurally evoked spike is almost always initiated at the outer end of the cell (Fig. 2 *C*). The response time of the postsynaptic potential is slightly shortened with increasing stimulation (Fig. 2 *A, B*) but remains constant thereafter.

The greater synaptic effectiveness of the neural volley at the excitable membrane of the outer part of the cell might be due to several factors: (i) The number of neural terminations at this end of the cell may be higher. This might come about relatively because the skin end may be supplied primarily by the larger fibers (as mentioned above) which would have the lowest threshold to weak electrical stimulation, or because of an absolute preponderance of nerve terminations in this region. (ii) On the other hand, the nature of the synaptic junctions may be such that those at the outer end of the cell have higher potency. Our experiments do not offer additional data on this matter, especially since the incoming neural volley was not observable at the amplifications employed (Fig. 13).

The discharge initiated by a weak neural volley may not be propagated although developing an overshoot (Fig. 2 *C*). The amplitude is then low and duration relatively short. With a stronger stimulus there is long latency be-

tween the onset of spike 2 and spike 1 (*D*), the latter rising slowly, as did spike 2 in record *C*. The slow rise (*D*) probably indicates a local response out of which the spike developed. When the stimulus strength is increased, the postsynaptic potential at electrode 1 is not markedly altered, but the onset of the spike is earlier (*E*). With maximal stimulation of the nerve spike 1 arose nearly simultaneously with spike 2 (*F*) or slightly earlier (*G*). Even then, however, the postsynaptic potential remained distinguishable. Analysis of the events associated with the postsynaptic potential will be dealt with in sections VIII and IX.

Once evoked by a weak neural volley, the spike in turn sets up excitation of the cell by means of the local circuit, as seen from the subsidence of postsynaptic response 1 before onset of the spike (Figs. 2, 12). However, the local circuit of the spike neurally initiated at the skin end of the cell acts upon adjacent regions of excitable membrane which had also been subliminally excited by the neural volley. The velocity of propagation for a spike initiated by a threshold neural volley should therefore be somewhat greater than for one caused by weak direct stimulation. This appears to be the case when comparisons are made between direct and neural excitation of the same cell, but the differences are small and do not reveal themselves in the averages of Table I. The nearly simultaneous discharge of the entire cell by a strong neural volley would appear to be an extension in the degree of generalized excitation produced by the widely disseminated innervation, similar to the effects of strong direct excitation.

The forms of the neurally evoked and directly elicited spikes do not appear to differ significantly within the large variation observable in the experiments. The falling phase of the neurally evoked spikes often is steeper (Fig. 2 *F, G*) than in the directly elicited responses (*H*), but this and the shorter, irregular peak were probably caused by potentials of opposite sign from the responses of other neurally excited cells. Smaller distortions ascribable to this are seen in *A* to *C*. The amplitude of the response with maximal neural excitation is usually slightly larger than with direct stimulation, probably as a consequence of the circuit properties (section X).

Comparison of the forms of the spikes initiated locally by weak direct or neural stimulation and of those initiated by a generalized discharge of the cell with strong stimuli is of interest. The latter case is analogous with "membrane" spikes (30, 37). The forms of the "propagated" and "membrane" spikes are not significantly different. This finding is in agreement with the results obtained in squid giant axons (26). The time course of the response in both appears to be determined by membrane events which are relatively independent of the amount of membrane surface involved, the exceptions being the cases of small "abortive" spikes noted earlier (Fig. 2 *C*).

V. Response to Indirect Local Stimulation

When a stimulus is applied to the innervated cell through local electrodes, as for direct stimulation, but in the reverse direction (*i.e.* current flow inward through the excitable membrane) the response of the cell is always later than

for direct excitation. It is mediated (2, 3) by stimulation of the nerves in the region of their termination in the caudal face of the cell (Fig. 3). This form of stimulation will henceforth be designated as indirect (20). As with stimulation of the nerve trunk, weak stimuli first produce a prefatory potential. The time of onset of this is always later in comparison with the onset of the directly elicited local response. This latency ranged from 0.96 to 2.43 msec. in different preparations. It is nearly constant for a given preparation, decreasing only a

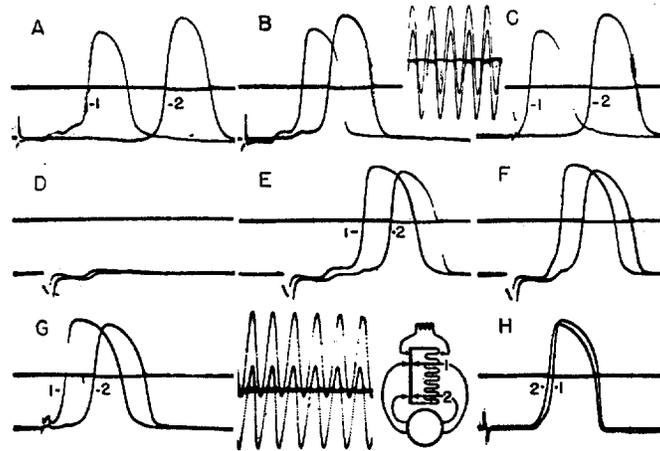


FIG. 3. Responses to indirect stimulation in two preparations. A postsynaptic potential appeared only at site 1, nearest the stimulating electrode (A). Spike 1 developed after a local response and spike 2 arose by propagation. With stronger stimulation (B) other nerve fibers were also stimulated giving rise to a postsynaptic potential on trace 2 and earlier onset of both spikes. C, direct stimulation showing absence of postsynaptic potentials and early onset of spike 1. Distances 0.83 and 4.5 mm., XI-16-13. D to H, Another experiment. Weak stimulation (D) caused activity in another cell. A stronger stimulus (E) elicited postsynaptic responses in both loci, that at 2 slightly later, followed in each case by spikes. With stronger stimuli (F) both spikes developed earlier on the postsynaptic potential. G, Responses to direct stimulation and H to a neural volley. Distances 0.59 and 2.13 mm., XI-13-14.

few tenths of a millisecond with strong stimuli. The response to indirect stimulation by local electrodes is therefore easily distinguishable from that to direct stimulation of the cell. As the strength of the stimulus is increased the sequence of bioelectric events is similar to that caused by the stimulation of the nerve trunk, with two notable differences: (i) The discharge of the cell begins in the region of the stimulating electrode and not, as with neural stimulation, usually at the outer end of the cell. (ii) Strong indirect stimulation never activates the whole of the cell (Fig. 3 B, F) as does strong direct excitation or a maximal neural volley (Fig. 3 H). The excitation produced by the electrical stimulus is

therefore primarily confined to the nerve fibers in the region of the stimulating electrodes. It may be expected, however, that some of these nerve fibers supply parts of the cell at some distance from the site of stimulation. Very strong indirect stimuli might then activate more than a local region of the cell. That this is sometimes the case is seen in Fig. 3 *B, F*. The latency of response 1 decreased, but the onset of spike 2 was earlier than by propagation (*A, E*).

When excitability of some nerve terminals is high a weak cathodal stimulus can also produce the response described above which is characteristic of indirect stimulation of the cell (Fig. 4 *A*). On increasing the stimulus, however, there is a transition to direct excitation indicated by the time relations of the activity (*B, C*), the latency decreasing abruptly to the low value characteristic

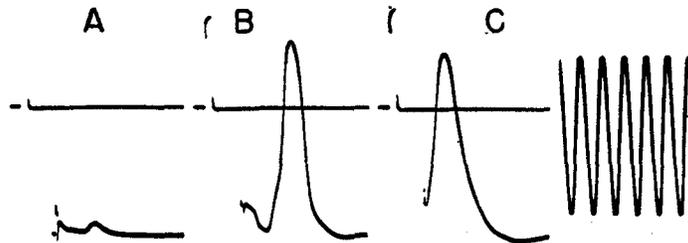


FIG. 4. Transition from indirect to direct stimulation. A weak direct stimulus first excited nerve terminals, and this gave rise to a postsynaptic potential (*A*). A stronger stimulus (*B*) caused a small local response, followed by a large postsynaptic potential and the indirectly evoked spike. The latter was absent when the directly elicited spike appeared with short latency (*C*) but the postsynaptic potential is detectable on the falling phase. The zero reference line also carries a monitor signal of the strength of the applied stimulus. VI-9-16.

of direct excitation. The falling phase of the spike is then distorted (*C*) by addition of the postsynaptic potential. The latter therefore develops independently of the prior discharge of the cell.

Excitation of the nerve terminals also occurs (Fig. 5) with strong direct stimuli which had already caused direct excitation of the cell. Under these conditions, the cell is refractory to discharge by the secondary neural volley. The synaptic potential nevertheless is then superimposed (*B*) upon the directly elicited spike and in the tempo of the initial response (*C, D*) to indirect stimulation.

VI. The Components of the Neurally and the Directly Evoked Responses

Sections III to V have described the types of electrical activity produced by single electrical stimuli to the cell itself, to its nerve trunk, or to the innervating terminations at the cell surface. The directly elicited electrical activity is composed of a graded local response and an all-or-nothing spike. That which is

neurally evoked also includes a graded postsynaptic potential. The experiments of Figs. 4 and 5 have indicated that the postsynaptic potential is not subject to absolute refractoriness of the cell, previously activated by direct excitation. The data of this section will demonstrate that the postsynaptic potential does indeed have different properties from the local response. The additional information on the excitable systems of the electroplaque was obtained by

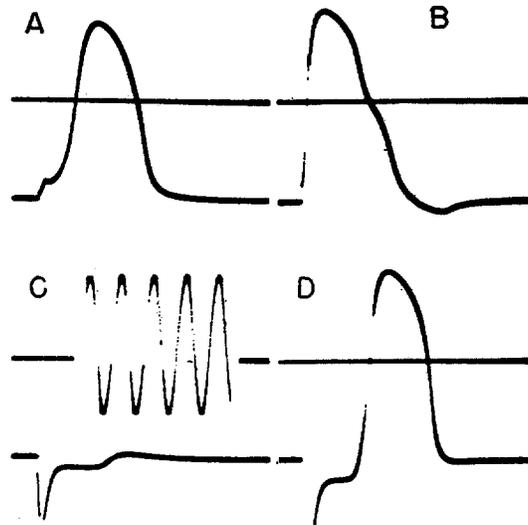


FIG. 5. Comparisons of responses to direct and indirect stimulation. *A*, moderate direct stimulation. *B*, very strong direct stimulation. The spike rises with extremely small latency but its falling phase is distorted by a postsynaptic response. The later distortion at the base line is due to activity of another cell. The postsynaptic response in *B* occurs with the same time relations as that to a weak indirect stimulus (*C*). Very strong indirect stimulation initiates the spike only after relatively long latency (*D*).

paired stimulation of one type or by the interaction of different modes of stimulation.

The absolutely refractory period of the electroplaque lasts about 4 msec., and relative refractoriness another 6 msec. (Fig. 6). Recovery of the cell from refractoriness, as tested by a maximal stimulus to the nerve, may take place as rapidly as when the testing response is produced by a strong direct stimulus. A neural volley therefore can excite a cell very strongly. However, many cells innervated by a given nerve are not usually stimulated to discharge by a single volley (4). Therefore the potency of the innervation must vary from strongly supramaximal for excitation to minimal in different cells. Since a given cell may be supplied by nerve fibers from at least 3 different trunks (4)

the number of synapses maximally engaged even by the nerve of greatest stimulating effectiveness must be only a portion of the total number available. In the animal, the individual cells might be activated by synchronized volleys in all the innervating trunks. Discharge of the cell would therefore occur with certainty and probably simultaneously over the whole excitable surface.

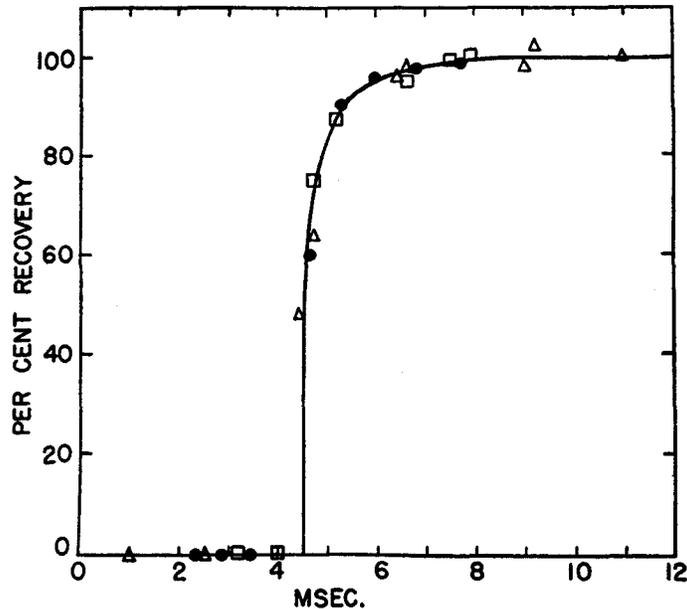


Fig. 6. Recovery of the electroplaque from refractoriness. Ordinate is the amplitude of the testing response relative to the unconditioned. Abscissa is the interval between conditioning and testing responses. Open triangles, conditioning and testing stimuli were very strong, direct. Open squares, the conditioning was neural and maximal. Filled circles, testing was neural and maximal. The responses of this cell are shown in Fig. 8. Propagation developed at about 9 msec. XI-11-11.

Responses of the cell during refractoriness are shown in Figs. 7 to 10. In the experiment of Fig. 7, the direct conditioning and testing stimuli were both very strong, as seen from the early onset of spike 1 (*E, F*). During the occurrence of conditioning spike 1 the testing stimulus caused no detectable response (*A, B*). The absolutely refractory period outlasts the spike as is also the case in some nerve fibers (28). Early in the relatively refractory period a small, brief response is elicited at electrode 1, but is not propagated (*C*). Still later, when response 1 is large, a small potential is recorded at electrode 2. This is probably caused by the combined currents from the spread of the stimulus and of local circuits from more proximal activated regions. Recovery of propagation takes

place thereafter (*E*). The difference in amplitude between spike 1 when it is not (*D*) and when it is propagated (*E*) is small, though the latter response

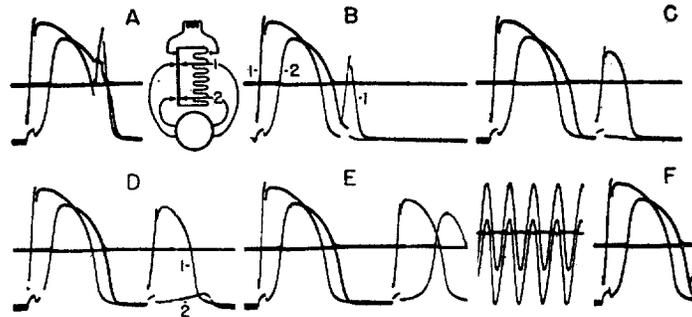


FIG. 7. Refractoriness to very strong direct excitation. During absolute refractoriness, the testing stimulus evoked no activity (*A*), but later (*B*) a small local response probably occurred at site 1. During early recovery (*C*) a local but large response developed without corresponding activity at 2. Later a small response occurred at 2 (*D*). Propagation returned (*E*) when there was little additional increase in spike 1. *F*, the unconditional testing responses. Distances 0.59 and 2.13 mm., XI-13-0.

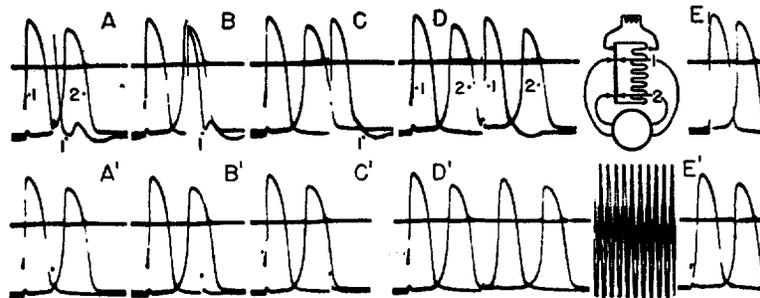


FIG. 8. Recovery of direct excitability and of propagation. *A* to *E*, the testing stimulus was strong (*E*). Early in refractoriness (*A*) this caused only the postsynaptic potential at site 1. Later (*B*) it produced a local and brief response though of high amplitude at 1, followed by a postsynaptic potential at 2. The latter is also seen (*C*) after the larger second response of 1. Earliest onset of propagation occurred at about 9 msec. (*D*). *A'* to *E'*, the testing stimulus was weak (*E'*) and caused no responses until nearly the end of relative refractoriness (*D'*). Data of the experiments on this cell furnished the curve of Fig. 6. Distances 1.09 and 3.6 mm., XI-11-8.

lasts longer. Inability of the cell to respond to extremely strong direct stimulation during the absolutely refractory period, or to propagate activity during early recovery is further shown on a slower time scale in Fig. 8 *A* to *C*. The events of recovery will be analyzed in section VII.

While the cell was inexcitable directly, a small, late response often appeared

(Fig. 8 *A*) at electrode 1 and persisted when the direct stimulus produced large but local activity at this site (*B, C*). The late potential may still be seen after testing spike 1 in record *D* when propagation returned. It is probably also present, though masked, in the longer lasting spike 1 of the testing stimulus in isolation (*E*).

All similar experiments have confirmed that during early recovery from refractoriness the cell responds with graded, local activity. Recovery of propagation occurs later. These experiments have also demonstrated, however, that during the period of unresponsiveness to direct stimulation, a small late poten-

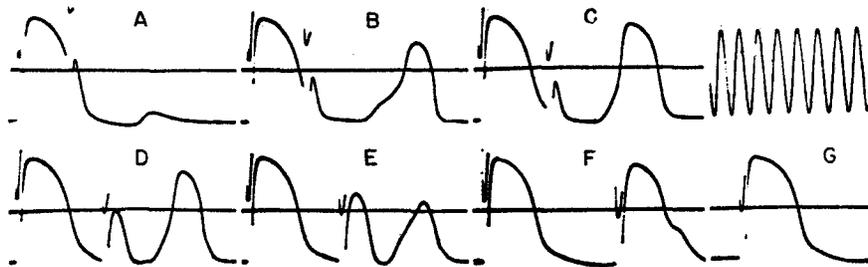


FIG. 9. Competitive excitation of the cell by the postsynaptic potential and by direct stimulation. A testing direct stimulus falling in the absolutely refractory period of the cell did not elicit direct activity of the cell (*A* to *B*), but produced the later, indirectly evoked postsynaptic potential (*A*). This in turn elicited a response of the cell during its relatively refractory period (*B*) which grew to spike-like proportions (*C*) later in recovery. As recovery to direct excitation developed (*D* to *F*), the directly elicited response decreased the synaptically evoked (*D*), grew at the expense of the latter (*E*), but the initial late postsynaptic potential remained obvious on the falling phase of the recovering spike (*F*). The testing response in isolation, *G*. XI-11-0.

tial is elicited by a strong stimulus, and persists (Fig. 8 *B* to *D*) even when prior local activity develops. The time of its onset corresponds to the time of development of the postsynaptic potential on stimulation of the terminations (Figs. 3 to 5).

Fig. 9 indicates the validity of this identification. A strong direct stimulus (*G*) elicited a spike, the terminal phase of which was distorted by the presence of a later potential. During absolute refractoriness caused by an antecedent stimulus, the direct testing response was absent (*A*) but the small late response was evident. When the testing stimulus was applied later, but still in the absolutely refractory period of the cell, and therefore before directly elicited activity was possible, the later small potential fell in the relatively refractory period and caused at first (*B*) a belated and small response. This grew in amplitude as recovery of the cell progressed (*C*). The testing stimulus itself caused a direct response of the cell (*D*) when applied in the relatively refrac-

tory period. This in turn produced refractoriness and, as a result, the subsequent response of the cell diminished, becoming smaller as the direct response increased (*E*). The diminution did not affect the initial component originally seen in *A*, which now appeared on the falling phase of the second spike (*F*). This component therefore is capable of initiating a spike and has the latency of the indirectly evoked postsynaptic potential.

The ability of the cell to respond to neural stimulation while refractory to a direct stimulus is further demonstrated in Fig. 10. The maximal testing neural

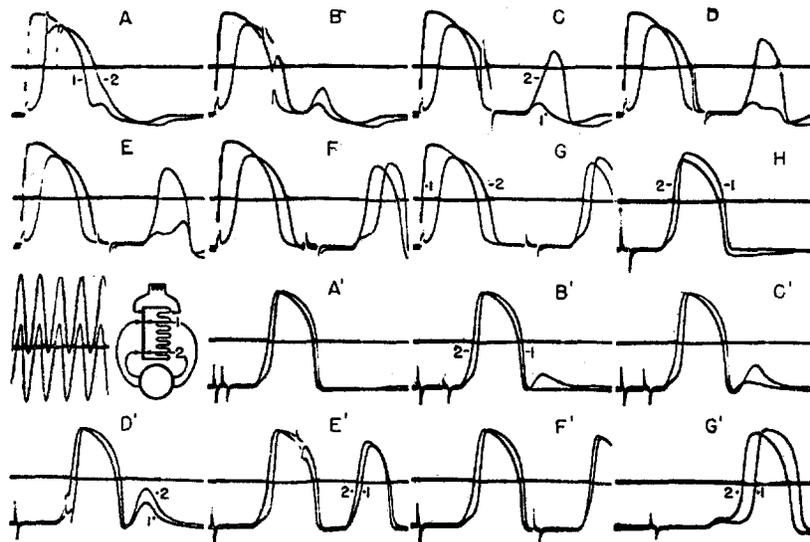


FIG. 10. Excitability to neural stimulation during the course of recovery of the cell from refractoriness. The same cell as in Fig. 7. *A* to *G*, direct conditioning stimulation. *A'* to *F'*, homosynaptic conditioning. *H*, *G'*, the unconditioned testing responses. The neural testing volley was maximal for the upper records, weaker for the lower set. Distances 0.59 and 2.13 mm., XI-13-5.9.

volley in isolation produced nearly synchronous discharge of the cell (*H*) prefaced by a postsynaptic potential. After a direct conditioning stimulus, and while the cell was still refractory to strong direct stimulation (Fig. 7 *A*, *B*, which are from the same experiment), the neural volley produced postsynaptic responses at both recording electrodes (*A*). These fell on the descending limbs of the spikes. The postsynaptic response at electrode 1 remained small as the neural volley arrived later in refractoriness (*B*, *C*), but this was also noted for single neural stimulation (Fig. 2 *B-E*). The response at electrode 2 became larger, developed an overshoot (*C-E*), but response 1 was still impaired. Later in recovery, activity developed at both recording sites (*F*, *G*) but the

onset of spike 1 was delayed. Analysis of the excitability changes during refractoriness will be postponed in order to emphasize at this time that the postsynaptic response is not subject to refractoriness of the cell (A, B). Furthermore the responses elicited in the directly conditioned cell by the neural testing volley (Fig. 10) are similar to the responses caused in the same cell by a direct testing volley (Fig. 7). Records C, D, E of Figs. 7 and 10 represent approximately the same times in the recovery from direct conditioning.

In the same experiment (Fig. 10) paired stimuli were also applied to the nerve (homosynaptic excitation, A' to F'). When these were closely spaced (A' , 0.6 msec.) the nerve was refractory to the second. At an interval of 1.2 msec. the second stimulus caused a postsynaptic response either at electrode 2 (B') or at both (C'), that at electrode 1 then being smaller as in the unconditioned response (Fig. 2). The absence of a response at 1 in record B' may be ascribed to small differences in recovery of nerve fibers from absolute refractoriness. In frog A fibers the absolutely refractory period at room temperature is about 1.2 msec. and it is likely that in the electric eel at least a similar value obtains. Slowed conduction in the nerve during its relative refractoriness probably accounts for the delay in the response time of the postsynaptic potential after the conditioned second stimulus, which is not present in the unconditioned responses (Fig. 10 G'). The delay may also be due in part to refractoriness of the postsynaptic potential after a preceding response of this type. However, because of the facilitation produced by homosynaptic volleys this possibility is unlikely, but the matter requires further study. The delay is still to be found at a longer interval between the stimuli (D').

The propagated testing responses in E' appeared earlier in the refractory phase of the cell than did the responses in F when the conditioning stimulus was direct, yet the former are much more nearly synchronous than the control (G'), while the latter are less synchronous than in the isolated testing condition (H). The same effects are to be seen on comparing F' and G . The neural stimulus preceded by homosynaptic conditioning activity therefore evoked more nearly synchronized spikes than did the same stimulus alone or preceded by directly excited conditioning activity. This result may be accounted for on the basis of homosynaptic facilitation (4). The facilitation is exerted also in another way. The smaller spikes occurring during the relatively refractory period of the cell (E', F') arise earlier on the postsynaptic potential than does the maximal unconditioned response (G'). It had been noted previously (4) that the prolonged homosynaptic facilitation takes place without a detectable electrical concomitant. This is also seen in Fig. 10 E', F' .

Independence of the postsynaptic potential from the excitability changes of refractoriness to which the directly excitable response of the cell is subject might be accounted for if the neurally evoked prepotential did not arise in the cell membrane. However, as pointed out earlier in this paper, the sign

relations of the spike, the local response, and the postsynaptic potential indicate that all occur at the excitable membrane of the cell. Another explanation might be that those regions of the membrane which develop the postsynaptic potential are not activated by an electrical stimulus and for this reason are not subject to the refractoriness following earlier activation of those portions which produce the directly elicited spike.

The validity of the latter explanation was demonstrated by superimposing the effect of anodal polarization (current inwardly directed across the excitable membrane) or cathodal (current outward) upon the excitation produced by

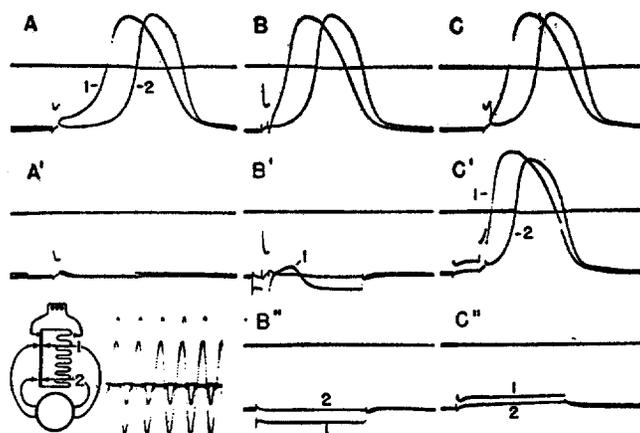


FIG. 11. Alteration of direct excitability by electrotonic polarization. *A, B, C*, responses to direct stimulation. Weak anodal polarization applied through the stimulating electrodes abolished the response (*A'*). Stronger anelectrotonus (*B''*) was required to block (*B'*), stronger direct stimulation (*B*). Cathodal polarization (*C''*) enhanced direct excitability (*C'*), decreasing latency of spike 1, and response time of spike 2. Denervated preparation. Distances 0.83 and 2.56 mm., XI-17-0.

direct or neural stimuli. Activity of the cell initiated by a threshold direct stimulus is abolished by weak anodal polarization in the region of stimulation (Fig. 11 *A, A'*). Stronger anodal polarization (*B''*) was required to abolish (*B'*) the spike produced by a strong direct stimulus (*B*) but in this case a local response appeared at the site of stimulation. Cathodal polarization of the stimulated region (*C''*) enhanced direct excitation (*C'*) as seen by the decreased latency of spike 1 and earlier onset of spike 2 (*C* and *C'*). The directly excitable membrane of the electroplaque therefore behaves in the manner to be expected of electrically excitable tissues.

This is not the case with the neurally activated component (Fig. 12). The anodal polarization which had been sufficient to block strong direct excitation (*A''*) did not affect (*A'*) the postsynaptic potential recorded at electrode 1

(A), either in its time of onset or magnitude. However, propagation through the polarized region was slowed as denoted by the delay of spike 1. The postsynaptic potential was also unaffected by a threefold increase of the polarization ($B-B''$) although spike 1 was further delayed (B'). Therefore it may be concluded that the postsynaptic potential, unlike the directly excitable spike, is not affected by electrical alterations of the excitable membrane.

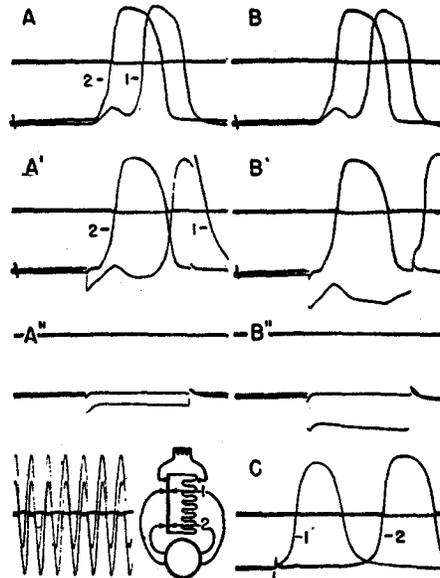


FIG. 12. Independence of the postsynaptic potential from anodal polarization. *A*, *B*, responses to a strong neural volley. Strong anodal polarization (*A'*) did not affect the postsynaptic potential, but delayed spike 1 (*A'*). The synaptic potential persisted unaltered (*B'*) with a threefold increase of the polarization (*B''*) which delayed spike 1 until the break of anelectrotonus. *C*, the directly evoked spike. Distances 0.83 and 4.5 mm., XI-16-0.

VII. Events of Electrical Excitability

Analysis of recovery from refractoriness in the electroplaque presents a special problem. Strong direct or neural stimulation can excite the cell nearly synchronously (Figs. 1 *G*, 2 *G*). The longer intervals between spikes 1 and 2 in Fig. 10 *F*, *G*, than between the unconditioned testing responses (*H*) indicate not slowed propagation but lowered excitability of the cell. The neural volley was probably strongly supramaximal at site 2 (as in the experiment of Fig. 6). This is indicated by the rapid rise of response 2 on the postsynaptic potential even early in recovery (Fig. 10 *D*). At site 1 neural excitatory action is lower (as indicated by Fig. 2 *C* to *E*). This weaker excitation at first leads to small

local responses (Fig. 10 *D, E*). When an overshoot appears in spike 1 (*F, G*) the records give the appearance of propagation, but this is belied by the high calculated conduction rates (3.2 m.p.s., *F*; 6.0 m.p.s., *G*). The delays are therefore reflections of lowered excitability of the cell to the neural volley. As recovery of this proceeds, response 1 moves forward on the postsynaptic potential (*F, G*).

Analysis of changes in propagative ability can be carried out more safely in experiments such as that of Fig. 8. The conduction velocity determined for the responses to a threshold testing stimulus (*E'*) was 0.75 m.p.s. This testing stimulus evoked no activity in the cell when delivered 8 msec. after the conditioning (*C'*). At a stimulus separation of 9.3 msec. (*D'*) both testing responses appeared though with prolonged latency. They were at least 95 per cent of maximal amplitude, but conduction velocity was 0.65, a slowing of 15 per cent. With the strong unconditioned testing stimulus (*E*) the apparent conduction velocity was 1.1 m.p.s., indicating that onset of spike 2 was speeded by electrotonic spread from the stimulus. At the 8.0 msec. interval (*C*) this testing stimulus produced a large but localized response at 1. Response 2 also occurred at the 9.2 msec. interval (*D*), although the spikes were evoked earlier in recovery than those in *D'* because the latency was shorter. Again, both responses had nearly maximal amplitudes. The conduction velocity was 0.75 m.p.s. so that direct initiation of the response must have occurred somewhat distally to site 1.

The strong testing stimulus itself caused brief electrotonic depolarization of the membrane almost as large as the maximum of the spike (*A*). Local response 1 (*C*) developed under the drive of this stimulus as did the local activity in Fig. 7 *C, D*, also under the drive of strong electrotonic depolarization (*A, B*). Therefore, during recovery from absolute refractoriness the cell is able to respond to very strong excitation from adjacent regions. In the experiment of Fig. 8 the stimulating electrodes were 1.09 mm. from site 1. However, the differences in amplitude and duration between the local response at site 1 and that which can cause propagation are rather small (Figs. 7 *D, E*; 8 *C, D*). The large non-propagating response 1 (8 *C*) therefore must have caused a response in regions of the cell immediately downstream, but this did not propagate. Why? One answer, which will be developed later might be that the all-or-nothing responsiveness of the cell had not yet returned although the local response, under the drive of an impressed stimulus, appeared as large as the later all-or-nothing spike.

The use of weak direct stimulation (Fig. 8) avoids the complication introduced by the development in the electroplaque of generalized but perhaps everywhere local, non-propagated activity caused by strong direct or maximal neural excitation (14, 48). However, use of this testing stimulus, though it reveals the recovery of propagation, may obscure the early events in recovery of responsiveness. This is seen in comparisons of the results with strong and

weak direct testing stimuli in Fig. 8. The strong stimulus caused a large localized response 1 (*C*) and propagation developed later (*D*) with little change in amplitude of spike 1. The weaker stimulus at the first stimulation interval caused no response at site 1, 1.09 mm. away (*C'*). When propagation returned (*D'*) both spikes 1 and 2 were initiated in this manner as seen from the latency of 1.

Comparisons may be made of the conditioned responses of the same loci in one cell to different strengths of the excitant. The testing responses were produced by a strong direct or a maximal neural volley (Figs. 7 and 10). The directly elicited testing response 1 in Fig. 7 *C*, *D*, and *E*, developed at approximately the same time after conditioning response 1 as did the neurally evoked testing response 2 in Fig. 10 *C*, *D*, and *E*, after conditioning response 2. Therefore the excitability of the cell at the two loci was probably undergoing similar changes. The stronger membrane depolarization produced by the direct stimulus (Fig. 7 *B*) at each comparable time (Fig. 7 *C* to *E*) initiated a larger local response than did the smaller postsynaptic potential (Fig. 10 *B* to *E*). The weak direct electrotonic stimulus at 2 in Fig. 7 *C* and *D* (about 7 mv. membrane depolarization) evoked smaller activity than did the larger postsynaptic potential at 1 (Fig. 10 *E*), which caused activity such as is often seen on the verge of propagation. Thus, the changes in responsiveness of the cell undergo essentially the same course when tested by strong or weak stimulation, whether this be direct (Fig. 7) or neural (Fig. 10). The largest responses were produced by the strongest stimulation (Fig. 7, site 1) and the smallest by the weakest (Fig. 7, site 2). At two loci of the same cell (1 in Fig. 7, 2 in Fig. 10) the stronger stimuli first caused graded responses which became large without leading to propagation. At both sites the latter was initiated rather abruptly with little change in the proximal response.

The sequence of excitability changes during recovery from absolute refractoriness may now be summarized. At first, strong excitation elicits a local reaction of the cell. Tests with very strong stimulation reveal that the maximum electromotive force of this reaction increases from zero during the absolutely refractory state nearly to full amplitude of the all-or-nothing response (Figs. 6 to 8). Tests with different strengths (Figs. 7 and 10) reveal that the excitability also increases during this time. Propagation does not return until nearly the end of the refractory period, when the local response has reached nearly maximal amplitude (Figs. 6 to 8 and 10). In this condition of the cell the safety factor is 1 or slightly larger, whereas in the normal state it appears to be 3 to 4. The difference probably reflects the critical level of depolarization needed for initiating the regenerative explosive process which is necessary for propagation. Prior to recovery of propagation, the threshold for this process must have been higher than the depolarization of neighboring regions produced by even the maximal electromotive force of activity. Therefore propagation

did not take place (Figs. 7 *D*, 8 *C*, 10 *E*). As the threshold for the regenerative process decreased in recovery, propagation could set in (Fig. 8 *D*, *D'*). At first the conduction rate was low (Fig. 8 *D'*), probably because this threshold was high.

Three different events therefore appear during recovery from refractoriness. Recovery of the degree of reactivity of the membrane, and recovery of its excitability may be linked as parts of one process which causes a gradually increasing local response. The recovery of regenerative reactivity, however, appears to be a distinct process, following a different time course and leading to a distinction between large local responsiveness and ability to propagate this responsiveness. The neural excitant acts in the same way as does electrical stimulation in initiating the response of the electrically reactive portion of the excitable membrane.

VIII. Initiation of the Postsynaptic Potential

Thus far the experiments have been too few to define the events which may intervene between arrival of the neural volley at the membrane and the initiation of the postsynaptic potential. The possibility that a delay exists between arrival of neural impulses and of the postsynaptic potential has not been tested directly. The incoming neural spikes are not to be seen in Fig. 13, although a change of about 300 μ v. would have been detected. However, existence of a delay between the arrival of the neural impulses and onset of the postsynaptic potential may be inferred. Indirect stimulation of the cell excites nerve fibers in the immediate vicinity of the stimulating electrodes. It probably causes this at their terminals close to, or at the caudal surface of the cell. The neural spikes would therefore reach the excitable membrane after very small conduction. Conduction velocities of the fibers in the vicinity of the electroplaque have not been measured. However, strong indirect stimulation in some preparations elicits a later postsynaptic response at site 2 (Fig. 3 *B*, *E*, *F*) than at 1. If this delay is a function of conduction distance in the nerve terminations the velocities in these 2 experiments were 4.5 M.P.S. (*B*) and 5.7 M.P.S. (*E*, *F*) respectively. The conduction velocity of intercostal nerves ranges between 4 and 10 M.P.S. (1). The intervals between the stimulus and the synaptic potential at site 1 were respectively 1.5 and 1.9 msec., whereas with the velocities just calculated the conduction times in the nerves should have been 0.2 and 0.1 msec. The observed latencies are not shortened by more than a few tenths of a millisecond by very strong indirect stimulation (Fig. 3 *A*, *B*, *E*, *F*). There are, of course, several assumptions underlying the indirect evidence, but these do not appear to be improbable, and, if the calculations are justified they indicate that a very considerable part of the delay between the indirect stimulus and the postsynaptic potential is occupied with initiating that activity. The shortest latency observed has been 0.96 msec. and the longest 2.43 msec.

The long facilitation evidenced in homosynaptic stimulation and its ex-

tremely short duration (2 msec.) in heterosynaptic stimulation or when a direct stimulus is paired with a neural volley (4) were confirmed in the present work, and were pointed out in connection with Fig. 10. Another difference between homosynaptic and direct excitation which may in the future throw some light on the nature of neural activation of the cell was mentioned in the earlier paper and was also confirmed in the present work. The cell becomes irresponsive to repetitive stimulation of the nerve trunk or of its terminals after a few or up to 100 spikes, and this effect develops even at stimulation frequencies as low as 5/sec. Interruption of the stimulation leads to slow recovery. However, during irresponsiveness to neural stimulation the cell is directly excitable. Direct stimulation at rates as high as 50/sec. is effective for as long as 5 minutes.

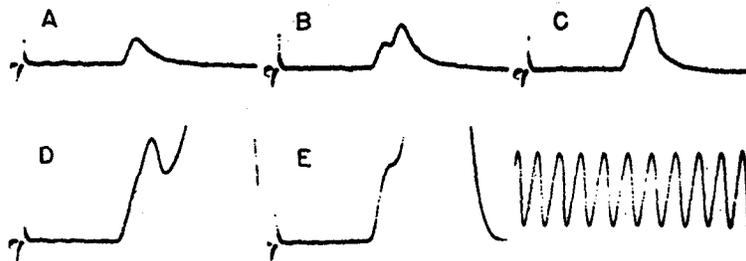


FIG. 13. Development of the postsynaptic potential with increasing neural stimulation. Increase of the stimulus to the nerve caused stepwise increments of the postsynaptic response (A to C) and its synchronization (C). The response lasts about 3.5 msec., but its major component is ended in less than 2 msec. The strength of the stimulus was the same in C, D, and E. Only the bases of these responses are seen at the amplification employed. Calibration 10 mv. and 1000 c.p.s., XI-4-7.

IX. Mode of Excitation of the Cell by the Postsynaptic Potential

Although that portion of the membrane which generates the postsynaptic potential has properties different from those of the electrically excitable part, activity in the former generates a response in the latter. How this is achieved is the subject matter of this section.

Increasing strength of stimulation of the nerve adds almost equal incremental steps of postsynaptic response (Fig. 13 A to C). These synchronize and fuse (C) but the total potential is nevertheless small compared with that of the membrane change of the spike, which arises out of it with a clearly seen delay (D, E). The stimulus was unchanged for records C to E of Fig. 13, the differences therefore reflecting threshold play of excitability and perhaps also homosynaptic facilitation. The additional increment of potential seen in D which is not present in E may have been an additional postsynaptic response, or an abortive spike. The latter would appear to be more likely, since a larger postsynaptic response might be expected to cause an earlier initiation of the spike, whereas

that in record *D* arose later than did the spike in record *E*. The postsynaptic potential thus consists of increments of depolarization of the membrane, produced by an increasing number of active nerve fibers. When it reaches an amplitude of about 15 mv. it is capable of initiating the events leading to the spike. A delay of about 0.5 msec. is to be observed in *E* between the time the postsynaptic potential reaches its peak and onset of the spike.

The transition from postsynaptic potential to spike cannot be distinguished in the records of Fig. 13, because the amplification used carried the initial deflection of the spike off the screen of the tube. This transition is shown in the experiment of Fig. 14. A single maximal neural volley did not discharge the

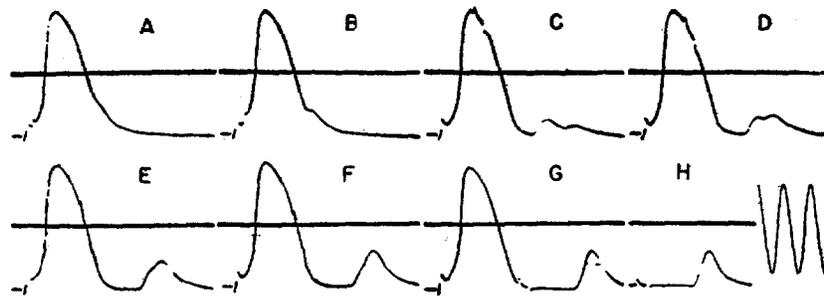


FIG. 14. Components of the neurally evoked graded response. The cell was not discharged by a maximal neural volley. The unconditioned response is shown in *H*. A component of this response is developed during the spike produced by a direct conditioning stimulus (*A*, *B*). A later, small component develops during recovery of excitability (*C*), grows (*D*), and arises earlier (*D* to *F*), to fuse with the first (*G*), except for a slight change in slope (*F*, *G*, and *H*). The first component (postsynaptic potential) is about 20 mv. and does not augment (*C* to *E*). The latter component (local response) has about equal amplitude. The maximal response (*G*, *H*) was about 40 mv.

cell of this experiment, but caused a prefatory response (*H*) about 30 mv. in amplitude. A notch may be noted in this response. When the neural volley arrived during the refractoriness caused by a direct conditioning response (*A*, *B*) only a small postsynaptic potential developed on the falling phase of the spike. During recovery of excitability of the cell, a small, late potential was added (*C*). This grew and arose earlier (*D*, *E*) to fuse with the postsynaptic potential (*F*, *G*). The neurally evoked prefatory potential therefore appears to be composed of two elements. The earlier, which is not subject to absolute refractoriness of the cell and does not increment during recovery from refractoriness, has the properties identified above with the postsynaptic potential. The second, which is subject to refractoriness of the cell and gains in amplitude during recovery, probably is to be identified with the local response of the cell. If this be correct, then the depolarization produced by a neural volley in the electrically non-responsive parts of the excitable membrane in turn causes local

depolarization of nearby electrically excitable segments. The summated depolarization, about 40 to 50 mv. at the time the spike is initiated (Fig. 2), is of the same order of magnitude as the critical membrane depolarization when the spike is initiated by direct stimulation of the denervated cell (Figs. 1 and 11).

Evidence to support the conclusion that the neurally evoked prefatory response is composed of the neurally initiated electrically inexcitable postsynaptic potential and of a local response generated by the latter is shown in Figs. 15 and 16. A threshold direct stimulus which caused the responses shown in the

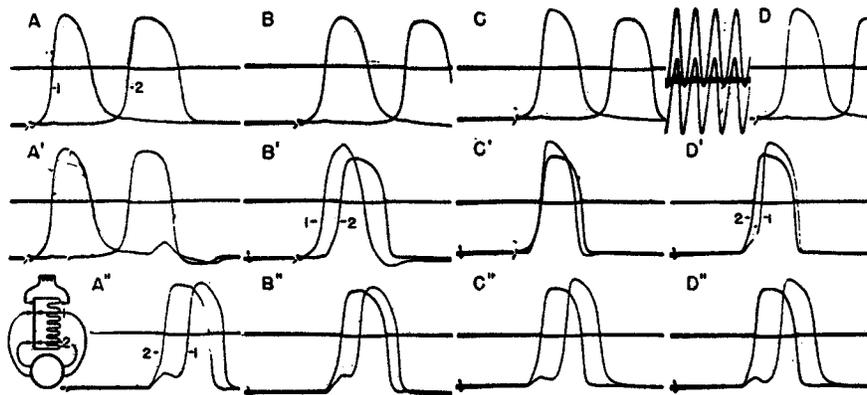


FIG. 15. *Upper row*, responses to threshold direct stimulation. *Lower*, responses to a neural stimulus. *Middle*, (*A'*), the direct stimulus applied early caused both spikes 1 and 2 before these could be evoked by the neural volley. (*B'*), the direct stimulus initiated spike 1 while spike 2 was evoked by the neural volley. (*C'*), spikes 1 and 2 simultaneous, but each evoked by a different excitant. The responses in *A'* and *C'* arose in the time specific for their excitant. (*D'*), spike 1, arising by excitation from the nerve, developed earlier when the excitatory effects of the direct stimulus were superimposed on the prefatory potentials. Distances 0.83 and 4.5 mm., XI-16-4.6.

upper row of Fig. 15 was delivered at various intervals before or after strong stimulation of the nerve (middle row). The responses to the neural volley alone are shown in the lower row. The spikes caused by the two kinds of stimulation appeared in reversed sequence and are easily identifiable. When the direct stimulus precedes the neural (column A) the responses elicited with shorter latency by the former than were the neurally evoked, cause refractoriness to the neural volley. Only the postsynaptic potential then develops at recording electrode 1 (*A'*). That at electrode 2 is lost in the large response of the directly elicited spike 2 which is shortened by the reversed potentials of other active cells. The direct stimulus was then spaced so as to initiate spike 1 shortly before the earlier stimulus to the nerve caused spike 2 (*B*). Each spike then appears at a time determined by the latency to its excitant. The

peak of the directly elicited spike 1 is distorted by superposition of the postsynaptic potential evoked neurally at that site. The falling phase of spike 1 is now also shortened as is spike 2. In column C the two stimuli were so spaced that both spikes occurred simultaneously, but spike 1 is the response to the direct and spike 2 to the neural stimulation. The former again carries the postsynaptic potential on its rising phase and peak. The time relations of the two responses (*C'*) therefore show that the direct stimulus delivered to the cell

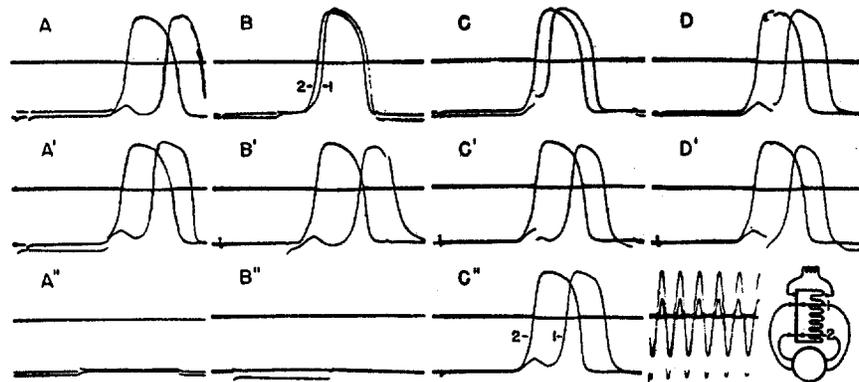


FIG. 16. Polarization and neurally initiated excitation. *Upper row*, effects of cathodal polarization on neurally evoked response 1. *Middle*, the effects of anodal polarization. *Lower*, *A''*, the cathodal polarization; *B''*, the anodal and *C''*, the testing responses in isolation. *A*, the cathodal polarization ended as the postsynaptic potential began. The latter was unaffected but spike 1 was delayed by postcathodal depression. *B*, later onset of polarization caused spike 1 to arise sooner. *C*, the excitatory effect of the polarization became less marked as the latter began at the peak of the postsynaptic potential. *D*, the onset of polarization at the end of the postsynaptic potential when spike 1 was beginning had no excitatory effect. *A'* to *D'*, reverse sequence of effects by anodal polarization. Spike 1 was prolonged by cathodal polarization and shortened by the anodal. Same experiment as in Fig. 15.

less than 1 msec. before onset of the postsynaptic potential did not interact with the latter. However, when the direct stimulus is applied during the postsynaptic potential (column D) interaction does occur (*D'*). In this record, the neurally evoked spike 2 precedes 1, but the latter arises earlier than following either stimulus alone. Thus, during the course of the electrically inexcitable postsynaptic potential, an electrically excitable component is produced which summates with the effects of a direct stimulus to facilitate discharge of the cell. This summation lasts about 2 msec.

Similar interaction, for brief times only, is obtained between a neural volley and a polarizing current (Fig. 16). The responses to the neural volley in isolation are seen in *C''*, and the magnitudes of polarization in *A''* (cathodal) and *B''*

(anodal). Since the polarizing current was applied nearest electrode 1, the magnitude of the potential change is largest at that electrode and almost absent at electrode 2. When a cathodal polarization is terminated at the time the postsynaptic potential begins to develop (*A*) postcathodal depression causes delay in onset of spike 1, though the postsynaptic potential is itself unaffected. Postanodal enhancement probably was not large enough to make spike 1 arise appreciably earlier (*A'*). When polarization begins before the postsynaptic potential develops, cathodal excitation (*B*) or anodal depression (*B'*) of conduction velocity is most marked. The effects decrease as polarization begins later in the postsynaptic potential (columns C and D). The effects of polarization probably take time to develop and therefore are not yet maximal when discharge of the cell takes place. Once the spike began the strength of anodal or cathodal polarization used did not prevent continued development, but did alter its form.

X. Alterations in the Form of the Spike

1. Effects of Polarization.—Inspection of Fig. 16 shows that the spike which arises in a region of depolarization is prolonged (*B* to *D*) and is shortened by hyperpolarization (*B'* to *D'*). Decrease of the duration of spike 1 by anodal polarization is also to be seen in Fig. 12 (*A'*). The opposite effects might be expected. Anodal polarization decreases sodium inactivation and cathodal polarization increases it (29). Cathodal polarization should therefore cause lowering of the spike amplitude and shortening of the duration and the reverse changes should occur with anodal polarization. The spike amplitude does not appear to have been markedly affected by the applied currents used, but the changes in spike duration are notably the reverse. Further study of polarization effects may therefore prove a valuable test of the Hodgkin and Huxley theory.

Anodal polarization initiated before or during the local response suppresses the latter and in consequence also the spike. The same, or several times stronger polarization applied once the spike has begun does not affect this response. This interesting phenomenon may be predicted from considerations regarding the safety factor, but detailed studies may serve to cast further light on the kinetics of response reactions.

2. Effects of Recording Conditions.—Propagation is slow in the electroplaque and the membrane resistance in the resting state is low (33). The space constant of the cell is therefore small. These factors may operate to cause distortion in the recorded form and amplitude of the potential of the spike (Fig. 17). Column A shows the responses to threshold direct stimulation (*A'*), strong neural (*A''*), and strong indirect (*A'''*) recorded in the standard manner at two sites (*A*), each internal electrode being provided with its own external reference electrode, carefully placed directly opposite the latter across the caudal membrane. A common reference was then used for both internal electrodes, its

position being at 2 (column B) or at 1 (column C). The synchronous (B'') or nearly synchronous (C'') neurally evoked spikes were distorted only slightly. The asynchronous spikes elicited by the direct (B' , C') or indirect (B''') stimuli were recorded in severely distorted form. These distortions occurred in the potential at the asymmetrically paired internal electrode.

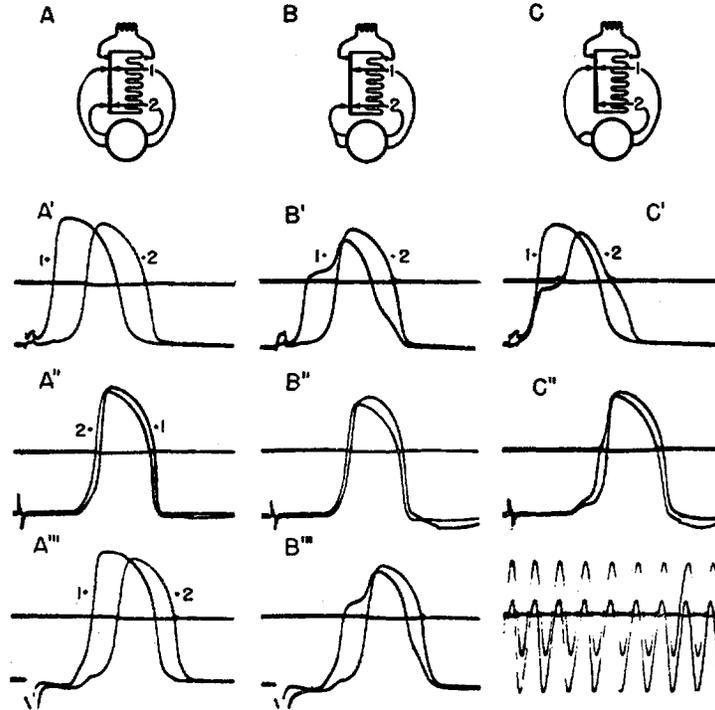


FIG. 17. Change in form of the spike with recording conditions. The diagrams of the top row show the arrangement of leads for the records of the respective columns. A' to C' , weak direct; A'' to C'' , maximal neural; A''' , B''' , strong indirect stimulation. Analysis of the records in text. Distances 0.59 and 2.13 mm., XI-13-0.

When this electrode is nearest the site of the onset of activity (B) the response of spike 1 is at first recorded correctly, but fails to continue rising until a response develops at the distal recording site (B' , B'''). The proximal potential then rises but its amplitude is smaller and duration longer than in the control (A' , A'''). The reverse is true when the asymmetrically paired internal electrode is that recording at the distal site (C , C'). The potential on trace 2 then rises too early, again reaches a plateau and begins to climb when spike 2 develops. The amplitude is again smaller, but the duration is now shorter. When the neurally evoked spikes (C'') developed less synchronously (as seen also

from their later onset in relation to the postsynaptic potential), response 2 developed first, as it does normally, but there was a break in its rise, similar to the plateau in B' and B''' . The further rise was then continued as activity developed at site 1.

The early deficit of potential recorded at electrode 1 in B' and B''' is largest as the rising phase and peak of spike 1 develop and before spike 2 is initiated. Therefore, it is not due to subtraction of a potential of the same sign caused at the common reference electrode by activity at site 2. The explanation offered for the distortion is based on the equivalent circuit conditions shown diagrammatically in Fig. 18.

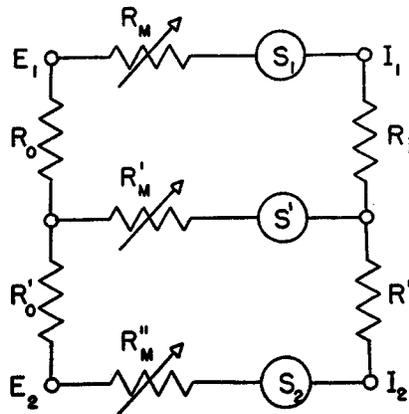


FIG. 18. Equivalent circuit of electroplaque. E_1 - E_2 , locations of external microelectrodes, I_1 , I_2 , of the internal. R_o , R'_o , resistance at exterior of cell; R_i , R'_i , of interior. R_M , R'_M , R''_M , variable membrane resistances. S_1 , S_2 , the bioelectric generators of spikes 1 and 2. S' , the propagating generators of intermediate loci. Discussion in text.

When each internal electrode (I_1 , I_2) is paired with its own external reference (E_1 , E_2) the potentials recorded by the pairs are essentially those produced by the local generators at the recording sites (S_1 , S_2) as these are brought into activity by propagation. The observed potentials are certainly smaller than the electromotive forces of the generators because of short circuiting by the relatively low resistance of R'_M , but should be somewhat larger when all generators are synchronously active. When E_2 is common for both internal electrodes (as in Fig. 17 B) the developing electromotive force of S_1 is recorded with a series-parallel arrangement of interposed resistance (R_o , R'_o , R'_M , R_i , R'_i). At first, however, R'_M , the lumped resistance of interposed inactive membrane, is relatively high. The potential across E_2 and I_1 should therefore be about as high as if the reference electrode had been at E_1 . Recording of spike 1 therefore

begins normally. Soon, however, activity develops at S' and R'_M decreases. This combination causes short circuiting of the potential difference across S_1 recorded between E_2 and I_1 . The potential does not fall, but assumes a value determined by the net potential of the complex of parallel generators $R_M S_1 - R'_M S'$. The level of the potential varies with experimental conditions (Fig. 17 B' , B''').

It might be expected that the potential would begin to increase again as the electromotive force of S' increases. However, the change in S' and R'_M continues to shift progressively toward site 2 since activity is propagating toward this site. This shift tends to maintain the relative short circuit and a plateau of potential until S_2 itself becomes active. Response 1 then rises again. Now, however, conditions are to some extent reversed. R'_M serves as a short circuit for $E_2 - I_1$, of the electromotive force generated at S_2 . The peak of response 1 is smaller and occurs when S_1 and S_2 are in the range of isopotential. The falling phase of response 1 is, however, prolonged by an addition from S_2 , the smallness being a reflection of the fact that electrotonic spread of activity is small in the electroplaque.

In the reverse condition of asymmetric recording (Fig. 17 C , C') the electromotive force of S_1 (Fig. 18) appears in full not only between E_1 and I_1 , but at first also between E_1 and I_2 , giving rise to the early potential of the distorted response 2. As short circuiting by $R'_M S'$ becomes operative a plateau begins to develop in response 2 and out of this rises the potential due to the change of electromotive force at S_2 . The peak of this potential again comes when S_1 and S_2 are isopotential, but the response begins to fall off rapidly as the electromotive force across S_1 declines. This is also a consequence of the short circuiting effects of R'_M . The conditions for recording the spike without distortion are met when S_1 , S' , and S_2 are activated simultaneously. This is almost the case in B'' , and slightly less so in C'' . In the former, the distortion is hardly noticeable, except at the peaks of the spikes. It is clear in the latter, but to a much smaller degree than in the propagated asynchronous responses (B' , B''' , and C').

The changes in the stimulus artifacts (A' , B' , and C') may be also analyzed in the same way. Under symmetrical recording conditions (A') the artifact of trace 1 is larger than that of 2, because electrode pair 1 is nearer the stimulating locus and the current across the membrane is larger. Referring to Fig. 18, the IR drop across $E_1 I_1$ is then larger than across $E_2 R_2$. The small stimulus artifact in trace 2 of B' (Fig. 17) is unchanged while that on trace 1 becomes larger, since the IR drop between E_2 and I_1 is larger. In C' , the artifact on trace 1 is unchanged but that on trace 2 is smaller and reversed, since the potential difference between E_1 and I_2 is in the reverse sense.

The foregoing analysis has been detailed because it offers a particularly clear illustration of the distortions in form and amplitude which may be obtained in the relatively simple case of a longitudinally propagating impulse

in a single large cell. Under other circumstance or in other tissues, particularly when recording with a single pair of electrodes, the plateau obtained in the records of Fig. 17 might be interpreted as block of conduction, or as a synaptic potential with synaptic delay. The secondary discontinuity on the falling phase (B' , B''' , and C') might be construed as activation of a new component. It is not clear whether or not the discontinuities in the falling phase of spikes in the slowly conducting and long skeletal or cardiac muscle fibers (14, 40) may be in part ascribable to similar distortions. It may be noted in this connection that the increased membrane conductance during the spike is surprisingly absent during the long plateau of cardiac fibers (47).

The changes in amplitude in Fig. 17 are to some extent comparable with those obtained in recording the spike of striated muscle fibers under the active end plate. Fatt and Katz (17) have ascribed this to short circuiting and in the light of the data of Fig. 17 and of the accompanying analysis this explanation would appear to be tenable. The amplitudes of the neurally evoked electroplaque spikes do not appear to be lower than the directly elicited spikes of the same cell. Often, indeed the reverse appears to be the case (Fig. 2). The circuit conditions analyzed in the foregoing make for ready distortion in spike amplitude, as well as form, and the variations in responses illustrated in this paper indicate that absence of the parallelism between responses of muscle fiber and electroplaques may not be significant. Furthermore, the electroplaque membrane even at rest has a very low resistance (33). Additional short circuiting by active but disseminated neurally excitable components might therefore cause only insignificant loss of spike amplitude as compared with the effects of the relatively massive zone of the end plate. Finally, the postsynaptic potential of the electroplaque itself causes only small depolarization of the membrane. Since it is an electrically inexcitable response the mechanism for regenerative driving (29) is probably absent and the change of membrane resistance at the sites of the postsynaptic potential may be too small to contribute significantly to short circuiting.

When the activity in the electroplaque preparation is recorded with the reference electrode grounded, the responses take on unpredictable and sometimes bizarre form, with lower amplitude and very long duration. This is probably caused by variations in the resistance between the grounded reference electrode and various parts of the cell through the fluid surrounding the preparation. This resistance may be rather important since the membrane resistance of the electroplaque is low (33). Even when paired recording microelectrodes are used, the recorded amplitude of the spike is diminished when the external reference electrode is moved away from the caudal face of the cell. Keynes and Martins-Ferreira (33) also observed the same effect. Use of a grounded reference electrode also increases the pick-up of activity from other cells than the unit under examination. This pick-up, in reverse sign, is seen in most of the

illustrations of neurally evoked activity, and is absent in records with threshold direct stimulation because only the one cell was then activated. On the other hand, Keynes and Martins-Ferreira (33) used general direct stimulation of up to 3 layers of cells, arranged in series-parallel. The currents of the activity of the large number of cells may have summated to give recorded potentials of the spike which are larger by about 25 mv. than the values of the present experiments. Keynes and Martins-Ferreira recognize this possibility and also discuss other types of distortion.

DISCUSSION

The Two Excitable Systems

Two distinct bioelectrically reactive systems are present in the electroplaque of the eel. One is electrically excitable and has the properties of this system found in axons and muscle fibers. It develops a graded local response and a propagated all-or-nothing spike. The second system is not electrically excitable, but is activated by neural impulses. It causes a postsynaptic potential which in turn can activate the electrically excitable components.

The Electrically Excitable System

The components of this system in the electroplaque have been revealed by analysis of responses elicited after conditioning activity. The recovery from absolute refractoriness apparently involves two changes. First are those leading to local reactivity of the membrane and later, those enabling return of propagation.

The threshold for reactivity is infinitely high during absolute refractoriness. At the end of this period strong stimuli can initiate membrane activity, but at first the generator produces only a small electromotive force. As the threshold decreases, weaker stimuli can also produce the minimal responses, but at the same time the maximal electromotive force of the membrane generator also increases and eventually reaches the level of the spike amplitude. The course of the latter recovery has been ascribed (29) to progressive decrease of sodium inactivation and potassium outflow. The decrease in threshold may also be related to these events.

A depolarization of about 40 mv. leads to initiation of a propagated response of the unconditioned electroplaque. Changes of the same magnitude are also required to set off a propagated impulse in the squid giant axon (29, 37), frog (17, 39), and crustacean (18) muscle fibers. During refractoriness of the electroplaque, however, the membrane develops local responses which have the amplitude of the spike. Propagation occurs with no further change of this, or with only small increase in amplitude and duration. The action on the cell of eserine, DFP, *d*-tubocurarine, procaine, and the tertiary analog of prostigmine (*m*-dimethylaminophenyldimethylcarbamate) appears to cause related effects

(5). First, the refractory period is prolonged. The electroplaque becomes neurally inexcitable though a single direct stimulus still evokes the propagated all-or-nothing response. Then propagation is eliminated, but for a long time thereafter increasing strengths of direct stimulation elicit graded local potentials which range from the minimal to full spike amplitude. Latent addition is also still present and upon repetitive weak stimulation the response also increases progressively from minimal to spike amplitude. Acetylcholine, and its quaternary and tertiary analogs (carbamylcholine and dimethylaminoethyl acetate), decamethonium, and prostigmine depress the amplitude of the neurally or directly evoked spike, but the latter maintains its all-or-nothing character and is propagated even when the amplitude falls as low as 50 to 60 mv.

The simplest assumption compatible with the data summarized above is that the regenerative process which drives the membrane into the all-or-nothing condition (29) is lost both when the cell is poisoned (as with eserine) and when it is in early relative refractoriness (6, 25). This is equivalent to the condition that the critical membrane potential required to initiate the regenerative process is greater than the maximum electromotive force of the membrane. During recovery from refractoriness this condition is temporary, and propagation returns soon after the response has attained or approached full spike amplitude. It is permanent after poisoning of the cell by substances which eliminate propagated responses. On the other hand, those substances which depress the spike but do not affect propagation of responses as low as 50 mv. apparently either do not raise the threshold for critical depolarization or do so only to a small extent. Although the maximum reactivity of the membrane generator is depressed, the explosive, regenerative process develops as long as the electromotive force of an active region is sufficient to cause critical, all-or-nothing depolarization of the next, previously inactive zone. Propagation therefore is retained until the electromotive force of the reactive generator falls below this magnitude. Similar results have been obtained in the squid giant axon, locally affected by microinjection of ions or drugs (25, 26).

A more complete description of the electrically excitable response is therefore possible on the basis of these results. An outward current derived from an external source or from the local circuit of activity first leads to a small depolarization of the excitable membrane which amounts to about 6 mv. (29, 30). This initiates a local response which represents a reaction of the membrane generator. The response is insufficient, however, for full activation of the generator and unless it is reinforced the membrane reaction proceeds no further, but may nevertheless be reflected in heightened excitability of latent addition. The local response is increased by stronger external or local circuit excitation, is proportional to this, and is therefore graded. At a threshold which is higher than for the initial reaction, a new process develops. This is regenerative and

explosive, causing the bioelectric generator to develop its maximal electromotive force rapidly. The value of the initial membrane reaction may be too small to change the membrane potential of a neighboring zone to the explosive level, and this may be caused either by decrease of the maximal response of the membrane or by increase of the threshold for the regenerative process. In either case propagation becomes impossible, except decrementally for a short distance, since the regenerative, explosive reaction implied in the all-or-nothing law is the *sine qua non* for decrementless conduction of the impulse. The differentiation between graded local responsiveness and all-or-nothing propagated activity also appears in mammalian A fibers (22). At the stimulating cathode responses may be elicited as frequently as 2000/sec., whereas the highest rates of propagated activity are about 1000/sec.

Inasmuch as the membrane generator can react to produce the maximum electromotive force of the spike either in an all-or-nothing, propagated action or in a graded, local response the two types of reaction are merely different pathways of membrane activity. The development of an overshoot through either pathway indicates that inward sodium flow takes place in both cases, but in one the self-driving sequence described by Hodgkin and Huxley (29) is absent. The transition from the non-propagated, graded pathway to an explosive, all-or-nothing response of the same bioelectric generator implies the onset of a different type of molecular activity, perhaps one involving a new component. However, speculation as to the precise nature of the molecular phenomena of the response would seem to be unprofitable at this stage.

The identification of two distinct components in the bioelectric response clarifies some problems emphasized in the work of Rosenblueth and his colleagues (42-44; summarized in reference 41, sections 18 and 23). That work pointed to difficulties in the concept of an absolutely refractory period. The existence of the latter appears to be confirmed by the results reported in this paper (Fig. 7) as well as by more recent work on squid giant axons (unpublished data). In the time interval between absolute refractoriness and the end of the "functional refractory period" (42), the response is graded, largest at the stimulating cathode and decrementally propagated from that region. The end of the "functional refractory period" would appear to correspond to recovery of decrementless propagation.

The present work also explains anomalies which led to the conclusion "that the local response and the spike potential are different in nature and properties" (reference 41, p. 56). The graded and all-or-nothing responses have many properties in common because both represent activity of the same bioelectric generator at the membrane. However, the transition from the graded response to the all-or-none involves an additional membrane change. Therefore modifications may be experimentally introduced in the threshold for graded activity, in the threshold for transition to an explosive response, in the maximum output

of the bioelectric generator, or in any combination of these. Such modifications might then be justifiably interpreted as indicating systems which are different in nature as well as in properties.

The Neurally Excitable System

The conclusion that a neurally excitable component of the response cannot be elicited by direct stimulation is based on four types of evidence. (i) The postsynaptic potential is caused by a neural volley when the electrically excitable response cannot be produced by strong direct stimuli. (ii) Anodal polarization, very much stronger than is needed to block the direct response, does not reduce the postsynaptic potential. (iii) The duality of the excitatory systems is further shown by the finding that an antecedent neural volley may facilitate the postsynaptic potential and thereby speed the time of onset and the degree of synchronization of the spike, whereas direct excitation does not facilitate the neurally evoked activity. (iv) This facilitation occurs only when activation of the cell is homosynaptic. Heterosynaptic excitation leads only to brief facilitation, similar in its time course (2 msec.) to interaction of direct and neural excitation.

Pharmacological data have confirmed (5) that the neurally excitable postsynaptic potential develops in a membrane component which is different from the electrically excitable. Those substances which inactivate electrical excitability of the membrane (acetylcholine, etc.) do not for a long time thereafter eliminate the neurally evoked post-synaptic potential. This response is elicited even when the membrane has become severely depolarized as well as electrically inexcitable. On the other hand, those substances (eserine, etc.) which block the explosive process of the membrane but not graded reactivity to electrical stimulation eliminate neural activation of the electroplaque and the postsynaptic potential.

Nature of the Neural Excitant of the Electroplaque.—It appears necessary to invoke a chemical transmitter to explain the neural initiation of the electrically inexcitable postsynaptic potential. Acetylcholine and its analogs, quaternary or tertiary, C₁₀, and prostigmine, depolarize the cell and inactivate the electrically excitable component, but do not affect the postsynaptic potential (5). The latter, on the other hand, is eliminated by compounds differing in anticholinesterase activity and chemical structure (eserine, the tertiary analog of prostigmine, DFP, *d*-tubocurarine, procaine), while the electrically excitable response remains intact for a considerable time. It would therefore appear (6) that the transmitter is not closely related to acetylcholine. This seems to be borne out by other pharmacological evidence (5). Though both eserine and prostigmine in low concentrations lower the threshold to neural excitation they do not alter the postsynaptic potential of the electroplaque and do not affect the course of homosynaptic facilitation. Different chemical excitants in

different tissues are not uncommon, as evidenced by the pharmacological differences of the adrenergic and cholinergic systems. Such differences may also be reflected in the shorter duration of the postsynaptic response of electric eel (about 2 msec.) as compared with the long neurally evoked response of the electroplaque of *Raia* (11) or of the frog muscle end plate (17).

Excitable Components in Other Cells.—Expanding knowledge of unitary activity in various excitable tissues suggests that these may be classified into three categories with respect to the nature of their activation. As far as is known at present, axons are electrically excitable everywhere along their length. Electroplaques of *Electrophorus* are endowed with a neurally but not electrically excitable system which is intermingled at the reactive membrane with an electrically excitable one. The former has different pharmacological properties from the latter (5) and in many ways its properties resemble those of the muscle end plate. Vertebrate striated muscle fibers of the “twitch system” (34) are electrically excitable everywhere except at their end plates (17) where apparently a neurally excitable and pharmacologically distinct system is concentrated. The end plate is generally considered to be a specialized structure although it is not clear whether it forms part of the reactive membrane of the muscle fiber or is a separate entity. In the electroplaque of the eel, the generator of the neurally initiated potential clearly appears to form part of the reactive membrane and for this reason its response has been termed a postsynaptic potential. On the same basis the end plate of muscle should also be considered as a specialized component of the excitable membrane of the muscle fiber, but unlike that in the eel electroplaque, concentrated in a zone of the fiber.

The electroplaques of rays and skates apparently belong to a third category in which the electrically excitable component is absent or nearly so (11, 20). The electroplaque of *Raia clavata* (11) is not electrically excitable and responds to neural stimulation with a long lasting potential, often lower in amplitude than the resting potential of 60 mv., but in some cells with an overshoot of a few millivolts. The cells of *Torpedo* also appear to be electrically inexcitable (20, 21).

The muscle fibers of the “slow” system of the frog, densely innervated over their entire length (34), react to neural stimulation with graded electrical and mechanical responses and may also be viewed as belonging to this category. The contractile mechanism of striated skeletal or cardiac muscle (of the “twitch” system) appears to have a regenerative component which leads to an all-or-nothing mechanical response. If this component be lacking in the contractile mechanism of “slow” fibers, as well as in their electrogenic membrane, graded, neurally evoked depolarization of the latter would cause graded mechanical responses in the former. On the other hand, these and other muscle fibers, particularly of invertebrates may constitute a group in which electrical

excitability only of the graded type is present. Direct as well as neural excitation might then produce only graded, non-propagated responses. Various types of excitability appear to exist in the diffusively innervated invertebrate muscle fibers (19, 28, 48). In some only neural excitability is present and the responses are graded (19, 27, 48). Others also respond to electrical stimulation, but with graded electrical activity (19, 27), while a third group develops all-or-nothing spikes (9, 13, 19, 27).

Electrical vs. Chemical Transmission.—Except for brief interaction with the local response caused by the postsynaptic potential, conditioning by direct stimulation does not affect the latter. This type of conditioning therefore cannot serve to test excitability changes of the neurally activated component. Homosynaptic stimulation, on the other hand, reflects the excitability changes produced by the initial neural volley, and also the responsiveness of the electrically excitable component as this recovers from refractoriness. Similar results have been obtained with autonomic ganglia (31, 35). Larrabee and Bronk (35) offered a very ingenious explanation which was based on the underlying assumption that the cell membrane is a single component electrically excitable system. They suggested that enhanced excitability after homosynaptic conditioning which is absent after direct (antidromic) conditioning was caused by presynaptic potentiation leading to increased excitatory action by the testing volley arriving in the preganglionic terminals. Facilitation is obtained in the electroplaque after neural conditioning tested by indirect stimulation. The results are then a combination of those seen in Fig. 8 *A* to *D* and Fig. 10 *A'* to *F'*. The strong testing stimulus excites nerve fibers close to or at the caudal membrane. Therefore, if presynaptic potentiation exists it must be confined to the extreme terminations of the nerve fibers.

Were there no evidence that the electroplaque membrane is endowed with two differently excitable systems the same explanation would also be applicable to the results obtained in this cell. However, excitability changes produced by direct and neural conditioning cannot be comparable in the electroplaque because the two kinds of conditioning affect different systems. Therefore, the demonstrated existence of a dual excitable system in the electroplaque, as in striated muscle (17),⁵ makes unnecessary the assumption of long lasting excita-

⁵ *Note Added in Proof.*—Fatt and Katz (17) inferred that the endplate is not electrically excitable. The demonstration has been provided by more recent work (del Castillo, J., and Katz, B., *J. Physiol.*, 1954, 125, 546) in which the directly excited spike was summed with the neurally evoked endplate potential. As in our experiments (*e.g.*, Fig. 10) the endplate potential enhances the falling phase of the spike. The overshoot, however, is diminished. This is taken to indicate that the neurally excited membrane reacts to increase ion permeabilities non-specifically. The depolarization produced in this way would be a Bernstein type (7) and cause short circuiting of the spike. The possibility that the neurally excited component of the electroplaque membrane

bility changes of nerve terminals too small to be detected in the rest of the electrically excitable axon (23) or in the electrically excitable component in the membrane of the cell body (35, 36).

The existence of an electrically inexcitable component demands that neural activation be initiated by a different mechanism and this is probably a chemical transmitter produced by activity of the nerve terminals. Long lasting homosynaptic facilitation may therefore reflect persistence of the excitant action of this transmitter or persistence of a lowered threshold in the neurally activated membrane component. Facilitation exists with no measurable accompaniment of depolarization of the post-synaptic membrane, and the first possibility—persistence of the transmitter—might seem more likely. However, during early action of substances which eventually block neural excitation (eserine, *d*-tubocurarine, etc.) the neurally evoked spike may develop when the postsynaptic potential is 2 mv. or less, and the resting potential of the cell is unchanged (5). Persistent heightened excitability of the neurally activated membrane component might therefore occur with no detectable electrical sign. Larrabee and Bronk (35) considered the possibility of localized postsynaptic facilitation in the cat stellate ganglion but rejected it.

The results of heterosynaptic stimulation are also similar in the autonomic ganglia (31, 35) and the electroplaque (4). Absence of prolonged facilitation under these conditions can be readily explained on the basis of preganglionic potentiation. On the other hand, the explanation offered above for homosynaptic facilitation could also account for these results. The chemical transmitter released at specific nerve terminals, or its effects, may not spread readily to membrane sites innervated by other nerve fibers, and the brief heterosynaptic facilitation (2 msec.) would take place by spatial summation (4) of the depolarizations produced by the different postsynaptic potentials and their local responses. Tests of the alternative explanations of homosynaptic facilitation could therefore be evolved from experiments with heteroneural stimulation.

Rapid fatigability of the eel electroplaque to repetitive neural stimulation while the cell is still, and for a long time, directly excitable may be ascribed to accumulation of blocking amounts of the chemical transmitter or to its exhaustion at the nerve terminals. Recovery to neural excitation takes place slowly after ending the repetitive stimulation of the nerve. This would imply on the one hand that the accumulated transmitter is removed fairly slowly or on the other, that its regeneration is slow. The explanation offered by Larrabee

also reacts in the same way was considered in section X of Results. On the other hand, inspection of spike 1 in Fig. 15 (*B'* to *D'*) shows that the peak is enhanced, not decreased by superposition of the postsynaptic potential. This might indicate that in the electroplaque the latter is caused by sequential switching of sodium and potassium permeability.

and Bronk (35) could also serve to account for these effects. Closer differential study of these phenomena and of their kinetics might, therefore, provide critical data in favor of one view or the other.

The junctional properties of synapses, and their pharmacological reactivity, coupled with the fact that the soma of neurons of the central nervous system are electrically excitable by direct or antidromic stimulation and develop all-or-nothing spikes (10) would appear to indicate that the cell bodies of neurons have a dual excitable system.⁶ If this be the case, the excitable membrane of nerve cells with its widely disseminated synaptic foci would resemble that of the eel electroplaque. Debate regarding electrical local circuit transmission (15, 24) *versus* chemical mediation (16) would become superfluous if the mechanism is in reality dual. Data obtained from experiments with direct electrical excitation which offer strong support for electrical transmission would become irrelevant for the behavior of a component which is only neurally excitable.

SUMMARY

1. Current flow outward through the caudal, reactive membrane of the cell causes direct stimulation of the electroplaque. The electrical response in denervated as well as in normal preparations recorded with internal microelectrodes is first local and graded with the intensity of the stimulus. When membrane depolarization reaches about 40 mv. a propagated, all-or-nothing spike develops.
2. Measured with internal microelectrodes the resting potential is 73 mv. and the spike 126 mv. The latter lasts about 2 msec. and is propagated at approximately 1 M.P.S.
3. The latency of the response decreases nearly to zero with strong direct stimulation and the entire cell may be activated nearly synchronously.
4. Current flow inward through the caudal membrane of the cell does not excite the latter directly, but activation of the innervated cell takes place through stimulation of the nerve terminals. This causes a response which has a latency of not less than 1.0 msec. and up to 2.4 msec.
5. The activity evoked by indirect stimulation or by a neural volley includes

⁶ *Note Added in Proof.*—Two types of neurally excited membrane component may be present in motoneurons. One reacts to produce depolarization and excitation as in the endplate or electroplaque. Another appears to react by enhancing chloride permeability (Coombs, J. S., Eccles, J. C., and Fatt, P., *Australian J. Sc.*, 1953, 16, 1). In the normal cell this would lead to chloride entry and inhibitory hyperpolarization. When the electrochemical gradient is changed, electrically by hyperpolarization or chemically by injection of chloride, the inhibitory postsynaptic potential diminishes or reverses to become excitatory depolarization. Some implications of these findings are discussed by one of us in the report of the 5th Conference on the Nerve Impulse (Josiah Macy, Jr. Foundation, in press).

a prefatory potential which has properties different from the local response. This is a postsynaptic potential since it also develops in the excitable membrane which produces the local response and spike.

6. On stimulation of a nerve trunk the postsynaptic potential is produced everywhere in the caudal membrane, but is largest at the outer (skin) end of the cell. The spike is initiated in this region and is propagated at a slightly higher rate than is the directly elicited response. Strong neural stimulation can excite the entire cell to simultaneous discharge.

7. The postsynaptic potential caused by neural or indirect stimulation may be elicited while the cell is absolutely refractory to direct excitation.

8. The postsynaptic potential is not depressed by anodal, or enhanced by cathodal polarization.

9. It is therefore concluded that the postsynaptic potential represents a membrane response which is not electrically excitable. Neural activation of this therefore probably involves a chemical transmitter.

10. The nature of the transmitter is discussed and it is concluded that this is not closely related to acetylcholine.

11. Paired homosynaptic excitation discloses facilitation which is not present when the conditioning stimulus is direct or through a different nerve trunk. These results may be interpreted in the light of the existence of a neurally caused chemical transmitter or alternatively as due to presynaptic potentiation.

12. The electrically excitable system of the electroplaque has two components. In the normal cell a graded reaction of the membrane develops with increasing strength of stimulation until a critical level of depolarization, which is about 40 mv.

13. At this stage a regenerative explosive reaction of the membrane takes place which produces the all-or-nothing spike and propagation.

14. During early relative refractoriness or after poisoning with some drugs (eserine, etc.) the regenerative process is lost. The membrane response then may continue as a graded process, increasing proportionally to the stimulus strength. Although this pathway is capable of producing the full membrane potential the response is not propagated.

15. Propagation returns when the cell recovers its regenerative reaction and the all-or-nothing response is elicited.

16. Excitable tissues may be classified into three categories. The axon is everywhere electrically excitable. The skeletal muscle fiber is electrically excitable everywhere except at a restricted region (the end plate) which is only neurally or chemically excitable. The electroplaque of the eel, and probably also cells of the nervous system have neurally and electrically excitable membrane components intermingled. The electroplaques of *Raia* and probably also of *Torpedo* as well as frog muscle fibers of the "slow" system have membranes which are primarily neurally and chemically excitable. Existence of a

category of invertebrate muscle fibers with graded electrical excitability is also considered.

17. In the eel electroplaque and also probably in the cells of neurons, tests of the mode of neural activation carried out by direct or antidromic stimulation cannot reveal the neurally and chemically activated component. The data of such tests though they appear to prove electrical transmission are therefore inadequate for the detection and study of the chemically initiated process.

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