REVIEWS OF MODERN PHYSICS VOLUME 31, NUMBER 2 APRIL, 1959

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Initiation of Nerve Impulses in Receptor and Central Neurons

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THE objective of this paper is twofold: to point out some of the problems which represent the present state of physiological analysis of some sense organs as detectors and transducers, and to point out a current view of the complex chain of events between transducing a stimulus and initiating a nerve impulse. It will be argued that this chain is similar in receptor and central neurons and that information about either one is relevant to the other.

Biophysicists long have been interested in sensory receptors, since they present in such a conspicuous form the conversion of physical events of the inanimate world into physiological events in the organism.[1] Some of the best-known achievements of biophysical science lie in this realm. Indeed, a vast literature exists on sensory capacities, analysis of the parameters of reception, and quantitative description of the subvarieties of unit receptors within modalities[2]. There continue to appear in the literature new kinds of receptors physiologically identified, and there remains a host of histologically known sense organs and diffuse receptors, differentiated visibly but not yet defined physiologically (e.g., pectines of scorpions, esthetes of chitons, osphradia of snails and clams, etc.).

This situation, virtually unique among organ systems, is not surprising if one thinks of behavior as the principal attribute in which the great wealth of animal types is differentiated, if one ascribes behavior to differentiation in the central nervous system and remembers the intimate and perhaps causal correlation between differentiation in the central nervous system and that in the sense organs. One has learned to expect startling discoveries in each new batch of journals—polarized-light detection, wind-speed indicators, gyroscopic-deflection sensing, infrared directional devices, olfactory separation of optical isomers, hydrostatic-pressure detection in supposedly gas-free organisms, ultrasound reception—not to speak of systems achieving a complex degree of analysis of signals such as the ultrasonic-pulse-reflection analysis in bats, the amplitude-modulation frequency analysis of the ears of crickets, the integration of proprioceptive information in the strike of a praying mantis, and of we do not know what information in many cases such as orientation of fish to turbulence and to other unseen fish. This list furthermore omits mention of eyes of various kinds and their fantastic achievements, of which Hartline writes (p. 515).

Since a systematic survey is patently impossible, I offer here only a small selection of some recent instances of progress in the *identification and characterization of receptors* with respect to their capacities and properties. A closer look is taken later at the *parameters of impulse initiation.*

ELECTRORECEPTORS

Recent work[3–6] has opened up a new sensory modality in the sensitivity to normally occurring electric fields, found in many species of fish of at least three unrelated families, the Gymnotidae, the Gymnarchidae, and the Mormyridae (Fig. 1). These fish have electric organs which emit pulses of low voltage—of the order of 1 v—in some species in brief, low-frequency bursts, and in other species continuously, hour after hour, with characteristic frequencies and pulse form and duration, diagnostic of the species (Fig. 2). The frequencies commonly lie between 60 and 400/sec, but in some cases exceed 1000/sec. The pulse duration—from the whole animal, representing activity of many electroplates—ranges from 10 msec to less than 0.2 msec. What is significant for us is that the behavioral evidence clearly shows in some species the use of these signals in orientation with respect to objects, apertures, and other emitting fish in the immediate environment. Clearly, the fish detects alterations in the pattern of the electric field in the water surrounding it (Fig. 3). Marked signs of agitation are elicited by a wire brought within range or by the discharges of other fish. Conditioned-reflex experiments show the ability to detect the presence of a stationary magnet outside the aquarium and to discriminate between conductors and nonconductors in the aquarium. The fish respond to the movement of a small electrostatic charge such as that produced by combing one's hair with a vulcanite comb. Sensitivity to imposed electric currents has been known, but what is of interest for us is the demonstration that a normally developed sensibility exists which is apparently used in nature to detect and to analyze in a complex way naturally occurring electric fields.

The sense organ is presumably the lateral line, heretofore regarded as a special form of mechanoreceptor. We will have to make room in our lists of modalities for a new category—electroreceptors. It is perhaps more curious that this modality is not more widespread. Muscle-action potentials can be recorded in the water at some distance from fish as well as from other animals, but so far the tests outlined in the foregoing, when applied to fish of other families than these three, have

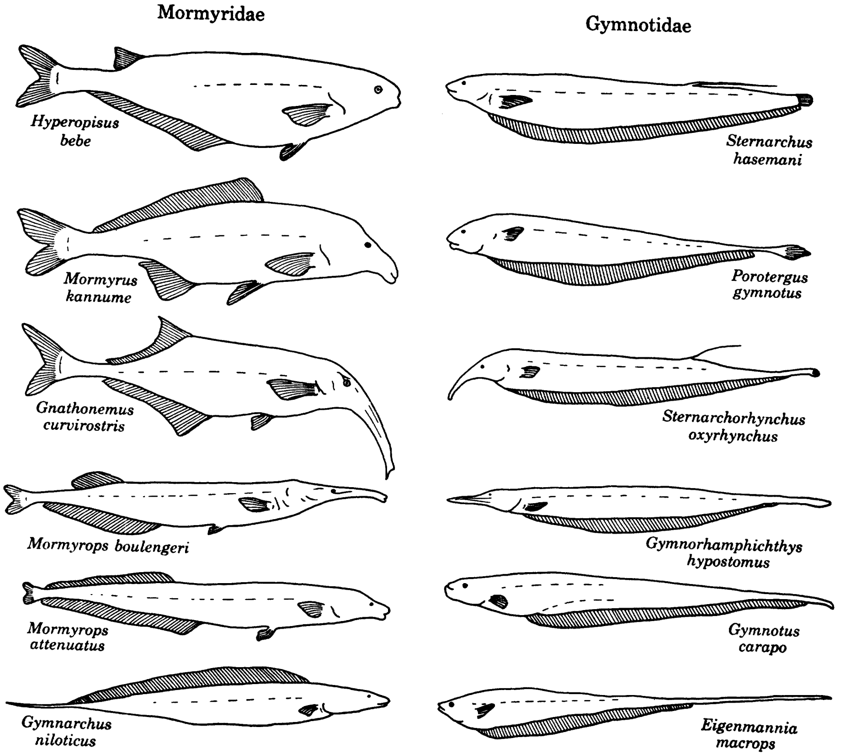
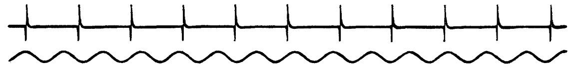


FIG. 1. Examples of continuously discharging, low-voltage electric fish. Representatives of the Mormyridae, Gymnotidae, and Gymnarchidae [from H. W. Lissman, J. Exptl. Biol. **35**, 156 (1958)].

indicated no such sensitivity (with the possible exception of the *Siluridae*). One may conjecture that it is perhaps not so much the deficiency in peripheral sensibility as the lack of central apparatus for making use of the signals, which distinguishes the ordinary from the electric fish. All electric fish share, among other things, the enormous development of the valvulae cerebelli, the so-called mormyro-cerebellum.

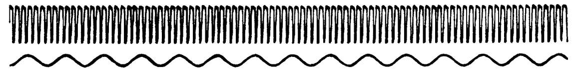
The sensitivity of the receptor has not been measured directly but Lissman and Machin[3] have calculated the intensities of the electric fields which are effective in altering behavior, for example in conditioned response tests to electrostatic charges or magnets outside the aquarium. The field in the water around the fish must be changed by about 0.003 μv/mm at threshold, in *Gymnarchus niloticus*. This corresponds to a current through the fish of 2×10^{-5} μA/cm^2, some hundred thousand times smaller than a still subthreshold stimulus current density across the membrane of a squid axon (2 mv across 10^3 ohms/cm^2). The problem of the possible meaning of such high sensitivity to electrical events already has been raised by Terzuolo and Bullock.[7] Our evidence suggests that even in ordinary nerve cells the sensitivity, while not nearly as high as in these electroreceptors, is several orders of magnitude higher than the usually recognized threshold changes of 10 to 20 mv required to excite a silent nerve fiber. In the first place, this high sensitivity is manifested only as an alteration of frequency of an already discharging cell. In the next place, to be sensitive to minute changes in membrane potential, both the critical potential for spike initiation and the rate of rise of the prepotential must be extraordinarily stable—and must be localized in a limited part of the cell. The basic problem of high electrical field sensitivity—of special interest because it may be the one case where one does not need to search for a transducing mechanism as we do in photoreceptors, mechanoreceptors, etc.,—is the matter of *stability*. What are the requirements in terms of stability of the threshold and of the prepotential such that a given channel can provide a useful signal within a reasonable time

(1) *Gymnotus carapo*



(2) *Staetogenes elegans*

(3) *Hypopomus*



(4) *Eigenmannia virescens*

FIG. 2. Examples of types of electric pulses produced by four species of the Gymnotidae. Both pulse duration and pulse frequency are different and characteristic for each species. The fish are continuously discharging day and night. Time marker 50 cps [from H. W. Lissman, J. Exptl. Biol. **35**, 156 (1958)].

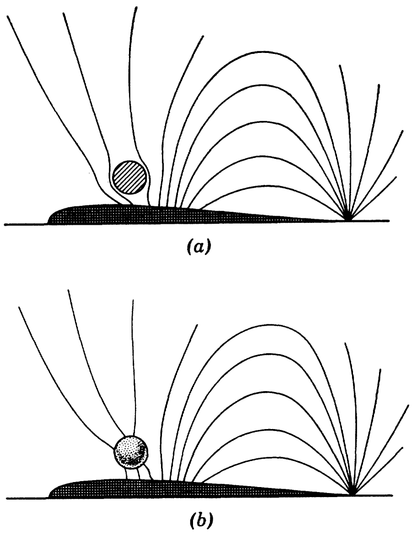


FIG. 3. The influence of objects upon the electric field around a fish. (a) An object of low conductivity, and (b) one of high conductivity. The fish detects the distortion of the field and reacts to both classes of objects [from H. W. Lissman and K. E. Machin, J. Exptl. Biol. 35, 451 (1958)].

(e.g., 1 sec)? At another level is the important problem of whether or not the high electric field sensitivity in the modulation of ongoing discharge plays a role in normal situations in the nervous system. If so, it permits effects not only of changes in electric fields normally present but also of changes in the chemical milieu which may act through the same mechanism, by changing the frequency of the spontaneous discharge for a given membrane potential at the critical locus.

MECHANORECEPTORS

Turning to the realm of mechanoreceptors, an attractive problem exists in the wide variety of arthropod hair-like exoskeletal projections (Fig. 4), each of which receives a single distal process of a primary sensory neuron and is specialized for signaling deflection of the hair in a certain direction.[8–10] In the lobster statocyst (an equilibrium sense organ where this is especially clear and for which recent analyses have been made), there are several specific types of hairs, differentiated for sensitivity to movement in one or another direction, or for sensitivity to a maintained position in one or another plane, or for sensitivity to vibration. Since the stimulus seems likely to be limited to a very restricted region—namely, the point of articulation of the hair near its base—one is faced with the general problem of transducing a mechanical event into a physiological one in perhaps a more simple and discrete form than in the more familiar mechanoreceptors such as stretch, touch, and auditory organs. Although the sensitivity must be high in terms of displacement, it cannot be estimated accurately, owing to the fact that, at the base of the long hair, where the relative movement that must act as a stimulus occurs, the mechanical disadvantage is maximal and the small threshold movements of the tip of the hair must be reduced enormously in absolute magnitude. Fundamental questions remain, regarding the ***ultrastructure and the local events*** in the region of the mechanical deformation of the sensory nerve ending.

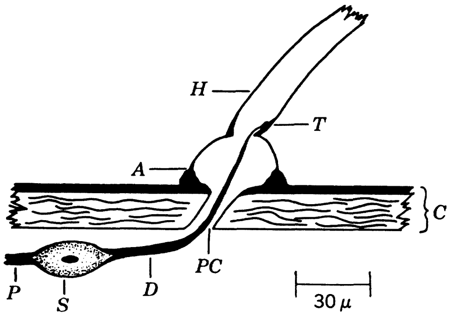


FIG. 4. Diagram of arthropod sensory exoskeletal hair of the type innervated by a single primary sensory neuron. The distal process of the sense cell ends near the region of articulation of the hair [from M. J. Cohen and S. Dijkgraaf in *The Physiology of Crustacea*, T. H. Waterman, editor (Academic Press, Inc., New York) (to be published)].

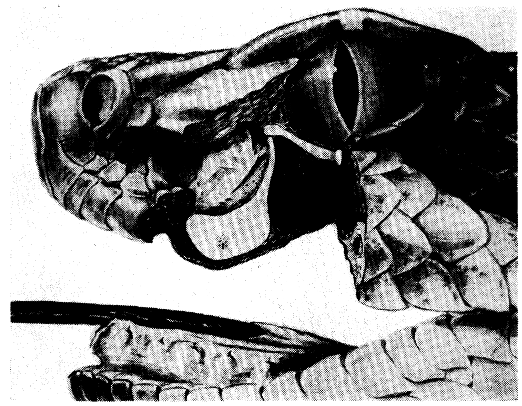


FIG. 5. The infrared sensitive facial-pit organ of rattlesnakes, *Crotalus*. Here, a wedge has been removed to show the deep pit and the sensitive surface—the thin membrane at its back (shown crinkled but normally smooth), with an air chamber both in front and behind it. This membrane, 10 to 15 μ thick, is heavily innervated and vascular [from T. H. Bullock and F. P. J. Diecke, J. Physiol. 134, 47 (1956)].

As an example of the specialization of which arthropod mechanoreceptors are capable, Burkhardt and Schneider[11] have found that units in the Johnston organ of the antenna of flies (*Calliphora*) are hardly less sensitive than the human ear to sound frequencies between 150 and 250 cps, the range of the wing-beat frequency. Even beyond this range the units follow the sound frequency faithfully, giving the animal sensory information corresponding to each wing beat with a delay of only about 1 msec. The speed of flight is controlled by this sense organ. For some other recent studies in this area, see references 12–17.

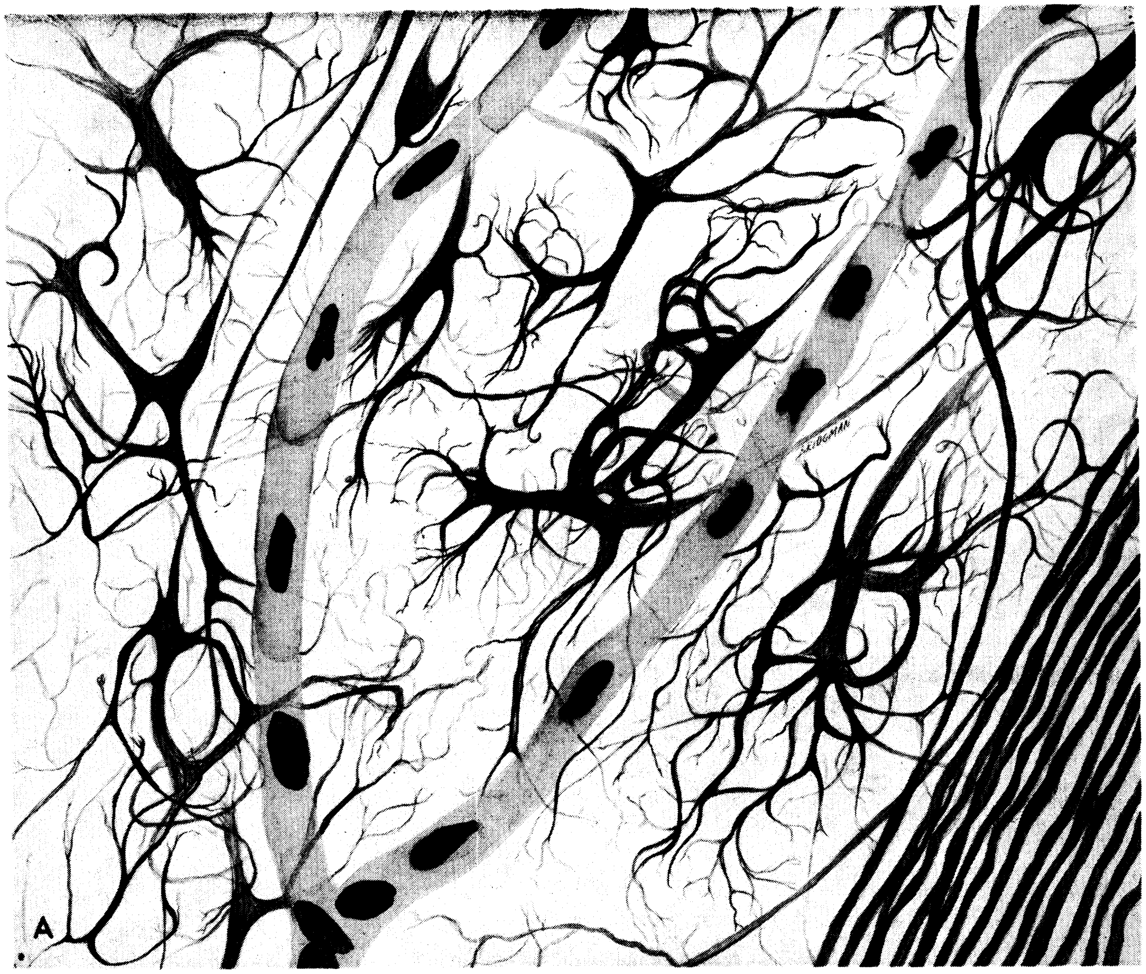


FIG. 6. The sensory endings in the pit-membrane of a rattlesnake. This is a faithful drawing from a silver-stained whole mount of the 10 to 15 μ membrane. The nerve fibers end freely in palmate expansions with branching processes. 500 to 1500 such expanded endings occur per mm². Apparently all are of one functional type—so-called warm receptors. Width of picture, 150 μ [from T. H. Bullock and S. W. Fox, Quart. J. Microscop. Sci. **98**, 219 (1957)].

TEMPERATURE RECEPTORS

Referring briefly to temperature receptors, there is some information regarding the extraordinarily developed long-infrared-radiation detectors found in rattlesnakes and other pit vipers in the thin membrane at the base of the facial pit (Figs. 5 and 6)[18,19]. This structure, richly provided with a special form of free nerve ending and specialized in other ways—such as, for the directional estimation of radiant sources—responds to very small doses, of the order of 10^{-11} small calories in $\frac{1}{10}$ sec on the area of the terminal ramification of one nerve fiber ($2000\ \mu^2$). The evidence indicates that this response is due to the change in temperature of the tissue, which both by indirect and direct methods is estimated to be of the order of 0.001°C, close to the value already found for the human.[20] Expressed as Q_{10}, the frequency of nerve impulses in a single fiber increases with temperature with a Q_{10} of about 10^{30}, a figure which offers considerable room for speculation about *high amplification with preservation of reasonable stability* (Fig. 7). Much more could be said about each of these cases with respect to physiological properties and characteristics of response. It is the purpose here only to call attention to the variety of opportunities and problems presented by a few recently studied receptors.

CHEMORECEPTORS

In the area of chemoreceptors, I choose only two recent reports. Hodgson and Roeder[21] have discovered labellar hairs in various flies in which two primary sensory neurons send distal processes to the chemoreceptive area at the tip of the hair. These two neurons have different modes of sensitivity: one responds only to sugars; the other to salts, acids, and alcohols. Not only is this preparation of interest because of the opportunity for the physiological *study of unit receptors of different chemical specificity*, but also, because of its additional sensitivity to mechanical stimuli and to tem-

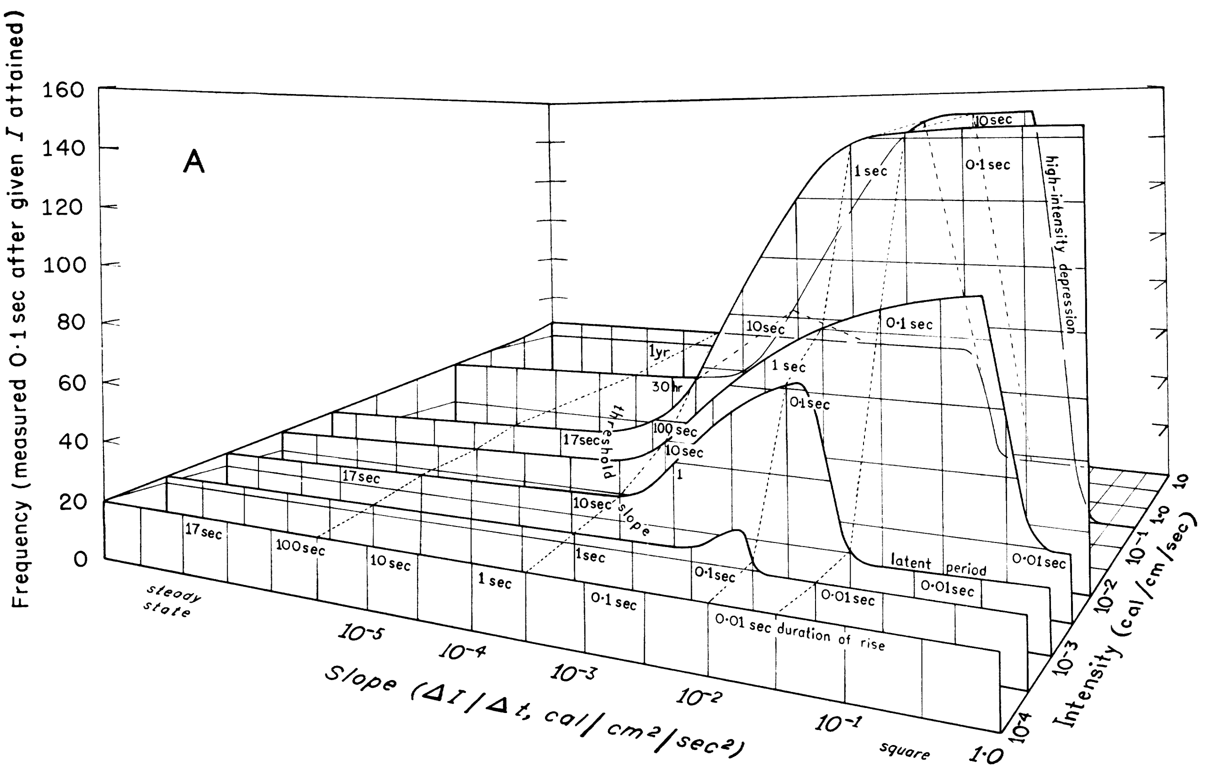


FIG. 7. Response of typical receptor unit of rattlesnake infrared sense organ, measured as nerve impulse frequency at different rates of increase of radiation and at different flux levels [from T. H. Bullock and F. P. J. Diecke, J. Physiol. **134**, 47 (1956)].

perature changes as small as $\frac{1}{10}$°C, it presents in a clear form the general ***problem of analyzing the world through receptors which are not unambiguously*** detecting one aspect of the environment only. Furthermore, these labellar hairs are remarkable, as shown by Dethier,[22] in that complete behavioral rejection or acceptance responses of the intact animal occur to stimulation by a microdrop which can reach only one single sensory neuron. Schneider[23] has discovered an electrophysiological response in the antennae of silk moths (*Bombyx mori*) in the males only, which is highly specific apparently to the naturally occurring odorous material produced by the female. This odorous material attracts the males from great distances. In microelectrode records from the isolated antenna, picked up extracellularly from many units, Schneider finds a slow wave of several millivolts which is negative for some substances and positive for others. Spikes are superimposed preferentially on the negative phase of the electroantennogram. As in the preceding case, there is here an apparently peripheral filtering of the normally specific stimulus, which filtering is achieved by a ***specific chemical sensitivity*** of a receptor and again one is faced with high sensitivity. de Vries[24] has calculated that at threshold there are far fewer stimulating molecules impinging upon the sensory epithelium in man than there are olfactory receptor cells in the same area.

ABSOLUTE RECEPTION

Extending the remarks by Rosenblith (p. 485), it is highly desirable that further attention be given the problems raised by the sensory reception of absolute stimulus values as opposed to reception where comparison can be made by the receptors with a status in the recent past. Besides body temperature, blood pressure, and the like in man, there are many other indications of reception of values which may be called absolute, e.g., preferred temperatures in cold blooded animals, levels of light which day after day induce a given response, CO_2 concentration reactions, and pitch recognition. Even in many humans lacking phenomenal ability, the cross-modality subjective-intensity matching and many psychophysical phenomena illustrate scales not dependent upon relative stimulation as measured against a just-preceding level. One faces here the problem of stability and drift correction—not only the control loops, intra- or inter-cellular, but the standard of reference for detecting error.

SEQUENCE OF LABILE COUPLINGS LEADING TO IMPULSE INITIATION

Consider now what makes a neuron fire—namely, the specification of the distinguishable processes within the neuron leading to an explosive firing of a propagated all-or-none nerve impulse. The interesting conclusion

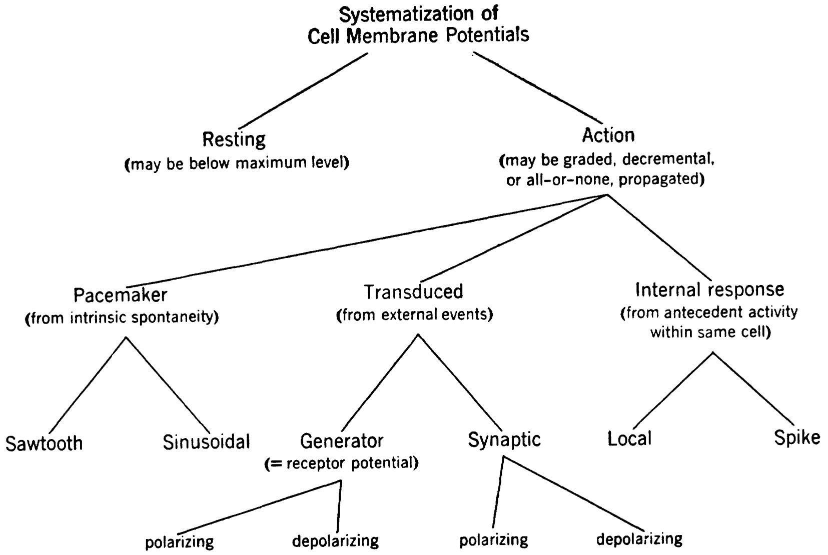


FIG. 8. The types of nerve cell membrane potentials.

permitted by current evidence is that *the determination of firing is not merely the build-up of an adequate stimulus* or its transduced and amplified resultant to a critical level, the threshold, but rather it is a sequence of labile couplings between graded (analog) events, each occurring in a limited fraction of the neuron. Normal firing is preceded by a series of steps, with alternative pathways. The several processes are separate, partly in space and partly in time, but causally are interconnected. Some of these processes are reflected in membrane-potential changes and some are not.

The *processes reflected in the membrane potential* include slow shifts of the average membrane potential, synaptic, generator, local, and pacemaker potentials. Figure 8 makes it clear that generator and synaptic potentials are parallel types of transduced potentials representing amplified response to external events whether from a presynaptic cell, from a non-nervous sense cell, or from the external environment. It also points out that both of these classes of response may occur either in polarizing or depolarizing directions. Depolarizing responses are commonly called excitatory because they are likely to increase the probability of firing. Polarizing, or as they are sometimes called, hyperpolarizing, responses generally decrease the probability of firing and are called inhibitory.

First among the processes which determine the initiation of impulses in receptor and central neurons, we should list what is actually no doubt a whole class of different and complex processes by which the level of the so-called *resting potential* is under the influence of the milieu and nonspecific agents (nonspecific to a given cell, though possibly specific to a cell type). Hormones, inorganic and organic constituents of the medium, deformation of the cell (even in neurons other than specific mechanoreceptors), and other factors are known to have some effects, although in most cases their importance in normal physiology cannot be assessed yet. In addition to these, we now can recognize as an important class the general shift in membrane-potential level accomplished through specific nervous pathways. For example, Otani and Bullock[25] found in certain cells of the 9-celled cardiac ganglion of lobsters that certain presynaptic fibers exert an influence, but without any discrete synaptic potentials. With repetitive stimulation they cause a slow, smooth shift of membrane potential (Fig. 9) as seen by an intracellular electrode in the soma. Terzuolo[26] has shown that certain neurons in the spinal cord of the cat similarly respond to stimulation of certain parts of the cerebellum by a shift of membrane potential level. This absence of discrete synaptic potentials even with a small number of incoming pathways possibly means that the main factor in determining the slow, smooth shift of potential is the distance of the synapses from the soma. If the distance is considerable, individual deflections would be smoothed out by the spread through leaky cables. But, if one believes that the membrane potential level at the basis of the axon or some such limited locus is crucial to the determination of cell firing, any such properties of the dendrites and soma—such as the electrotonic conduction of slow potential changes, the smoothing out of potential changes, or the discrimination against rapid deflections—would be of decisive importance in spreading the influence of the decrementally propagating activity of the much-branched dendritic processes to the region in which the spike originates. This may be one reason for having long dendrites. Among sensory neurons, there are many whose distal process divides to supply a considerable number of receptor regions of the periphery (Fig. 10, from the work of Meyer[27]) and in which the histology is suggestive that these loci are not alternative sources of full-fledged impulses but may, in some cases, make graded contributions to the probability of an impulse arising at some

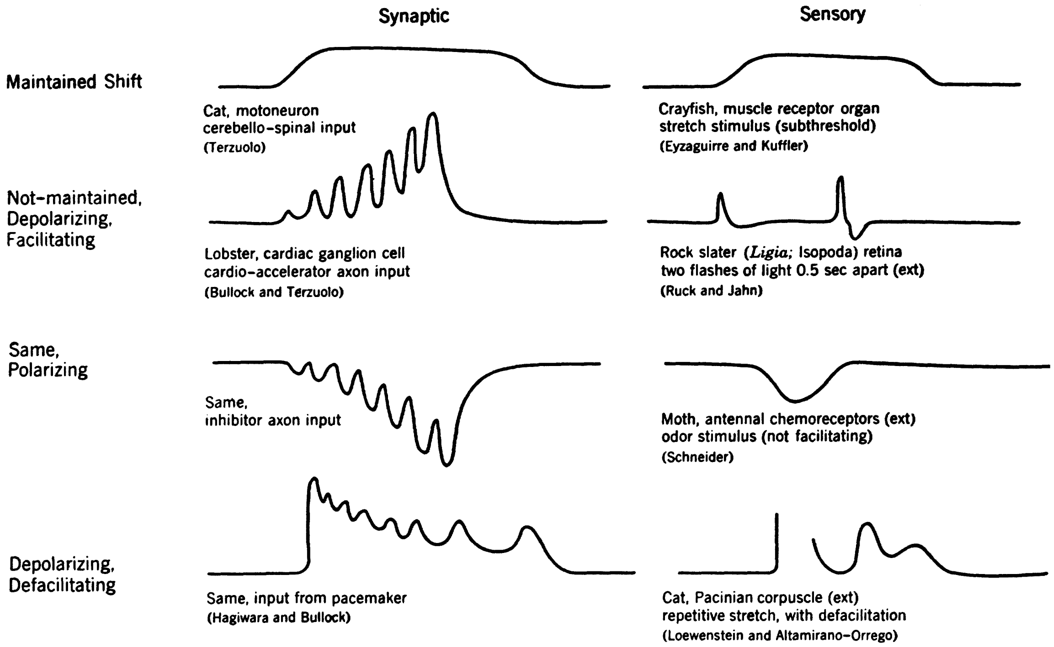


FIG. 9. Some of the types of subthreshold potentials, to show the correspondence between synaptic and sensory types. Hand-drawn approximations of original records, intracellular (except when marked ext = external); time scale is not uniform; upward deflection is depolarization or, in external recording, negativity of closer electrode.

point, such as a confluence of main branches of the distal process. So much for the processes that act upon the spike probability through the so-called resting potential.

More typically, or at least more familiarly, *the response to an external event is the transient deflection*, called a synaptic potential or a generator potential according to whether the external event is presynaptic or whether it is a sensory stimulus (Fig. 9). These responses arise in restricted portions of the neurons and have their significance in the effects they produce in other regions after nonregenerative spread—with decrement and phase delay. They may be polarizing or depolarizing, and this alternative may be decided by the nature of the external event or, in certain circumstances by the level of the membrane potential obtaining at the moment. Some normally polarizing, hence inhibiting, events are reversed readily to a depolarizing response which can be shown to increase the probability of firing and are thus at least in a measure excitatory. Such change in the response to a given input owing to different levels of membrane potential is not purely a laboratory phenomenon resulting from experimentally imposed polarization, but occurs normally, for example, in the aforementioned situation where Terzuolo found the membrane level of spinal motoneurons shifted by cerebello-spinal pathways, thereby altering the response to dorsal-root inputs. (In some accounts, emphasis has been laid on the fact that an inhibitory input drives the membrane potential from either side toward a certain equilibrium value and does not cause any synaptic potential if the membrane is already at that level. Even under the latter condition inhibition can result, because the lowered impedance tends to clamp the membrane. But this can inhibit only in the near vicinity. Upon spike-initiating regions or synaptic regions at some distance, the sign and magnitude of the potential change will determine the sign and magnitude of the effect.)

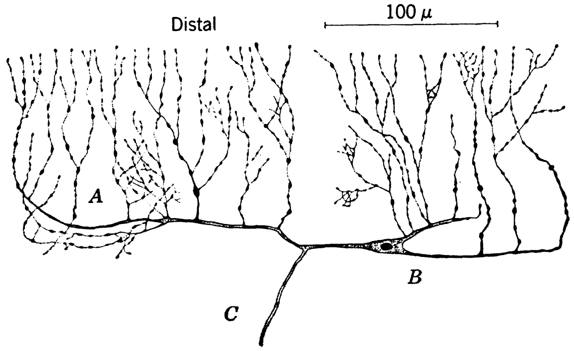


FIG. 10. Sensory nerve cell from the leg of a centipede, *Lithobius*; methylene blue. *A*—lattice of fine terminal fibers, *B*—cell body, *C*—axon [from G. F. Meyer, Zool. Jahrb. Anat. 74, 381 (1955)].

In at least some cases, there intervenes between the synaptic or generator potential and the critical level at the spike-initiating locus (which is a very restricted part of the neuron at some distance from the principal synaptic and generator loci) ***an intermediate graded potential*** which is usually called the *local potential* (Fig. 11, from the work of Bullock and Terzuolo[28]). This is presumed to arise in an electrically excitable membrane adjacent

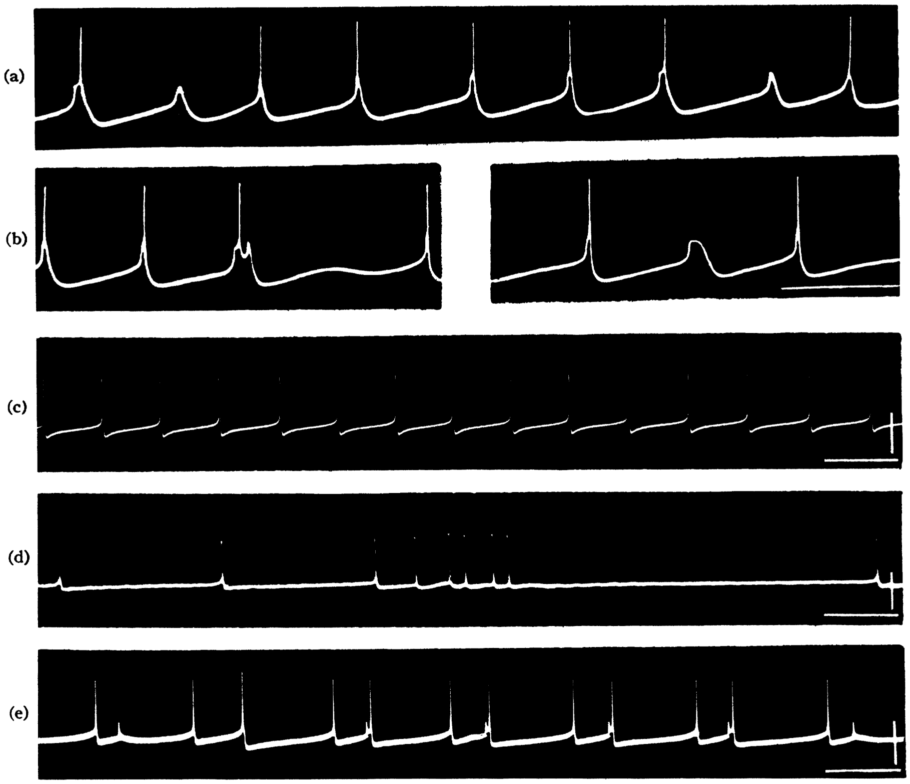


FIG. 11. Examples of true spontaneous activity as seen by an electrode inside a nerve cell. Cardiac ganglion cell of the lobster, *Panulirus* [(a) and (b)] or the crab, *Cancer* [(c) (d) (e)]. (a) and (b) show a large pacemaker potential, presumably arising nearby, and another prepotential, regarded as a local potential, before the spike. The local potential may fail to elicit a spike and then can alone cause repolarization. Note the failure of the local potential to arise following the third spike in (b) with instead an undulation leading to a new cycle. (c), (d), and (e) show different forms and permutations of pacemaker potential and repolarization. Scales: (a) and (b)—500 msec; (c), (d), and (e)—50 mv, 200 msec [from T. H. Bullock and C. A. Terzuolo, J. Physiol. 138, 341 (1957)].

to the synaptic or generator membrane, but distinct from it, and as a consequence of the local circuits from antecedent activity of the same cell. In this respect, the local potential is like the spike potential, which also arises by electrical excitation from the local circuits in neighboring regions of the same neuron. Indeed, local potentials can be made to have graded amplitudes all the way to the full amplitude of the spike potentials, and they differ only in being nonregenerative and, therefore, in spreading decrementally for distances of the order of a millimeter only. In some sensory neurons, we may conjecture that such local potentials occur in different branches of the distal process and sum in the stem process to determine the initiation of a spike. The experiments of Katz[29] on the summating subthreshold deflections in sensory terminals of muscle spindles may be so interpreted. Situations where distal sensory processes branch over a considerable area are quite common.

The nonregenerative property of some neuronal membranes is important in those cases where it results in the *inability of spike potentials, once initiated, to invade* those regions of the neuron. This preserves these regions from explosive depolarization and from subsequent strong repolarization, and thus enhances their integrative capacity. For example, the soma of the large cardiac ganglion cells in the lobster and perhaps the dendrites on some other types of neurons never experience an explosive all-or-none event. This characteristic is very likely true of most neuronal membranes, that is to say, of the vast forests of fine branching processes making up neuropiles. If this is so, one must think of all-or-none spike potentials as a peculiar development of a limited portion of the neuron whose function is the faithful propagation of signals over long distances rather than integration.

The familiar local and spike potentials are depolarizing in direction, but there are a few instances indicating that membranes are capable of equivalent *regenerative potentials in the opposite direction.* For example, during the plateau of an essentially completely-depolarized potential in the Purkinje tissue of the mammalian heart, during a heart beat, a threshold stimulus in the polarizing direction sets up a regenerative repolarization which grows explosively and restores a resting potential [Weidmann[30]]. Hisada[31] has recorded action potentials of the same polarity—that is, increasing polarization across the cell membrane of the protozoan *Noctiluca.*

Finally, we have *spontaneous activity* manifested in *pacemaker potentials* in some neurons including many sensory neurons. A background of continuous spike discharge in the absence of known stimulation or under steady-state stimulation may be called spontaneity. Of course, such activity depends upon permissive conditions of the metabolism and of the milieu of the cell. If these conditions are normal ones, however, steady-state firing is spontaneous in the sense that the origin of the intermittent activity lies within the neuron, rather than in the environmental mechanisms. Such activity has been explored in several cases (Fig. 11), and the significant finding from the present point of view is the *localization of the pacemaker process to a limited portion of the neuron* different from that in which spikes arise, and perhaps also different from that in which synaptic or generator activity occurs primarily. Of course, these regions must be within shouting distance of each other, for their normal significance lies in the summing of their effects to produce a threshold change in the membrane potential at the spike-initiating region. In lobster cardiac ganglion cells, evidence of more than one pacemaker locus is seen in the same neuron. This is possible because these loci apparently occur in different major processes of the cell, each of which has its own spike-initiating region; two different rhythms of spikes arise and can be seen in the same soma without interfering one with another since they cannot invade the soma. What this says is that the absolute dimensions of neurons are not accidental but are fixed by engineering requirements specified by the electrotonic parameters of the membrane.

There are very likely at least *two distinct types of spontaneous potential* change. The rarer is approximately sinusoidal. Spikes tend to occur during the phase of maximum depolarization. Several spikes may occur during this phase, but they are not necessary for and do not accelerate the repolarizing phase. In contrast, the more common type is similar to a relaxation oscillation, in that the pacemaker potential itself is a steadily depolarizing potential which must be interrupted by some new process with a threshold whose net result is to repolarize, thereby starting a new cycle. This threshold process is typically the spike potential, but it may be preceded by and indeed may be substituted by a local potential which is capable of repolarizing the membrane during its recovery phase, thus setting the stage for another pacemaker potential to begin.

There are other *permutations and complexities in the processes of spontaneous impulse initiation* as is suggested by the high rhythmicity of some receptors and the low rhythmicity of others. In fact, it may be characteristic that, in one and the same unit, rhythmicity is relatively high at high frequencies of discharge and low at low frequencies. Tokizane and Eldred[32] have found two distinct populations of stretch receptor fibers in the dorsal roots of cats. One population is consistently more rhythmic at any given average frequency than the other; that is, the standard deviation of intervals is smaller for a given average interval. They believe that the more-regular units come from flower-spray endings in the muscle spindles and that the less-regular units belong to annulo-spiral endings.

The various possibilities for understanding the *origin of nonrhythmicity* or randomness-within-limits of successive intervals cannot be discussed here. Nor can one discuss the interesting question of the central problem created by nonrhythmicity, the *distinction of signals from noise*—that is, of weak true stimuli from random changes in the frequency of firing. These problems have been discussed elsewhere (see references 33–35).

These separate processes reflected in membrane potential shifts, in synaptic, generator, local, pacemaker, and spike potentials are *sequentially coupled* in complex ways: partly because there are several alternative sequences; partly because much depends on the particular microanatomy of a given neuron, the spatial relations of the separate loci, and the possibilities of spread of the respective potentials; and partly because of the labile character of the coupling constants. The constants or transfer functions well may be nonlinear.

However, these do not exhaust the processes of which there is evidence that determine the initiation of impulses within the cell. Turning to *processes not manifested by membrane potential* changes—at least by changes detectable with the same methods used for measuring the just-discussed events—we are faced mainly with indications of excitability changes. These are brought to attention only by responses to subsequent stimuli. One is called *facilitation* (Fig. 9). The cell can be initially at a certain membrane potential and respond to a given arriving impulse (in this case, a single presynaptic fiber) with a small synaptic potential. After repetition, the response to the same presynaptic impulse is much greater even when the membrane potential is allowed to return to the initial value between stimulations. Another type, which may be seen in the same neuron may be called *defacilitating*; the response is less to successive presynaptic impulses the closer together they come. Here also, at the same membrane potential level, the cell or presynaptic terminals are altered in response or excitability according to the recent history. This is not a laboratory artifact but is an essential part of the normal mechanism of burst formation in the 9-celled cardiac ganglion of the lobster. Here, the large follower neurons, driven by a burst of arriving presynaptic impulses from the pacemakers, characteristically give the form of synaptic potential response shown in Fig. 9. A large, initial synaptic potential is followed by a series of small ones during the high-frequency portion of the burst and then by growing amplitude responses as the frequency of the arriving presynaptic impulses declines.

Here we have to deal with the *time course of excitability of graded, subthreshold events*. There must be a separate curve for each of the subthreshold events previously discussed and for each locus and type of synapse. The last two instances, of facilitation and defacilitation, recorded in the same neuron were both excitatory synaptic potentials, from different presynaptic pathways (one from the pacemaker in the cardiac ganglion, and the other from the cardioacceleratory nerve from the central nervous system). The same neuron also has facilitating inhibitory synapses. Each of these synaptic responses must have its own dependence of response upon input and of this relation (response per input) upon time after preceding activity. The local potentials also may have a separate time dependence of excitability and so may the spike-initiating locus where the excitability is measured by a sharp threshold. We note that the classical spike threshold, so easily measured any place in the axon, is normally a significant parameter at a very limited region only in the whole neuron. Beyond this region, the safety factor is normally sufficiently high that the spike threshold is without great significance.

Another degree of freedom not predicted by the membrane potential is the presence or absence of after-effects —either persistent response after the input ceases or of overshooting rebound (post-excitatory inhibition or post-inhibitory excitation). Cases of each kind are known for receptors and for central neurons.

Still another line of evidence for processes determining spike initiation, which cannot be seen in the usual membrane potential measurements, is that of significant *alteration of the frequency of already-active neurons by weak electric fields* in the surrounding medium, already mentioned. In a preliminary analysis, Terzuolo and Bullock[7] estimated the intensity of the voltage gradient in the saline surrounding a neuron (stretch receptor of the crayfish) when an imposed polarization was of a magnitude just sufficient to cause a noticeable change in the maintained frequency of firing. The results, as already mentioned, were that very weak fields (of the order of 1 $\mu v/\mu$ in the external medium around the cell) were sufficient. There will be a potential change in a given direction (for example, an excitatory direction) in certain parts only of the neuron, and since this will be graded in magnitude geographically, there must be a very limited region only wherein the imposed field actually exerts its effect. It can be concluded, therefore, that, even in the absence of average change in membrane potential, very localized regions may be critical in determining the firing frequency and may have an extremely high sensitivity to small voltage gradients. Thus, old ideas are confirmed in a certain sense that, among other things, the neuron is to a significant degree under the influence of differences of potential between one part and another of the surface of the neuron.

The general picture then is quite different from that of the axon. Grundfest[36] has given arguments for believing that adjacent patches of neuronal membrane are, respectively, electrically excitable and electrically inexcitable in a certain meaning of these terms. It may be emphasized that the evidence cited here is also strongly indicative of a *neuronal membrane consisting of a patchwork* of different kinds of subthreshold-response capacities. The responsiveness or the excitability—these two are different, but ordinarily are not easy to distinguish experimentally—vary separately one from another; some are manifest by electrical events, others are not.

One more degree of complexity must be added: the separate processes do not simply sum algebraically to determine the level of probability of impulse initiation, but at least some of them apparently interact: the presence of an increased magnitude of one subthreshold process may alter the magnitude of one of the others. For example, in the presence of a large generator potential, excitatory synaptic potentials may not simply add but will themselves be altered in size. The same may be true for inhibitory and pacemaker potentials. These interactions have been little studied, but there is no doubt that they will be found, in some cases, to contribute importantly to the integration of input to determine output (see, for example, the evidence of multiplicative neural events adduced by Hassenstein and Reichardt[37]).

SUMMARY

A selection of sense organs upon which recent progress has been made is reviewed in order to point out problems and opportunities of biophysical interest. The electroreceptors in electric fish, mechanoreceptor hairs in arthropods, infrared receptors in pit vipers, and specific chemoreceptors in flies and moths are the examples presented.

The general problem of absolute reception as opposed to reception of change is emphasized in terms of stability, drift correction, and reference standard.

The long series of subthreshold events which may intervene between transducing a stimulus and initiating a spike is reviewed. These include events which are reflected in the membrane potential and others which are not. Specific shifts of membrane potential, pacemaker, generator, synaptic, local and spike potentials, and some subvarieties are sequentially coupled in labile and perhaps nonlinear ways. They occur, in general, in restricted regions of the neuron which interact in complex ways not only because of the complex coupling constants between successive steps but also because of the profound influence of the anatomical distribution of the differently responding types of membranes.

Events not reflected in the membrane potential are the excitability cycles and the presence or absence of aftereffects. In addition to the spike threshold, each graded process preceding the spike has a curve of response against input and of this relation against time after preceding activity.

It is probably important that these several processes not only are sequentially related but that they interact —the amplitude of one may alter the responsiveness of another. Spike initiation is, therefore, potentially a highly derived and integrative result.

ACKNOWLEDGMENTS

Permission to reproduce figures from the *Journal of Physiology*, *The Journal of Experimental Biology*, and *The Quarterly Journal of Microscopical Science* is gratefully acknowledged.

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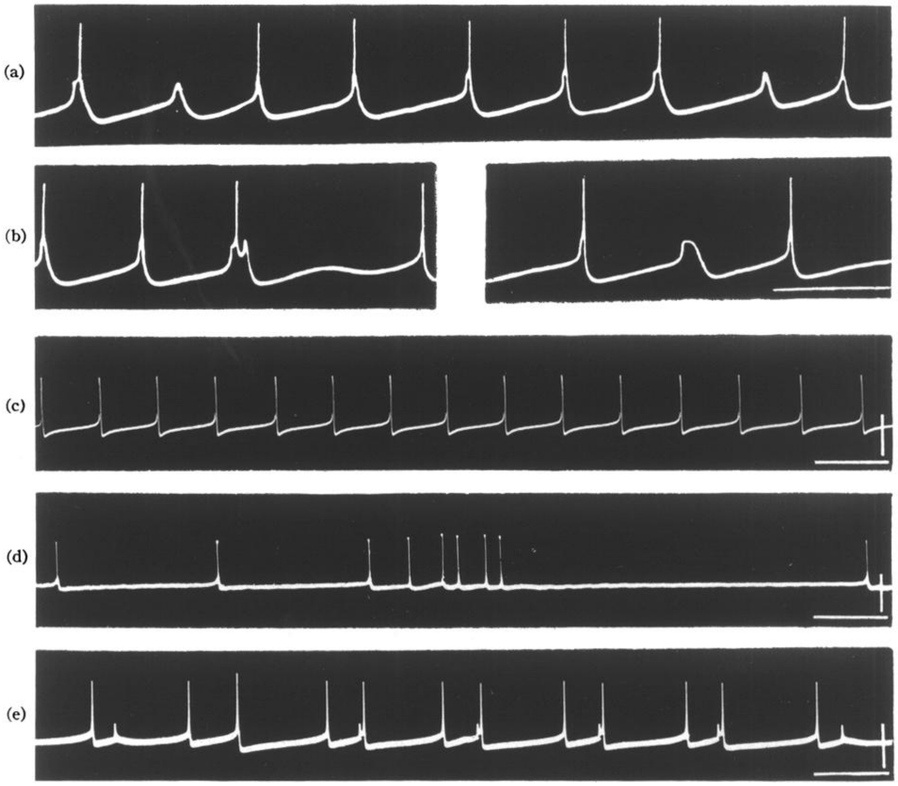
FIG. 11. Examples of true spontaneous activity as seen by an electrode inside a nerve cell. Cardiac ganglion cell of the lobster, *Panulirus* [(a) and (b)] or the crab, *Cancer* [(c) (d) (e)]. (a) and (b) show a large pacemaker potential, presumably arising nearby, and another prepotential, regarded as a local potential, before the spike. The local potential may fail to elicit a spike and then can alone cause repolarization. Note the failure of the local potential to arise following the third spike in (b) with instead an undulation leading to a new cycle. (c), (d), and (e) show different forms and permutations of pacemaker potential and repolarization. Scales: (a) and (b)—500 msec; (c), (d), and (e)—50 mv, 200 msec [from T. H. Bullock and C. A. Terzuolo, J. Physiol. **138**, 341 (1957)].



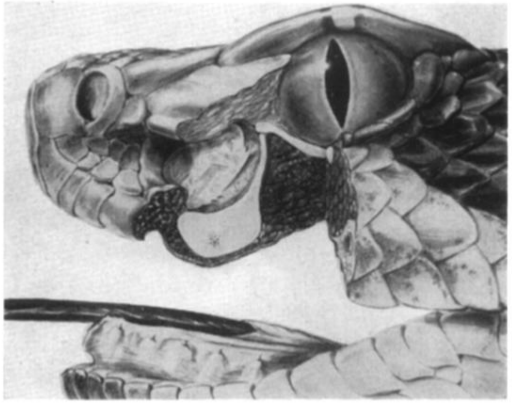


FIG. 5. The infrared sensitive facial-pit organ of rattlesnakes, *Crotalus*. Here, a wedge has been removed to show the deep pit and the sensitive surface—the thin membrane at its back (shown crinkled but normally smooth), with an air chamber both in front and behind it. This membrane, 10 to 15 μ thick, is heavily innervated and vascular [from T. H. Bullock and F. P. J. Diecke, J. Physiol. **134**, 47 (1956)].

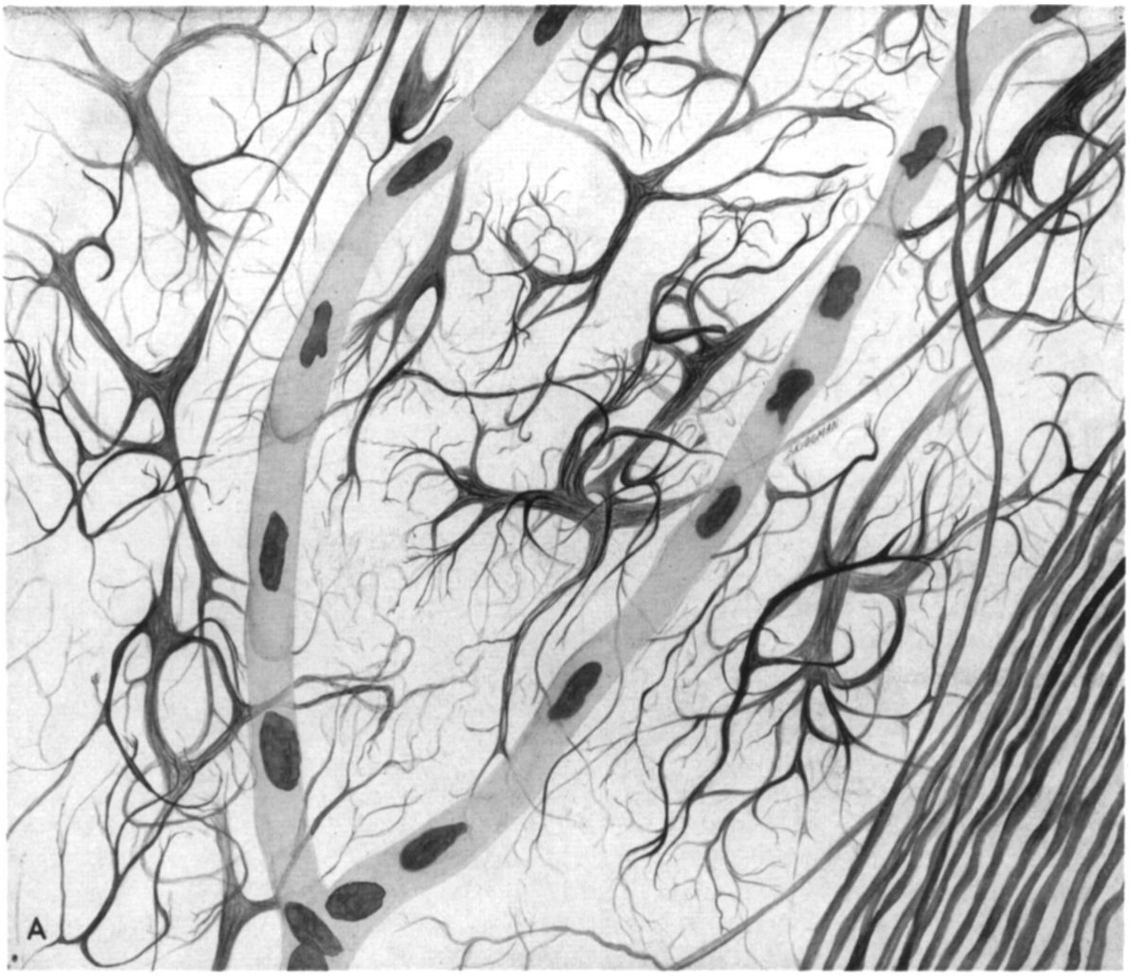


FIG. 6. The sensory endings in the pit-membrane of a rattlesnake. This is a faithful drawing from a silver-stained whole mount of the 10 to 15 μ membrane. The nerve fibers end freely in palmate expansions with branching processes. 500 to 1500 such expanded endings occur per mm^2. Apparently all are of one functional type—so-called warm receptors. Width of picture, 150 μ [from T. H. Bullock and S. W. Fox, Quart. J. Microscop. Sci. **98**, 219 (1957)].