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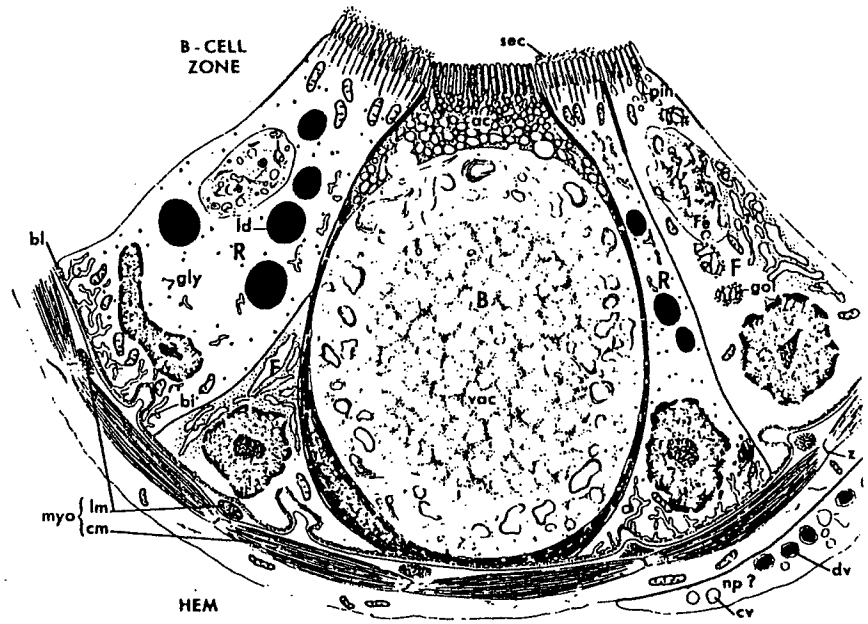

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Membrane Physiology of Crustacean Neurons

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(1982)

I. INTRODUCTION

The arthropods, Nature's most prolific and diverse group of multicellular animals and mankind's most serious competitors for available energy and food on this planet, are well worth our serious attention. We need to know not only how they operate so efficiently, but also the developmental basis for their machinery, how the genetic blueprint is translated. Knowledge of membrane physiology will help to answer these questions.

The crustaceans, particularly the larger decapod forms, supply some of our best models for investigation of membrane physiology. They are large and their individual cells often attain generous dimensions. This is especially true among neurons and muscle cells, where it is possible to apply sophisticated electrical and chemical techniques to study membrane performance of intact preparations. Thus, a great deal of the work on crustacean membranes has been concerned with description of membranes in large neurons and muscle cells. The present review will reflect this emphasis.

Recent reviews have dealt with membrane physiology of crustacean muscle fibers (Ashley, 1970; Atwood, 1972,1973; Blaustein, 1976) and synapses (Takeuchi and Takeuchi, 1972; Gerschenfeld, 1973; Atwood, 1976). However, membrane physiology of crustacean neurons has not been comprehensively reviewed and, in view of the important contributions to knowledge of membrane physiology provided by study of these neurons, an overview of them seems desirable.

One of the most striking features of crustacean neuronal membranes is the great diversity of mechanisms encountered not only from one neuron to another but also at different locations on a single neuron. Other invertebrate neurons also show diversification of membrane properties; it seems to be a common feature. This observation leads to the question of causation. How do these membranes come to be diverse, and what maintains them in their differentiated state?

At the present time, we can describe how crustacean neuronal membranes behave when we take them from an animal and subject them to various experimental manipulations. This knowledge will be reviewed here. When it comes to deducing how these well-studied membranes normally operate within the intact animal, we know less and have to guess more. I submit that we have a great deal to learn about them in a temporal sense. Are these membranes capable of altering their properties with time to meet new environmental or developmental challenges (Prosser, 1967)? Do they play key roles during development to determine the overall properties of the cells, perhaps by mediating feedback to the genome?

In the present review, I will attempt not only to outline some of what has recently been established about crustacean membranes, but also to advance some speculations about little-studied problems which may become important in the future.

II. DIVERSITY OF NEURONS

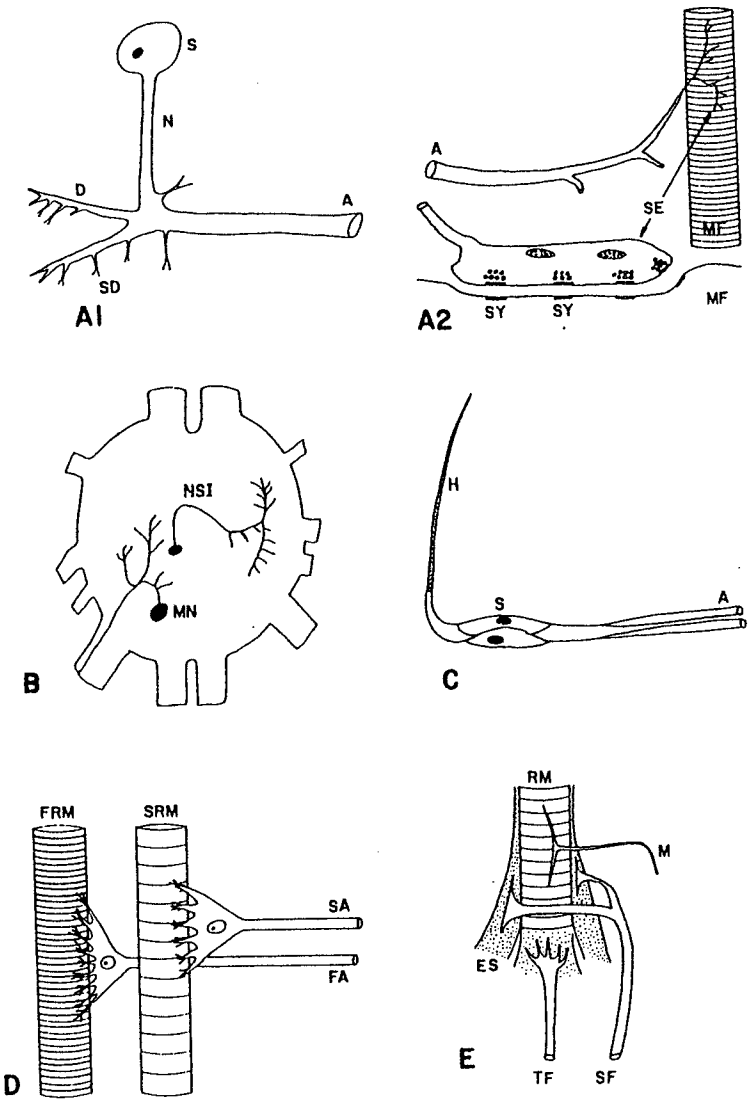
Nerve cells of crustaceans have been extensively exploited in studies of electrical membrane properties and mechanisms of sensory transduction. The discovery of "giant" neurons has permitted detailed studies with modern techniques. Giant axons of crustaceans can be used for some of the same purposes as the somewhat larger giant axons of squid, and the large sensory neurons provide excellent material for study of stimulus transduction at the membrane level.

An important point to note at the outset is that neurons, in crustaceans and elsewhere, are extremely diverse both in their morphology and in their membrane physiology. As a corollary, it is not possible to learn all about neuronal mechanisms by studying only one "type specimen" neuron, such as the squid giant axon. Comparison of different neurons has revealed a wide range of membrane performance, and has turned up important mechanisms not encountered in the type specimen.

A brief survey of some of the neurons studied in crustaceans will serve to illustrate neuronal diversity (see Figure 1).

Crustacean motoneurons (Figure 1), particularly their peripheral axons and synapses, have been a favorite material for investigation for some time. The early research on the peripheral axons and their role in motor control (reviewed in Wiersma, 1961; Atwood, 1972,1973, 1977) set the stage for a recent flood of work on the cell bodies and dendrites within the ganglia and neuropile (e.g., Takeda and Kennedy, 1964; Otsuka et al., 1967; Zucker, 1972a-c).

Although crustacean motoneurons are themselves diverse enough to make generalizations difficult, the "typical" pattern one finds can be illustrated using the fast flexor motoneurons of the crayfish abdomen as examples (Figure 1A). Morphologically, these neurons have been well defined by means of intracellular injections of the fluorescent dye Procion yellow (Zucker et al., 1971) and more recently by injections of cobalt chloride (Pitman et al., 1972a,b) which can be used to create an electron-dense precipitate, permitting study at the electron-microscopic level (Atwood and Pomeranz, 1974). The fast flexor motoneuron comprises a large cell body (soma) containing the nucleus, which emits a thin neurite leading to an enlarged dendritic arborization (large primary and smaller secondary dendrites) and to the main axon supplying muscles in the abdomen. Some dendrites receive synaptic input from lateral or medial giant fibers of the nerve cord (which are involved in escape reflex behavior: Zucker et al., 1971), while other dendrites receive synapses from, or supply synapses to, other neurons in the neuropile.



All parts of this system are of interest to membrane physiologists. The different parts of the neuron are specialized for different functions, and this is reflected in regional differences of membrane properties. The main axon conducts action potentials to the muscles, and its membrane is specialized for electrogenic changes in ionic conductance. The motor axons are surrounded by glial cells (Figure 2), which invest them loosely enough to leave channels and periaxonal spaces. The motor axon terminals in the muscle are specialized for release of the transmitter substance, and this entails further membrane specialization. In some fast flexor motoneurons, the cell bodies are able to support electrogenic responses, but in the largest of these neurons, the "motor giant" cell, this is not the case (Takeda and Kennedy, 1964). Furthermore, many other motoneurons of crustaceans are known to have electrically inexcitable somata. Spike-initiating regions of such neurons commonly occur in the enlarged dendrite-bearing region (Sandeman, 1969).

Among motoneurons, a variety of different types of membrane behavior which contributes to pattern formation of motor output has been

Figure 1 Examples of well-studied crustacean and other arthropod neurons. A1. Intraganglionic portions of a crayfish fast flexor motoneuron. A2. Axon terminal and neuromuscular synapse of a fast flexor motoneuron. The structures shown are soma (S), neurite (N), major dendrites (D), secondary dendrites (SD), axon (A), synaptic ending (SE) on muscle fiber (MF), and neuromuscular synapse (SY). B. Spiking neuron (motoneuron, MN) and nonspiking interneuron (NSI) of a cockroach ganglion. (After Pearson and Fourtner, 1975.) Similar neurons occur in crustaceans. The spiking neuron (motoneuron) sends an axon into a peripheral nerve, while the nonspiking interneuron is confined to the ganglion. C. Tactile sensory neurons of a crayfish, showing soma (S), axon (A), and sensory hair (H) innervated by neuronal dendrites. (After Mellon and Kaars, 1974.) D. Muscle receptor organs of crayfish, showing the sensory neurons with dendrites embedded in the muscle strands: FRM, finely striated "fast" receptor muscle; SRM, coarsely striated "slow" receptor muscle; FA, axon of "fast" receptor neuron; SA, axon of "slow" receptor neuron. E. Nonspiking neurons of the coaxal receptor organs of a crab. Only the peripheral parts of these neurons are shown; somata are located in the central nervous system: TF, the T fiber (in series with receptor muscle); SF, the S fiber (in parallel with receptor muscle); RM, receptor muscle; ES, elastic strands flanking the receptor muscle; M, motor axon supplying the receptor muscle. (After Bush and Roberts, 1968).

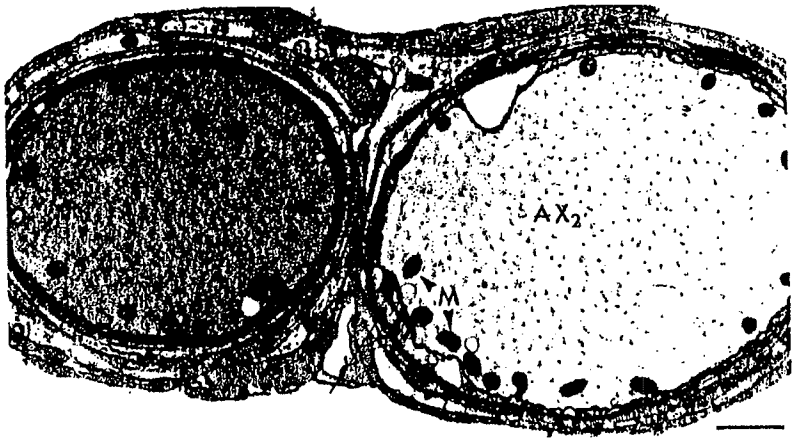


Figure 2 Electron micrograph of two motor axons in crayfish: the excitor (AX_1) and inhibitor (AX_2) axons supplying the claw opener muscle. A difference in electron density of the axoplasm can be seen, the significance of which is not known. A glial sheath (GS) surrounds the axons with a loose wrapping, and an invaginated channel (arrow) connects the periaxonal space to the external medium. Numerous mitochondria (M) occur near the inner surface of the axonal membrane. Scale mark: 1 μm.

described. Membrane properties involved in such behavior will be considered subsequently (Section VI.C).

Turning from motoneurons to interneurons, we can recognize several different types. For example, the "giant fibers" in the nerve cords of drayfish and other crustaceans are specialized for rapid longitudinal conduction. The somata of the lateral giant fibers and their dendrites reside in the segmental ganglia and give rise to the large impulse-conducting axon, whereas the somata of the medial giant fibers are found in the brain. Many other interneurons conducting impulses between ganglia have been described; these are all spiking types.

Recently, Wine (1975) has provided evidence that some crustacean interneurons conducting from one ganglion to another have electrogenic cell bodies and dendrites, while others do not. Those with electrogenic cell bodies and dendrites tend to fire longer bursts of impulses when activated. These observations suggest that dendrites and cell bodies of a given arthropod neuron share membrane properties, whereas the axon may have quite different properties.

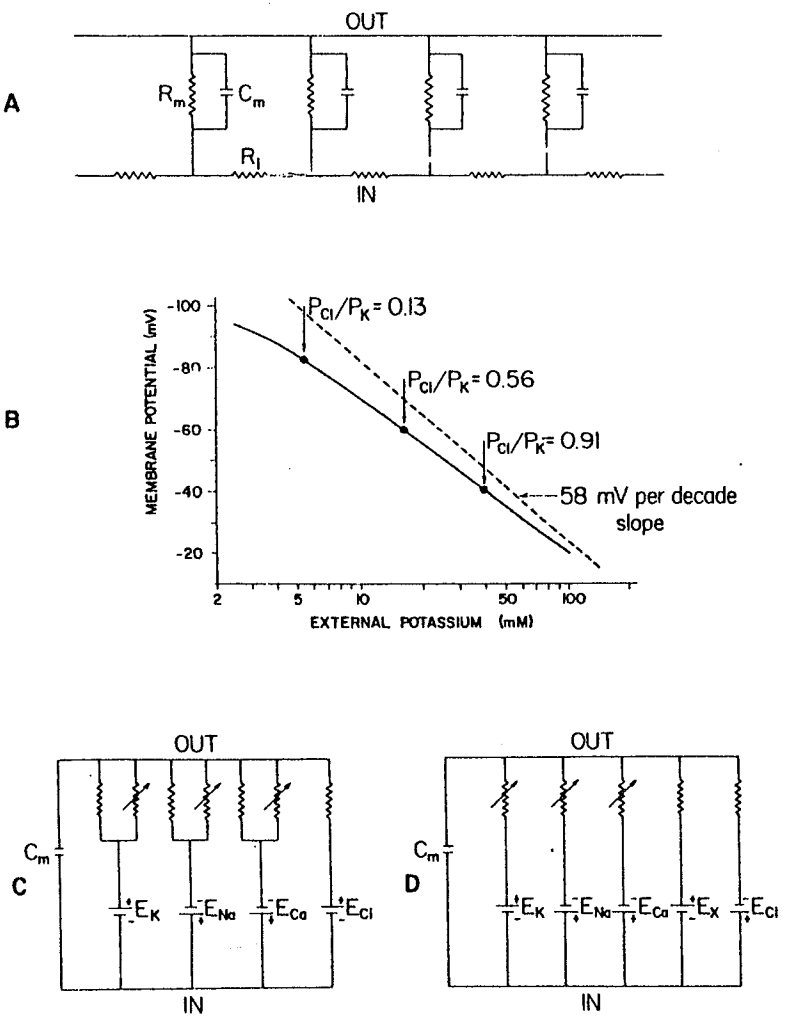
In contrast to multisegmental interneurons, a class of nonspiking interneurons occurs in crustaceans (Mendelson, 1971) and in insects (Pearson and Fourtner, 1975). They undergo changes in membrane potential and drive other neurons, including motoneurons, without generating any type of conventional nerve impulses. Morphologically, these neurons are distinguished by the fact that they send no processes out of their ganglion of origin (Figure 1B). Some are known to be important components in the control of rhythmic locomotory movements. Pearson (1977) estimates that a majority of the interneurons within the arthropod nervous system may be of this nonspiking type.

Sensory neurons also come in spiking and nonspiking forms. The numerous tactile receptors of the animal's surface (see, for example, Mellon and Kaars, 1974) have fairly simple associated neurons consisting of sensory dendrite, cell body, and spike-conducting axon (Figure 1C). The well-known abdominal stretch receptor neurons in crayfish and lobsters (Alexandrowicz, 1951) also have mechanosensitive dendrites associated with a large cell body and a spike-conducting axon (Figure 1D). By contrast, mechanoreceptors at the bases of the legs in certain crustaceans have been shown (Bush and Roberts, 1968) to be nonspiking and to conduct potential changes from mechanosensory end-processes decrementally along a large axon to the central nervous system (Figure 1E). These nonimpulsive electrical changes play an important role in the modulation of the animal's locomotor reflexes. Similarly, light-sensitive ocellar neurons in the barnacle's eye have been shown to conduct passively and to transmit information nonimpulsively to the brain over distances of 1-2 cm (Shaw, 1972). Other examples of both spiking and nonspiking sensory neurons have been studied in crustaceans.

This survey provides a starting point for further consideration of the membrane properties which are found in different types of neurons, and which give rise to regional membrane specialization of individual cells.

III. PASSIVE ELECTRICAL PROPERTIES

The nerve cell at rest can be represented by an electrical network of the type illustrated in Figure 3A. The membrane opposes diffusion of ions across it, and the extent to which it does this in the resting condition can be determined by measuring the "specific membrane resistance" (R_m) which is usually expressed as the electrical resistance of a square centimeter of membrane (Hodgkin and Rushton, 1946; Fatt and Katz, 1951). The lipid bilayer of the membrane also imparts capacitative properties C_m, and the axoplasmic composition determines the axoplasmic resistivity R_m. The membrane time constant τm, which



determines the rate at which the membrane can be charged or discharged, is the product of R_m and C_m. The length of the space constant or the neuron λ which describes the longitudinal spread of a nonregenerative electrical event, is a function of R_m, R_i, and cell size: λ decreases as cell size or R_m decrease, and as R_i increases.

Knowledge of the passive electrical membrane properties is extremely important for the study of signal conduction in the neuron and also provides insight into the relative permeability of the membrane to ions in solution.

The isolated axons of crabs and lobsters formed the material for some of the very first determinations of electrical membrane properties of neurons; the classic paper of Hodgkin and Rushton (1946) was the result (see Table 1). Since then, a number of other determinations of the passive electrical properties of various crustacean neurons have been made; some of these are included in Table 1. The majority of the studies have used the classical methods of "square pulse" analysis, in which the amplitudes and shape of membrane voltage changes produced by a rectangular pulse of injected current are analyzed (Hodgkin and Rushton, 1946).

In examining the various studies, one is struck by the variability of the results. For example, in Hodgkin and Rushton's (1946) study of large axons from lobster leg nerves, a wide range of values for R_m is reported. In part, this reflects the variable physiological conditions of the axon, since sequential determinations on the same axon showed appreciable variability. However, another factor could be the nonuniform nature of the axons themselves. Among large motor axons, "fast" and "slow" types are known to be physiologically different (Atwood, 1976). So far, no one has made careful determinations of the passive membrane electrical properties of leg nerve axons identified as to type.

Figure 3 A. Electrical model of "passive" neuronal membrane properties, showing the resistive and capacitative elements: R_m, specific membrane resistance; C_m, specific membrane capacitance; R_i, axoplasmic resistivity. B. Variation of the ratio, membrane Cl^- permeability (P_{Cl}) to membrane K^+ permeability (P_K), as a function of K^+ level, in crayfish giant fiber. (After Strickholm and Wallin, 1967.) Membrane potential decreases with external K^+, but approximates the slope predicted by the Nernst equation only at high external K^+ levels. C and D. Two models for ionic permeability channels of the crayfish giant fiber. (After Yamagishi and Grundfest, 1971.) Voltage-sensitive channels are shown as variable resistors, and unreactive channels as fixed resistors; equilibrium potentials for the various ions are shown as batteries. Model C differs from model D in having parallel reactive and unreactive channels for K^+, Na^+, and Ca^{2+}.

Table 1 Passive Electrical Properties of some Crustacean Neurons

Species	Preparation	Time constant τ_m (ms)	Length constant λ (mm)	Specific membrane resistance R_m (Ωcm^2)	Axoplasmic resistivity R_i (Ωcm)	Reference
Homarus (lobster)	Axons of leg nerve	2.3 (0.76-5.4)	1.61 (0.81-2.95)	2290 (564-7330)	60 (43-83)	Hodgkin and Rushton, 1946
	Giant axon of nerve cord	–	–	1250	60	Julian et al., 1962a
				2434	97	Tobias, 1960
				8300	80	Brinley, 1965
Carcinus (shore crab)	Axons of leg nerve	5	2.5	5000	60	Katz, 1966
Callinectes (blue crab)	Axons of leg nerve	–	1.66-2.35 (20°C)	1150-2420 (20°C) 1368-3498 (10°C)	64-191 (20°C) 89-225 (10°C)	Machne and Orozco, 1970

Procambarus (freshwater crayfish)	Sensory cell body	2.5	–	630	–	Mellon and Kaars, 1974
	Sensory cell axon	8.29	0.31-1.43	2617	160	
	Motor giant neuron, soma	3	–	300	–	Takeda and Kennedy, 1964
	Nongiant motoneuron, soma	2.0-2.5	–	200	–	
	Medial giant fiber axon	–	1.8 (1.6-2.6)	870 (726-1210)	97 (80-108)	Yamagishi and Grundfest, 1971
Balanus	Visual neuron	12	4.9	57,000	90	Shaw, 1972

Reported values for membrane capacitance in these neurons are in the range 1-2 $\mu F\ cm^{-2}$, as is the membrane capacitance for many other neurons. Some variation in values for R_i is apparent in Table 1; this could reflect variability of ionic content of the axons (Katz and Freeman, 1972).

An interesting report by Mellon and Kaars (1974) indicated that the specific membrane resistance R_m of the soma of a crayfish sensory neuron is considerably lower than that of the axon. This factor is important for impulse initiation and conduction in sensory neurons. In addition, this feature appears to be the reverse of the situation reported for some molluskan neurons, in which the axon has a lower R_m than the soma (Carpenter, 1973). Takeda and Kennedy, (1964) reported similarly low values for R_m of the somata of both motor giant and motor nongiant neurons supplying the fast flexor muscles of the crayfish abdomen (Table 1). The results seem to imply that the soma membranes of crustacean neurons are generally more highly permeable to ions (at rest) than are the membranes of the axons. In turn, this could explain the rather small soma potentials recorded in some of these neurons during activity (Takeda and Kennedy, 1964; Selverston and Remler, 1972).

It is worth noting also that rather high values of membrane capacitance (10-12 $\mu F\ cm^{-2}$) were obtained by Takeda and Kennedy (1964) for somata of fast flexor motoneurons. This could indicate a high degree of membrane folding (as in muscle: Fatt and Katz, 1953; Selverston, 1967; review, Atwood, 1972), or, for some unknown reason, a spuriously low value for calculated R_m. So far, no case is known in which an unfolded neuronal membrane has an intrinsic C_m as high as 1-10 $\mu F\ cm^{-2}$.

Recent work on motoneurons of the lobster stomatogastric ganglion (Miller, 1975) using Procion dyes to mark recording sites has shown that postsynaptic potentials originating in fine neuropile processes can be recorded in the soma of the same neuron with relatively little decrement. Effective length constants of 1.2-2.5 mm were determined. Assuming a value of 80 Ω cm for R_i and an average diameter of 25 μm for neuropile processes and the neurite leading to the soma (King, 1976b), a tentative calculation of R_m can be made from the formula:

$$\lambda = \sqrt{\frac{aR_m}{2R_i}}$$

where a is the radius of the neurite. A value of 2600 $\Omega\ cm^2$ results, which is within the range of values reported for axons (Table 1). Thus, the passive electrical membrane properties of the neuropilar processes are not strikingly different from those of the more easily studied larger axons.

By far the largest values for membrane resistance among crustacean neurons were determined by Shaw (1972) for specialized sensory neurons of the barnacle's lateral eye. These values (Table 1) indicate unusual membrane properties. Indeed, the visual signal in this structure is transmitted passively from one end of the neuron to the other, a distance of 1-2 cm. It would seem that the use of action potentials to convey information in the nervous system, involving as it does a considerable expense of energy to maintain ionic gradients, is resorted to by crustaceans for signaling between ganglia or over relatively long distances, but within ganglia or over shorter distances, passive conduction is used. Some of the neurons utilizing passive conduction appear to have relatively impermeable membranes.

IV. RESTING POTENTIALS

Analysis of passive electrical membrane properties is necessary for an understanding of signal conduction along neurons, but in itself does not permit analysis of the membrane's ionic channels.

Measurements of membrane potentials in the presence of various ions, coupled with determinations of intracellular ionic content, provide more detailed information about the ionic channels involved in establishing the resting membrane potential. Additional techniques, such as injection of current into the cell to produce sudden changes of membrane potential, and the use of voltage clamp techniques, are necessary to provide information about the ionic channels which are voltage-dependent (activated or inactivated by changes in membrane potential).

The large size of many crustacean neurons has permitted application of all of these techniques, with much resultant information about the ionic channels of these neurons. The giant axons of crayfish and lobsters are an off-season substitute for the giant axons of the squid and can be voltage-clamped, internally perfused, or experimentally manipulated in various other ways. Although these operations are less easily performed with crustacean neurons than with the squid giant axons, they are entirely feasible. Recently, interesting work has been done with large crustacean sensory neurons (Brown, 1976).

An interesting point of contrast between crayfish and squid giant axons is that ionic concentrations in the former remain stable in isolated preparations over a considerable period of time (Wallin, 1967a), whereas they change with time in the latter (sodium increases, potassium decreases), indicating progressive deterioration (Steinbach and Spiegelman, 1941; Hodgkin and Katz, 1949). Despite their smaller size, crayfish fibers offer advantages in physiological stability.

In considering first the resting membrane potentials of crustacean neurons, one can ask at the outset whether or not these potentials are

determined entirely by the passive distributions of ions and by relative membrane permeabilities for the different ions, or whether additional factors, such as electrogenic ion pumps (see Chapter 1), contribute a significant fraction of the resting potential.

If ionic distributions and the resting membrane permeabilities (leakage channels) predominate, then the membrane potential should be described by the "constant field equation" (Goldman, 1943)

$$Em = \frac{-RT}{F} \ln \frac{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o + P_{Ca}[Ca^{2+}]_i^{\frac{1}{2}}}{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i + P_{Ca}[Ca^{2+}]_o^{\frac{1}{2}}}$$

where P_K, P_{Na}, P_{Cl}, and P_{Ca} are the permeability coefficients for K^+, Na^+, Cl^-, and Ca^{2+}, respectively; $[K^+]$, $[Na^+]$, $[Cl^-]$, and $[Ca^{2+}]$ have activity coefficients assumed constant; subscripts i and o denote intracellular and extracellular concentrations; T is absolute temperature; F is the Faraday unit; and R is the gas constant.

The model for the resting potential described by the equation predicts a low temperature coefficient for this potential and assumes a passive movement and distribution of the relevant ions.

The extent to which the equation is valid, and its predictions and assumptions justified, has been tested for a number of crustacean neurons. Most of the published work deals with large axons, usually either the giant fibers of crayfish or lobster nerve cords or the large axons of crustacean leg nerves.

At least one study, however, was performed on the somata of neurons in a lobster abdominal ganglion (Zollman and Gainer, 1971). In this study, the cell bodies were exposed by desheathing the ganglion. Applications of solutions varying in ionic concentration showed that the membrane potential of these cells was responsive to changes in K^+ and Cl^-, and that the membrane was apparently highly permeable to these ions and also to larger anions such as $CH_3SO_4^-$. The membrane potential agreed closely with the Nernst equilibrium potential for K^+, $E_K = (-RT/F \ln ([K^+]_i/[K^+]_o)$, over a wide range of external K^+ concentrations, whereas changes in external $[Cl^-]$ at constant $[K^+]_o$ produced only a transient alteration of membrane potential ("chloride transient"). The situation is somewhat similar to that in frog muscle fibers (Hodgkin and Horowicz, 1959) in which P_K and P_{Cl} are both high. The chloride transient is thought to result from rapid alteration in E_{Cl} as chloride redistributes across the membrane to satisfy the Donnan equilibrium condition: $[K_o^+]\,[Cl_o^-] = [K_i^+]\,[Cl_i^-]$. When this condition obtains, $Em = E_K = E_{Cl}$.

The resting potentials of these cells had a very low temperature dependence and were insensitive to the actions of metabolic inhibitors,

the Na^+-pump inhibitor ouabain, and low oxygen. Thus, there is no evidence for involvement of an electrogenic Na^+ pump or any other metabolically driven ion pump in their resting potentials.

An entirely different story emerges from the work on crustacean axons and sensory neurons. The resting potentials of some of the large axons show a much higher temperature dependence than predicted from the Goldman equation (Dalton and Hendrix, 1962; Senft, 1967). In addition, metabolic inhibitors such as 2,4-dinitrophenol and cyanide, and the Na^+-pump inhibitor ouabain bring the resting potential much more closely into line with the predictions based on ionic distributions and relative permeabilities. Thus, there appears to be a contribution of the Na^+ pump to the resting potentials of these axons.

Further support for this idea comes from measurements of intracellular concentrations of Na^+, K^+, Cl^- (Wallin, 1966,1967a,b; Brown, 1976). Not only is there a wide scatter of values for these ions (Katz and Freeman, 1972), but the distributions do not seem to be in electrochemical equilibrium across the membrane. This is best shown by the measurements of Wallin (1967a,b), who was able to extract small samples of axoplasm from crayfish giant fibers for analysis while simultaneously measuring the membrane potential. The mean values of intracellular $[Na^+]$, $[K^+]$, and $[Cl^-]$ were 17.4, 265, and 12.7 mM liter^{-1} axoplasm, respectively. For electrochemical equilibrium, in van Harneveld's solution containing 5.4 mM K^+, the expected $[K^+]_i$ would be 168 mM, and the expected $[Cl^-]_i$ would be 7.8 mM.

A further piece of evidence in this study is that the distributions of sodium and potassium (but not of chloride) are altered by application of ouabain, indicating involvement of the sodium pump in the distribution of cations.

It can be argued that some of the variability in ionic concentrations noted above may be due either to errors in estimating the extracellular space trapped by Schwann cells (see Figure 2) or to contributions of the Schwann cell cytoplasm itself to the measurement, since this component is known to differ from axoplasm in its composition in well-studied examples (Villegas et al., 1965). However, Wallin's measurements are immune to this criticism since he extracted axoplasm from within the axon. It could be argued further that activity coefficients of the ions are not the same inside and outside the axon, necessitating a correction of the measured concentrations (see Strickholm and Wallin, 1965). However, Wallin (1967a,b) argues against a drastic difference between intracellular and extracellular activity coefficients, at least for Cl^- and K^+. Among his arguments he adduces the observation that Cl^- comes to be distributed at electrochemical equilibrium when $[K^+]_o$ is increased. It would be difficult to explain this finding on the basis of a difference in external and internal

activity coefficients without postulating a change in the intracellular activity coefficient for Cl^- as $[K^+]_o$ is raised. It is more reasonable to assume that there is no drastic difference in the activity coefficients.

Additional evidence comes from experiments by Strickholm and Wallin (1967), who recorded membrane potential simultaneously with intracellular Cl^- activity, using an Ag-AgCl electrode, while changing the external solution to keep $[K^+]_o$ $[Cl^-]_o$ constant, as required for electrochemical equilibrium. When the membrane was depolarized in high $[K^+]_o$ at constant $[K^+]_o$ $[Cl^-]_o$, a loss of intracellular Cl^- was observed. If electrochemical equilibrium had initially existed, no change in $[Cl^-]_i$ would have occurred.

From the above discussion we can see that the "passive membrane" model for resting potentials does not apply as well to crustacean axons as it does to the crustacean ganglionic cell bodies studied by Zollman and Gainer (1971).

However, the ionic batteries do in fact contribute a large part of the resting membrane potential and, as in many other cells, the equilibrium potential for K^+ is dominant, owing to relatively high resting P_K. Unlike the situation in some other neurons, P_{Cl} is not negligible in certain crustacean axons; in fact, P_{Cl} increases in value relative to P_K when the neuron is depolarized in high $[K^+]_o$ (Strickholm and Wallin, 1967; Figure 3B). The results of Strickholm and Wallin (1965, 1967), obtained by analyzing responses of the membrane potential to rapid changes of ionic concentration, showed that the close agreement between membrane potential and E_K in the steady state cannot be used as evidence for a predominance of P_K over other ionic permeability coefficients (this point was made also in an earlier work with striated muscle fibers: Hodgkin and Horowicz, 1959).

Brinley (1965), using radioisotopes to measure ionic fluxes, obtained mean fluxes of 13 pmol cm^{-2} sec^{-1} for K^+, and 10 pmol cm^{-2} sec^{-1} for Cl^- in lobster lateral giant fibers. This measurement, like the preceding, indicates a substantial resting membrane Cl^- permeability. Similarly, Freeman et al. (1966), using osmometric methods, found that lobster axons had a substantial P_{Cl} whereas squid axons did not.

In sensory neurons, the recent measurements by Brown (1976) with intracellular ion-selective microelectrodes also indicate a substantial chloride permeability. Furthermore, his measurements cast doubt on the validity of previous approaches employing measurements of membrane potential to estimate ionic permeabilities (see Section VII.C).

A recent study by Yamagishi and Grundfest (1971) has extended the range of ionic substitution experiments on crayfish medial giant fibers. These authors have shown that Na^+ can contribute to the

membrane potential when $[K^+]_o$ is reduced to zero; addition of Na^+ depolarizes the membrane, and subtraction of Na^+ hyperpolarizes it. These effects of Na^+ are not blocked by tetrodotoxin, indicating a "leakage channel" for Na^+ distinct from the voltage-dependent Na^+ channel operating during the action potential. Furthermore, increase in $[Ca^{+2}]_o$ can depolarize the membrane and increase its resistance when $[Cl^-]_o$ is absent. Yamagishi and Grundfest (1971) conclude that the resting ionic permeabilities of the crayfish medial giant fiber are in the order $P_K > P_{Na} > P_{Ca} > P_{Cl}$. This conclusion conflicts with that of Strickholm and Wallin (1967) on the same fiber from the same genus (*Procambarus*). The latter authors estimated that, at the resting potential, P_{Cl}/P_K was 0.14 and P_{Na}/P_K was 24×10^{-4}. Similarly, Wallin (1967b) estimated $P_K:P_{Na}:P_{Cl}$ as 1:0.001:0.1. It is worth noting, however, that the latter authors studied this fiber in the abdominal nerve cord, whereas Yamagishi and Grundfest (1971) studied it in the circumoesophageal and thoracic nerve cord; regional membrane differences have been reported for this fiber (Grundfest and Yamagishi, 1972).

From their observations, Yamagishi and Grundfest (1971) proposed two possible (and provisional) models for the medial giant fiber membrane (Figure 3C and D). In one (Figure 3C), leakage channels for Na^+, K^+, and Ca^{2+} are considered to be different from the electrically excitable (voltage-controlled) channels operating during an action potential. In the other model (Figure 3D), the leakage channels for all cations are lumped together, and some leakage can also occur through the electrically excitable channels. In the case of the Na^+ channels, there is evidence that the leakage channels are not blocked by tetrodotoxin, which suggests at least two channels for Na^+ entry. For other channels in this preparation, the evidence is not complete. The models are useful in pointing out that ionic species may have more than one pathway available to cross the membrane.

Resting membrane permeabilities of crayfish medial giant axons were determined at different pHs (6.0, 7.5, and 9.0) and with different levels of potassium depolarization by Strickholm et al. (1969). Both P_K and P_{Cl} were pH and depolarization dependent, whereas P_{Na} was relatively independent of both factors. The finding of depolarization-dependent changes in P_{Cl} (Figure 3B) appears to contradict the models of Figures 3C and D, in which Cl^- channels are represented as unreactive. It has not yet been shown whether depolarization alone, or high $[K^+]_o$, is necessary to cause changes in P_{Cl}. Strickholm et al. (1969) considered the possibility that ionized fixed changes on the membrane surface are involved in regulation of membrane permeability, but found some of their data inconsistent with this notion. Instead, they suggest that some type of protein-phospholipid conformational change

may be involved. Probably this type of problem can be fully resolved only with more complete knowledge of membrane biochemistry.

In conclusion, it is worth noting that the situation described for crustacean neurons, in which the axons have a resting potential partly dependent on an electrogenic ion pump, whereas somata do not, is the opposite of that seen in molluskan neurons in which the electrogenic pump contributes more to the resting potential in the soma than in the axon (Carpenter, 1973). The degree to which the electrogenic pump can contribute is related to the passive electrical membrane resistance: the higher the resistance, the greater the potential contribution of the electrogenic pump (Carpenter, 1973). Crustacean axons generally have a higher R_m than do somata (Table 1); this is in accord with the above postulate. At the present time we do not know whether the dendrites of most crustacean neurons most resemble the somata or the axons in their membrane performance. In crustaceans, the sheath surrounding the central nervous system appears to regulate the ionic concentrations of extraneuronal spaces, whereas the perineural sheath of peripheral nerve seems to provide little barrier to ionic diffusion (Abbott et al., 1975). From the standpoint of system design, this may impose different demands on neuronal membranes in different parts of the crustacean nervous system.

V. STEADY-STATE MAINTENANCE

Related to the problem of the resting potential is that of the participation of membrane transport processes in establishing ionic and osmotic properties of the intracellular milieu.

It has already been noted that the resting potentials of many crustacean axons are partly attributable to activity of an electrogenic Na^+ pump. Historically, crustacean nerves played an important role in the elucidation of the mechanism of the Na^+ pump; crab nerves were used by Skou (1957) in the first demonstration of cation-sensitive membrane ATPase activity. Since then, a number of other important studies have been done on the properties of crustacean membrane ($Na^+ + K^+$)-activated ATPase (Baker, 1964,1965; Baker and Connelly, 1966), but most of the work on this membrane transport system has since been done using other preparations, in particular squid giant axons and erythrocytes (Baker and Willis, 1972; Post, 1974). The crustacean enzyme appears similar in most respects to those obtained from other preparations.

Maintenance of amino acid composition of crustacean nerves has also received attention. Glutamate uptake by crab nerves is an active transport process stimulated by external Na^+ and inhibited by external K^+ (Baker and Potashner, 1971); kinetic analysis indicates that two Na^+ or K^+ are required for activation or inhibition, respectively.

In addition to active transport, metabolic mechanisms contribute to the steep gradient of amino acids which is maintained across crustacean neuronal membranes (Gilles and Schoffeniels, 1969; Evans, 1973). In some crustaceans, amino acid concentrations in nerves respond to osmotic stress in an adaptive fashion. Thus, in the euryhaline crab, *Callinectes*, amino acids decrease in concentration in axons subjected to hyposmotic solutions, whereas an increase in amino acid concentration is seen in axons subjected to hyperosmotic solutions (Gilles and Gerard, 1974). These changes are correlated with volume changes characteristic of these axons (Gerard, 1975). Both metabolism and changes in membrane permeability are involved in regulation of amino acid concentrations. Hyposmotic exposure increases passive efflux of alanine and partially inhibits active transport. In addition, changes in catabolism appear to be involved.

It seems clear that many crustacean neurons can respond to environmental challenges by alterations in membrane permeability, membrane transport, and metabolism. The observed effects appear adapted to steady-state maintenance, and it is likely that longer-acting processes involving altered protein synthesis may also come into play to meet certain environmental changes.

VI. ACTION POTENTIALS

The giant axons in the central nervous system of lobsters and crayfish, and the large axons in crustacean leg nerves, provide excellent experimental material for analysis of action potentials. Although these axons are not as large as those of squid, they have been used successfully for experiments similar to those performed on squid axons and for many years were the next best axon available. Recently, the large axons of the annelid worm, *Myxicola*, have become more favored by experimentalists. However, the crustacean axons have contributed, and continue to contribute, much useful information about conduction. In crustaceans, large axons of several distinctive physiological types are available, inviting comparative studies.

A. Ionic Mechanisms

Significant early papers on crustacean axons, in which information was obtained by external recording techniques, include the studies by Hodgkin (1938) on local subthreshold electrically excited responses of crab axons, and by Hodgkin and Rushton (1946) on electrical properties of lobster axons. With the advent of microelectrode recording and voltage-clamping techniques, numerous other studies appeared.

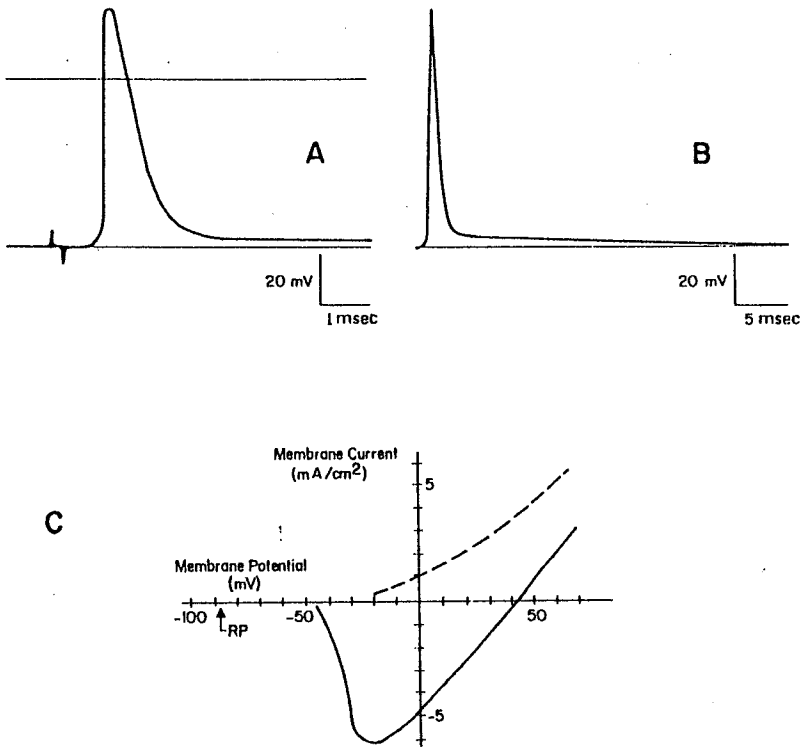


Figure 4 Action potentials. A and B. Action potentials recorded from a crab motor axon with an intracellular microelectrode, showing the depolarizing after-potential; the upper horizontal line in A indicates zero membrane potential. (From Takeda, 1976.) C. Voltage clamp records from a lobster giant fiber (sucrose gap method), showing early peak inward current (solid line) and late outward (steady-state) current (dotted line) as a function of the clamped membrane potential. RP, resting potential. (After Julian et al., 1962b.)

Action potentials recorded from crustacean axons with microelectrodes (Figure 4A and B) show a characteristic depolarizing after-potential of long duration following the positive-going peak of the event. This feature distinguishes these action potentials from those of squid, where a hyperpolarizing after-potential is seen.

The maximum rate of rise of the action potential is 650-925 V sec^{-1} in crayfish giant axons (Hartz and Ulbricht, 1973), which is slightly greater than the value of 630 V sec^{-1} reported for squid (Hodgkin and Katz, 1949).

The ionic mechanism of the crustacean action potential has been analyzed by voltage clamp methods by a number of workers; some have used sucrose-gap techniques, whereas others have been able to use axial wire electrodes inside the axon.

Experiments on the ionic dependence of the action potential showed clearly that the amplitude of the positive peak was determined mainly by the Na^+ equilibrium potential (Dalton, 1958,1959). Thus, the ionic mechanism of this event appeared to be similar to that analyzed by Hodgkin and Huxley (1952) in squid axons and to involve an increase in Na^+ conductance (giving the positive peak), followed by an increase in K^+ conductance (giving the return to resting potential). Voltage clamp experiments on lobster giant axons (Julian et al., 1962a,b) showed an early inward membrane current (Na^+ current), activated at a membrane potential of about -45 mV and rapidly inactivated with maintained depolarization, and a delayed outward current (K^+ current) which showed no inactivation with maintained depolarization (Figure 4C). A difference from the action potential of the squid axon was that total membrane conductance returned to resting level before repolarization was complete. This suggests that there is not a large increase in K^+ conductance (g_K) at this time, and that the termination of the action potential could be highly dependent on the rate of inactivation of Na^+ conductance (g_{Na}).

Confirmation of the early inward current as a Na^+ current and the delayed outward current as a K^+ current has been obtained in more recent voltage clamp measurements (using axial wire electrodes in crayfish giant fibers) by Shrager (1974). The early inward current is blocked by tetrodotoxin (TTX) and the delayed outward current by internal application of tetraethylammonium chloride (TEACl) (Shrager et al., 1969a).

The prolonged depolarizing after-potential, on a priori assumptions, could be due to accumulation of K^+ in the periaxonal space (Figure 2). However, experiments by Julian et al. (1962b) failed to show any evidence for K^+ accumulation (See also Abbott et al., 1975). A subsequent analysis of the after-potential by Yamagishi and Grundfest (1971) suggested that it could be due to an increase in Ca^{2+} conductance (g_{Ca}); increase in external Ca^{2+} altered the after-potential, making it larger (more positive). Thus, it is possible that in this axon a relatively long-lasting increase in g_{Ca} follows the action potential. In fact, it has recently been claimed (Fuchs and Getting, 1976) that the more terminal regions of crustacean motor axons have

an increasingly greater Ca^{2+} component of the action potential, which could be important in considering synaptic transmission and facilitation. It is possible, therefore, that the ionic mechanism of the action potential is not uniform along the length of the axon. An analogous situation has been reported in some molluskan neurons, where ionic mechanisms for action potentials in axon and soma are different (Kado, 1973; Junge and Miller, 1974).

Other effects of Ca^{2+} have been described for crustacean axons. Dalton (1958,1959) observed a decline in resting and action potentials in low external Ca^{2+} concentration, and Julian et al. (1962b) showed that the current-voltage relation obtained with voltage clamp was shifted by changing the external Ca^{2+} concentration, as in squid axons. In crayfish giant axons, Mg^{2+} can substitute partially for Ca^{2+} in maintaining the amplitude of the action potential but not in maintaining the resting potential; in lobster giant axons, Mg^{2+} is a much poorer substitute for Ca^{2+} (Dalton, 1958,1959; Julian et al., 1962). The reasons for this difference between species have not been ascertained.

Observations by Machne and Orozco (1967) on crayfish medial giant fibers indicated that alterations of external Ca^{2+} had a greater effect on the Na^+ conductance mechanism than on the K^+ conductance mechanism of the axonal membrane. Effects of Ca^{2+}, lanthanum, and other polyvalent cations have also been studied by Hafeman (1969a,b), D'Arrigo (1973,1974), and Hartz and Ulbricht (1973). Ca^{2+} and other polyvalent cations appear to form complexes with binding sites on the membrane. These sites can also be protonated and, at low pH, excitability disappears; Ca^{2+} and lanthanium prevent the loss of excitability (Hafeman, 1969b). The acidic dissociation pK of the Ca^{2+} binding site was estimated at about 6, suggesting possible involvement of a phosphate ester (Hafeman, 1969b).

Studies on spike threshold (D'Arrigo, 1973,1974) and maximum rate of rise of the spike (Hartz and Ulbricht, 1973) in crayfish giant axons have shown pronounced effects of Ca^{2+}, La^{3+}, and other ions. These ions raise the spike threshold; a tenfold increase in concentration of divalent cations makes the threshold membrane potential about 30 mV more positive (D'Arrigo, 1973). D'Arrigo (1973,1974) has interpreted his results in terms of an hypothesis involving screening of negative charges at the axonal membrane surface (McLaughlin et al., 1971), rather than binding of these ions to specific negatively charged groups at the outer surface near Na^+ channels (Tasaki, 1968; Hille, 1968). Furthermore, he has calculated a surface charge density of one e^- per 43 $Å^2$ for crayfish axons in the region of the Na^+ gates. The main evidence in support of the hypothesis is the observation that equivalent amounts of different species of divalent (or trivalent) cations have the same effect on threshold membrane potential.

It should be noted that the crayfish axons studied by D'Arrigo appear to differ from other axons in which specific binding of cations to negative surface charges, rather than nonspecific "screening" effects, appear to be more important (Frankenhaeuser and Hodgkin, 1957; Hille, 1968). D'Arrigo (1974) suggests that the main difference between axons which show screening and those which do not is the net density of ionized acidic groups on the membrane surface near the Na^+ gates; this density is higher for axons which show screening than for those which show "binding." Crayfish axons can be converted from one condition to the other by changing the pH (D'Arrigo, 1974). At low pH, the specific binding effect becomes evident, whereas at pH 6-8, nonspecific screening predominates. The overall conclusion emerging from this work is that extracellular polyvalent cations influence spike threshold mainly by altering the negative potential in the region of the Na^+ gates.

A somewhat different approach was adopted by Hartz and Ulbricht (1973), who had in mind the theory advanced by Lettvin et al. (1964) on control of ion flux through nerve membranes, and specifically the prediction that La^{2+}, with an ionic radius similar to that of Ca^{2+}, but with a higher valency, should bind more strongly to membrane sites. As predicted, La^{3+} was found to be equivalent to a much higher concentration of Ca^{2+} in its effects on action potential parameters (Takata et al., 1966; Blaustein and Goldman, 1968). Hartz and Ulbricht (1973) studied the effects of Ca^{2+} and La^{3+} on steady-state inactivation (the Hodgkin-Huxley parameter h_α: Hodgkin and Huxley, 1952) by measuring the maximum rate of rise of the action potential following conditioning pulses of various values in the Ca^{2+}- and La^{3+}-containing solutions. The maximum rate of rise V_A was assumed to represent the availability of "active" Na^+ channels. At conditioning membrane potentials of about -70 and greater, V_A was maximal in normal crayfish solution. Addition of Ca^{2+} and/or La^{3+} shifted the conditioning potential at which V_A was maximal to a more positive value (typically -50 to -40 mV), and lowered the absolute value of V_A^{max}. In comparing the actions of Ca^{2+} and La^{3+}, Hartz and Ulbricht (1973) concluded that the concentrations of the two ions required to produce a given shift of the steady-state inactivation curve were not in agreement with the "screening hypothesis" of D'Arrigo (1973). However, as D'Arrigo (1974) pointed out, two different parameters (inactivation and threshold) were examined in the two studies. Evidently, the screening and binding hypotheses require further attention. It is worth noting at this point that inactivation may be governed by membrane components on the inner surface (Bezanilla and Armstrong, 1975; Sevcik and Narahashi, 1975), whereas threshold effects are determined by many factors, among which charged groups on the outer surface of the

membrane as well as other factors, such as degree of inactivation, could play a role. Until these things are taken more fully into account, and properly controlled, the question is far from settled.

As noted above, effects of low pH were interpreted as arising from protonation of specific membrane binding sites (Hafemann, 1969a, b) and/or from reduction of surface charge density (D'Arrigo, 1974); the end result of these effects was thought to be a modification of the action of Na^+ channels. However, a voltage clamp study by Shrager (1974) on crayfish giant axons showed slowing of potassium currents (I_K) with "little effect" on Na^+ currents (I_{Na}) as pH was lowered below 7 (in fact, I_{Na} was reduced in amplitude by 20%, and time to peak was increased by 50%; the term "little effect" must be qualified). The slowing of I_K was attributed by Shrager (1974) to protonation of a histidine group at low pH, which could alter the ability of K^+ gates to change from nonconducting to conducting configurations. Thus, the evidence from studies in which pH was varied has been interpreted in various ways, and it may be that the methods used in these studies are not sufficient to analyze the types of membrane changes postulated.

General evidence for conformational changes in proteins or protein-phospholipid complexes during excitation and ionic movements is provided by several additional techniques, including measurements of changes in fluorescence, turbidity, light scattering, and birefringence, (Tasaki et al., 1968,1969; Cohen and Keynes, 1971). Also of interest are measurements of membrane current "noise" under voltage clamp (Poussart, 1971).

Biochemical approaches to crustacean membrane channels have included studies of TTX receptors in isolated membranes (Barnola et al., 1973. One can extract fairly large amounts of membrane from crustacean nerves for analysis. Crustacean nerves are favorable material for analysis of effects of various chemicals, e.g., alcohols (Houck, 1969), DDT (Shrager et al., 1969a), and aldehydes (Shrager et al., 1969b). The application of these various methods demonstrates the utility of crustacean axons for basic studies of membrane function.

The actions of pharmacologic agents on the ionic channels of nerve membranes is a major topic in itself and, in view of extensive recent reviews (e.g., Narahashi, 1974), will not be dealt with at length here. However, it is worth noting that many significant studies have been made on crustacean axons using chemicals to analyze the nature of the ionic channels. For example, the effect of tetrodotoxin on Na^+ channels of lobster axons was analyzed in voltage clamp studies (Narahashi and Moore, 1968) and shown to occur when the drug was on the outside, but not when it was on the inside, of the membrane. Thus, the conclusion was drawn that the Na^+ channel is polarized, with an external

opening which can be blocked by TTX (more recent studies on gating currents of squid axons have shown that TTX does not eliminate the gating current, hence the "gate" and the "channel" of the Na^+ conductance system appear to be different entities: Armstrong and Bezanilla, 1973,1974).

In other studies, actions of veratrum alkaloids (Ohta et al., 1973), DDT (Narahashi and Haas, 1968), and other compounds have been analyzed, mainly on lobster giant axons. In general, results of these studies show that various compounds can selectively affect opening or closing of Na^+ or K^+ channels. For example, DDT prolongs the Na^+ conductance increase and suppresses K^+ conductance increase, thereby leading to the characteristic repetitive firing of crustacean axons. These studies, and others, have provided much valuable information on the behavior of axonal membranes under various conditions, but as yet we still do not have detailed knowledge about the chemical structures of the various membrane components, and it is necessary to rely on best-fit hypothetical models (e.g., Smythies et al., 1974) for some insight into the ultimate molecular events underlying membrane conductance changes.

B. Conduction

Crustacean axons provide some interesting examples of conduction of membrane excitation. Although most crustacean (and other invertebrate) axons are "unmyelinated" and conduct action potentials by discharging the entire axonal membrane, some "giant fibers" in the nerve cords of shrimp (and possibly some giant and nongiant fibers in other crustaceans) are functionally myelinated and display saltatory conduction analagous to that seen in vertebrate myelinated axons (Kusano, 1966; Kusano and LaVail, 1971). Among other invertebrates, only earthworms have been found to possess this functional feature (Gunther, 1976).

The shrimp fibers do not have nodes of Ranvier, yet they conduct impulses at velocities of 90 m sec^{-1}. Functional "nodes" have been found at giant fiber-motor giant axon synapses and at branch points in the abdominal ganglia where the "myelin" is reduced, allowing ionic movement across the axonal membrane. This interesting example shows convergent evolution of saltatory conduction in vertebrates and crustaceans.

Recent studies on branch points of crustacean axons (Parnas, 1972) have shown that these regions are of interest as possible sites for differential channeling of impulses. In crustacean abdominal extensor muscles, a "common excitor" axon supplies one muscle (medial deep extensor, or DEAM) with a large branch, and another muscle

(lateral deep extensor, or DEAL) with a smaller branch (Parnas and Atwood, 1966). At low frequencies of excitation both branches conduct, but at high frequencies of excitation the larger branch blocks while the smaller branch continues to conduct (Parnas, 1972). Parnas (unpublished observations) believes that conduction of the smaller branch may be maintained by more rapid entry and accumulation of Ca^{2+}, which may modify conduction processes, and by more rapid activation of the Na^+ pump, which would remove K^+ lost during impulse activity from the periaxonal space, as well as extruding Na^+. These mechanisms remain to be more thoroughly examined, but it is clear that the problem is of fundamental importance in understanding the role of membrane processes in information processing by the nervous system.

A related (but controversial) finding is that of Smith and Hatt (1976), who have reported that impulses in the motor axon innervating the opener and stretcher muscles of the crayfish claw are blocked in the branch leading to the opener muscle (but not in that supplying the stretcher muscle) after several minutes of stimulation at a moderate frequency (20-30 Hz). The block is attributed to buildup of extracellular K^+ at a region of the axon surrounded by dense connective tissue which may prevent diffusion of the ion away from the axonal surface. Attempts to repeat these observations on lobster and crayfish muscles (Govind and Lang, 1977) were unsuccessful, and it is not clear at present why impulse blockage may occur in some cases but not in others.

Impulse blockade in finer axonal terminals of motor axons supplying limb muscles has been observed by Hatt and Smith (1976) in the crayfish opener muscle and by Govind and Lang (1977) in lobster claw closer muscles. The impulse blockade may occur at branch points or at bottlenecks of the terminals (Jahromi and Atwood, 1974) and occurs intermittently during high frequency activation of the motor neuron, or with two closely spaced impulses. The geometry of the terminals may contribute to the failure of conduction since branch points and bottlenecks constitute regions where the current generated by the action potential faces a relatively low impedance owing to the larger area of axonal membrane at the branch point or just beyond the bottleneck. Analyses and experimental work with such situations show that the geometrical factors contribute to the low safety factor of propagation at these regions (Parnas et al., 1976).

Similar regions of low safety factor have been studied in the dendrites of fast flexor motoneurons of the crayfish abdomen (Zucker, 1972c). Antidromic axon spikes fail to propagate actively into the dendrites, and orthodromic dendrite spikes (which normally excite the axon to fire an impulse) can be blocked by hyperpolarization or by

rapid repetitive stimulation. The points of impulse blockage are thought to correspond to the branch points of the dendrites where the safety factor for impulse propagation is lower than elsewhere (Zucker, 1972c).

A controversy has arisen over whether or not the synapse-bearing terminal regions of crustacean motor axons can conduct impulses. The original observations of Dudel and Kuffler (1961) suggested that the terminals are inexcitable since antidromic impulses were not elicited by stimulation at synaptic loci through a local extracellular microelectrode. Also, Dudel (1963) interpreted the monophasic, positive waveform sometimes recorded at such locations as an indication of decremental conduction in the terminals. More recently, Zucker (1974a) has been able to elicit antidromic action potentials by focal stimulation of the terminals, thereby rendering Dudel's (1963) hypothesis of decrementally conducting terminals less likely (see Atwood, 1976).

Although the examples above illustrate situations in which the probability of impulse conduction is lowered by certain properties of the neuron, it is worth noting that other cases have been found in which properties of the neuron are modified to favor impulse conduction. For example, certain bipolar mechanosensory neurons (Mellon and Kaars, 1974) possess a thin axonal segment near the soma (Figure 1C). Impulses initiated in the dendrites are propagated through the soma into the axon. The reduction in size of the initial axonal segment lowers the space constant of the axon at that point, thereby ensuring propagation by minimizing the resistive and capacitative load on orthodromic action potentials.

A further mechanism for ensuring propagation is found in the variable membrane properties of certain regions of neurons, particularly sensory neurons (Section VIII).

C. Axonal Differences

Although the primary mechanisms involved in production of action potentials appear to be very similar in all crustacean axons which have been carefully studied, secondary membrane differences are apparent under certain experimental situations.

Early work by Wright and his associates (Wright and Adelman, 1954; Wright and Coleman, 1954) showed that the "phasic" and "tonic" motor axons in crustacean leg nerves responded differently to a maintained depolarization: the phasic axons fired a short burst of action potentials and showed rapid accommodations, whereas the tonic axons gave a more prolonged discharge and showed less rapid accommodation.

Hodgkin (1948) originally defined three types of axonal firing response to prolonged depolarization, and his classification has been

followed by more recent workers (Chapman, 1966; Connor, 1975). *Class I* axons give prolonged repetitive discharges over a wide range of frequencies, *class II* axons give repetitive discharges over a restricted frequency range, and *class III* axons give only a short burst of impulses. Class III axons show a higher critical threshold for firing than do the others [3] 20-30 mV versus 10-15 mV) and a lower membrane resistance (Chapman, 1966). Hodgkin (1948) and Chapman (1966) noted that these differences may have been introduced by variations in the condition of the axons rather than by inherent membrane variations of the axons themselves. However, the results of Wright and Adelman (1954) and of Connor (1975) suggest that neuronal variation may also occur. This viewpoint receives additional recent support from the work of Wiens and Atwood (1976), who recorded from the intraganglionic regions of phasic and tonic motoneurons supplying the crayfish claw closer muscle and found that identical synaptic input to the two neurons would cause the tonic neuron to fire, while the phasic neuron remained silent or fired only a short burst. Clearly, differences in firing threshold and accommodation occur in these two neurons. Similar features have been reported also in "fast" and "slow" insect motoneurons (Burrows and Horridge, 1974).

Some of the membrane factors involved in bringing about the differences in firing patterns have been analyzed by Chapman (1966) and Connor (1975). Both authors were concerned with the reasons for the differences between the repetitive responses of the crustacean axons and the predictions of these responses generated by the squid axon-based Hodgkin-Huxley (1952) equations. One factor of importance in explaining the difference seems to be that restoration of normal membrane resistance following the action potential occurs more rapidly in crustacean axons than it does in squid axons (Section VI.A). In addition, lengthening of the relative refractory period during a repetitive response was related to the effects of membrane potential on restoration processes; depolarizing current was effective in slowing these processes (Chapman, 1966).

The recent voltage clamp study of Connor (1975) has produced additional observations on membrane differences. In particular, the outward current in oscillatory axons (class I and class II) observed during voltage clamp was found to have two components: a transient phase I_A not present in squid axons, and a more slowly developing prolonged phase I_K, similar to the voltage-activated potassium conductance of squid axons. I_A appeared at more negative membrane potentials than I_K and appeared similar to transients observed in molluscan central neurons (Nehre, 1971; Connor, 1975). I_A is inactivated at large positive holding membrane potentials, whereas I_K is not. Thus, I_A shares features with the voltage-dependent Na^+ conductance.

In high $[K^+]$ saline, I_A can still be demonstrated, but the direction of current is reversed and, therefore, I_A is probably carried by K^+.

The presence of I_A in axons giving repetitive responses can affect the firing pattern of the axon during maintained depolarization (Connor, 1975). For example, during a suprathreshold stimulus, I_A would be activated following the first spike, delaying the onset of the next, but progressive inactivation of I_A would allow subsequent spikes to occur at shorter intervals, speeding up the discharge rate. In axons where I_A is absent (class III, or depolarized class I axons), interspike intervals remain the same.

Although detailed analysis of repetitive firing has been carried out mainly in easily isolated walking leg axons, some studies have been made of the responses of interneurons to applied DC currents (Biederman, 1964) and of the mechanisms of impulse pattern generation in the interneurons (Takeda and Kennedy, 1965) and in motoneurons (Gillary and Kennedy, 1969; Smith, 1974; Tatton and Sokolove, 1975). These studies show that membrane variability is likely normally present in various types of crustacean neurons, and that the mechanisms elucidated for peripheral axons may well play a part in the overall performance of central neurons.

A final observation of interest in a comparison of axonal properties concerns the changes in action potentials of "phasic" and "tonic" motor axons during prolonged repetitive stimulation (Takeda, 1976). At rates of stimulation of 10-25 Hz, the conduction velocity of the action potential gradually decreases and its maximum rate of rise diminishes. In part, these changes may be brought about by accumulation of Na^+ ions during the action potential. However, the changes occur more rapidly in the phasic axons which are slightly larger, and reversal of the changes following stimulation is not significantly different in the two axon types. Unless more Na^+ enters per impulse in phasic axons, while extrusion rate by the Na^+ pump is the same, it is difficult to account for these observations solely on the basis of Na^+ accumulation. Instead, it seems possible that phasic axons may show some type of slowly developing inactivation to a greater extent than tonic axons. Recent observations on other systems have indicated the existence of "slow" inactivation in addition to the classic Hodgkin-Huxley (1952) inactivation described in squid axons. For example, Rudy (1975) has described a slow recovery of Na^+ conductance inactivation in *Myxicola* giant axons, and Fox (1976) has described an "ultraslow" inactivation of Na^+ conductance at the nodes of Ranvier in frog myelinated nerve axons. The existance of such effects should alert us to the possibility that progressive changes may take place in the membranes of crustacean axons during prolonged activity.

D. Adaptive Changes

The membrane mechanisms responsible for the action potential are not immutable. Within an individual neuron, membrane properties may respond to specific challenges by adaptive functional adjustments. The biochemical basis for such adjustments remains to be worked out, but descriptions of changes in neuronal properties exist for several different experimental manipulations.

One of these is transection of a peripheral axon. Pitman et al. (1972a,b) showed that, in cockroaches, transection of a peripheral nerve was followed by increased excitability of motoneuron somata, which become able to generate full-fledged action potentials. In caryfish, transection of the third root of an abdominal ganglion (which contains only efferent axons) produces a similar increase in excitability of the soma of the motor giant neuron (T. J. Wiens and H. L. Atwood, unpublished results, 1977). This neuron normally does not give a full spike (Takeda and Kennedy, 1964) but becomes able to do so a few days after transection of the third root. This change in excitability strongly suggests the appearance of additional voltage-dependent conductance channels in the neuronal membrane. Furthermore, it suggests that the neuronal genome is responsive to the challenge imposed by transection of its axon and is able to generate new membrane proteins or proteolipids, which alter the character of the membrane in a specific region of the cell.

Another example of adaptive change concerns the alteration of the action potential at different environmental temperatures (thermal acclimation). Crustacean leg nerves (Bullock, 1955) and individual axons isolated from the leg nerves (Atwood, 1960) normally show a decrease in conduction velocity when the test temperature is lowered. However, exposure of the animal to a lowered environmental temperature for a few days before the test shifts the conduction velocity-temperature curve towards lower temperatures, so that the conduction velocity at a given test temperature is higher. This change is adaptive; the nerve axon can conduct better at a lower temperature in a cold-acclimated animal.

In earthworm giant fibers, where similar changes have been found (Lagerspetz and Talo, 1967; Talo and Lagerspetz, 1967), further analysis has shown that the faster conducting action potentials of cold-acclimated axons have a markedly faster rate of rise and fall and a shorter total duration. In addition, threshold is higher and membrane cable properties are changed; input resistance is lower at 5°C in cold-acclimated axons (Dierolf and McDonald, 1969). These observations indicate that compensatory alterations in the kinetic properties of the ionic conductance channels have occurred. Whether these are due to changes in the composition of the membrane proteins, or solely to

alterations in membrane lipids which could in turn affect the performance of protein-containing ionophores, remains to be determined. In any case, the neuronal genome operates to change the composition of the membrane in response to an environmental challenge. The change could be triggered directly by temperature, or indirectly, perhaps by a transient ionic imbalance across the cell membrane induced initially by the temperature change.

It is worth noting that, in earthworm giant fibers, the resting potential is not altered by acclimation (Dierolf and McDonald, 1969). However, in molluskan neurons the resting potential shows an acclimation effect attributable to increased effectiveness of an electrogenic pump (Merickel and Kater, 1974). Such an effect may be difficult to detect in some giant axons even if present because of their low input resistance and the resultant small contribution of the electrogenic pump to the resting potential (Carpenter, 1973). The increased effectiveness of the electrogenic pump could be due to either increased membrane leakage resistance or increased pumping rate (resulting from either more membrane pump sites or higher performance rate for each site). Changes in both membrane leakage and pumping rate have been documented for red blood cells of hibernating mammals (Kimzey and Willis, 1971a,b).

The general topic of membrane alteration in response to environmental challenge can be further explored in crustacean neurons which afford preparation amenable to many different experiments.

VII. OSCILLATORS

Many crustacean neurons are known to undergo periodic fluctuations of membrane potential which can drive patterns of activity. Well-known examples include the nonspiking oscillator neuron responsible for driving respiratory movements (Mendelson, 1971), neurons of the cardiac ganglion (Hagiwara, 1961), certain neurons of the stomatogastric ganglion (Maynard, 1972; Selverston et al., 1976), and various interneurons, some of known function (Arechiga et al., 1974) and others of unknown function (Biederman, 1964).

Among motoneurons, a sequence of types can be discerned ranging from those which fire one or a few impulses when strongly depolarized, through those which fire in pairs (Smith, 1974) or bursts (Gillary and Kennedy, 1969), to those which undergo spontaneous oscillations and act as pacemakers (Selverston et al., 1976).

Unfortunately, the membrane mechanisms which distinguish oscillating neurons from nonoscillating neurons have not been well studied in crustaceans. More on this topic has been done in molluskan neurons where more detailed studies of possible mechanisms have been carried

out. For example, the work of Junge and Stephens (1973), Meech (1972,1974) and others suggests that oscillations in certain molluskan neurons are largely controlled by delayed increase in K^+ conductance initiated by influx of Ca^{2+} during spiking activity. Thus, membrane leakage for Na^+ and/or Ca^{2+} causes depolarization, leading to spiking activity and to accentuated influx of Na^+ and Ca^{2+}, which in turn results in a hyperpolarizing, long-lasting increase in g_K that terminates the burst and suppresses impulse formation for some time after the burst. The generality of this mechanism, and its applicability to crustacean neurons, remain to be tested. It is possible that several membrane oscillator mechanisms may be found; for example, the oscillator mechanisms of nonspiking interneurons (Mendelson, 1971) may turn out to be different from those in spiking neurons such as the pacemaker motoneurons of the stomatogastric ganglion.

Recently, work on modulation of crustacean oscillators by hormones has reached an interesting phase. Neuronal activity and responsiveness (particularly in visual and movement-detecting neurons) undergoes circadian variation, with reactivity lower during "expected" night (Arechiga et al., 1974). The variation appears to be correlated with release of a hormone from the eyestalk; injections of extracts of nervous tissue from the eyestalk (and, to a lesser extent, from lower ganglia) induce the day-phase characteristics. The active principle involved appears to be a peptide (Arechiga et al., 1977). The available information strongly indicates that neuronal membrane properties, particularly in oscillators, may be under hormonal control.

VIII. SENSORY NEURONS

Membrane specialization is a prominent feature in neurons adapted for transduction of stimuli from the environment. Sensory neurons of crustaceans, by virtue of the technical advantages afforded by their relatively large size and accessibility, have contributed greatly to the elucidation of sensory transduction processes. Techniques such as voltage clamp can be applied to these neurons to study the membrane currents appearing with stimulation. In addition, the occurrence of sensory neurons responding differently to a given stimulus modality allows comparative studies of membrane function and definition of parameters which modify performance.

Among the various crustacean sensory neurons which have been investigated, the stretch-sensitive neurons of the muscle receptor organs in the abdomens of crayfish and lobsters (Figure 1D; Alexandrowicz, 1951) and the visual neurons in the simple eyes of barnacles (Shaw, 1972) have contributed most to our understanding of membrane processes. Recently, important studies have also been

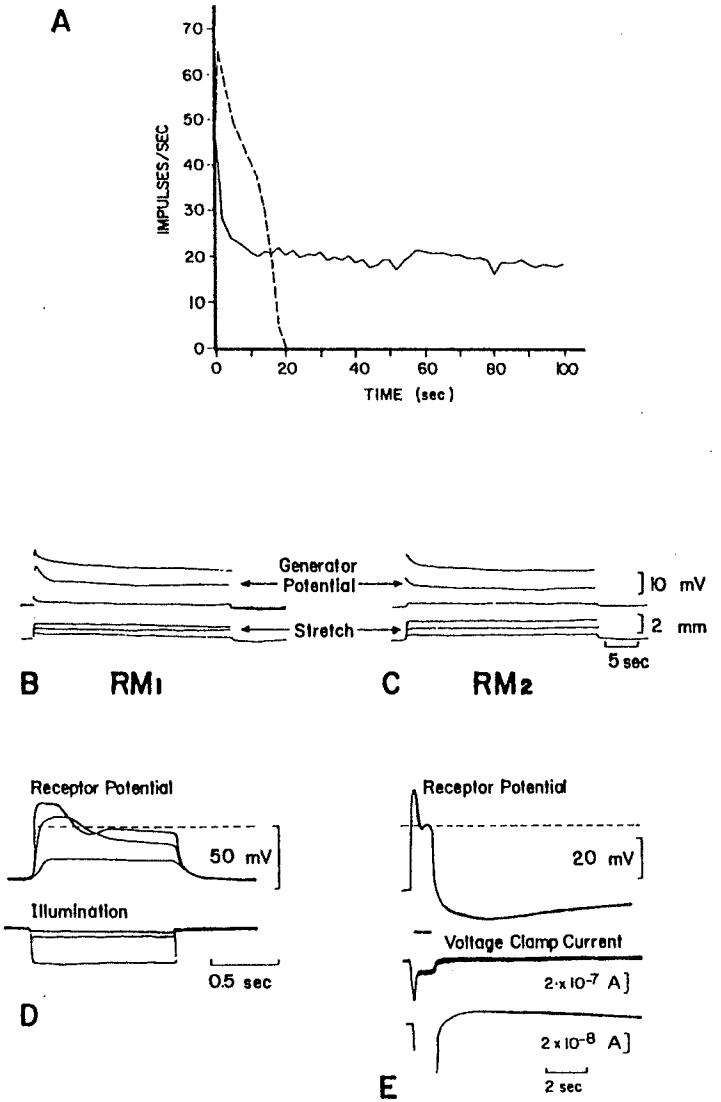
conducted on stretch sensitive neurons of coxal muscle receptors in crabs (Figure 1E; Bush, 1977). Brief reviews of the work on membrane properties of these structures will be given in this section.

A. Abdominal Stretch Receptor Neurons

Following Alexandrowicz's (1951) description of the abdominal stretch receptors, a number of physiological investigations outlined important features of performance and control. In each abdominal hemisegment, two parallel stretch receptors are found, each consisting of a large neuron attached to a thin muscle strand (Figure 1D); the neuron and the muscle strand are innervated by several efferent axons. Each neuron has its dendrites embedded in the muscle strand. One of these organs responds to stretch with a maintained discharge of impulses ("slowly adapting receptor," RM_1), whereas the other gives only a brief discharge at the beginning of the stretch ("rapidly adapting receptor," RM_2). The efferent innervation controls the sensitivity of the system. An excitatory axon regulates tension in the muscle strand, and its activity increases the firing rate of the stretch sensitive neuron to an applied stretch. Inhibitory innervation of the dendrites acts to decrease sensitivity and firing rate (Eyzaguirre and Kuffler, 1953a, b; Kuffler and Nicholls, 1976).

The early physiological investigations showed that a generator potential appeared in the dendrites of these neurons during stretch. This generator potential, if large enough, could trigger action potentials in the initial segment of the axon, which was found to have a particularly low spiking threshold. Thus, a regional membrane specialization exists in the neuron; nonregenerative, graded depolarizations are induced by stretch in the dendrites (although some regenerative Na^+ conductance increase has also been seen in the large dendrites (Washizu and Terzuolo, 1966). Transformation of this depolarization into action potentials occurs in the sensitive specialized initial segment of the axon. Subsequent studies (e.g., Nakajima and Onodera, 1969a; Ringham, 1971) have essentially confirmed the earlier observations on regional membrane specialization, although it has been shown that a slight shift (50 μm) of the impulse-initiating region closer to the soma occurs with strong depolarizations. This corresponds to an increase in voltage threshold for spike initiation during strong stimulation (Eyzaguirre and Kuffler, 1955a,b).

The neurons of the two receptors (RM_1 and RM_2) differ in voltage threshold for spike initiation (with RM_1 having a lower threshold), as well as in duration of the spike discharge during prolonged depolarization (Figure 5A; Eyzaguirre and Kuffler, 1955a,b; Nakajima and Onodera, 1969a,b).



The reasons for the physiological differences between RM_1 and RM_2 have been analyzed in elegant experiments by Nakajima and Onodera (1969a,b). With a maintained stimulus, RM_2 stops firing sooner than RM_1 (Figure 5A), and the question arises whether the site of adaptation lies either in the dendritic membrane responsible for the generator potential ("generator adaption") or in the axonal membrane responsible for spike initiation ("spike adaptation"). By inserting two microelectrodes into the soma of a stretch receptor neuron and applying constant current pulses through one while recording with the other, information on membrane properties and on spike adaptation could be obtained. Also, voltage clamping could be employed to study the kinetics of membrane currents during depolarization.

No striking differences in passive electrical membrane properties of the two neurons could be shown. However, a clear difference

Figure 5 Sensory neurons. A. Differences between slowly adapting stretch receptor (solid line) and rapidly adapting stretch receptor (dashed line) with application of constant current (approximately 35×10^{-8} A) to the spike-initiating zones of the stretch receptor neurons through external electrodes. The rapidly adapting stretch receptor is unable to maintain a prolonged discharge. (From Nakajima and Onodera, 1969a. Membrane properties of the stretch receptor neurone of crayfish with particular reference to mechanisms of sensory adaption, *J. Physiol., Lond. 200:*161-165, by permission of Cambridge University Press.) B and C. Comparison of generator potentials in slowly adapting (B) and rapidly adapting (C) stretch receptor neurons in response to constant stretches; spikes are blocked by tetrodotoxin. (After Nakajima and Onodera, 1969b. Adaption of the generator potential in the crayfish stretch receptors under constant length and constant tension. *J. Physiol., Lond. 200:*187-204, by permission of Cambridge University Press.) D. Receptor potentials of the barnacle visual neuron in response to three steps of illumination of different intensities (maximum intensity, 1.45×10^4 lux). The dotted line represents zero membrane potential. (Reprinted from *Fed. Proc. 30:*69-78, 1971. Figure 1 from Electrical characteristics of a barnacle photoreceptor, by H. M. Brown, et al.) E. Receptor potential of the barnacle visual neuron, and voltage clamp current records at low and high gains (membrane potential clamped at the resting potential during illumination). A marked postillumination hyperpolarization, associated with outward current, follows the 1-sec period of illumination (short solid line below receptor potential record). (From Koike et al., 1971.)

between RM_1 and RM_2 neuron responses to maintained constant current depolarizing pulses was shown; the slowly adapting neuron always gave a prolonged discharge and the rapidly adapting neuron a very brief one. Conversely, the time courses of the respective generator potentials, investigated after blocking action potentials with tetrodotoxin, were almost the same (Figure 5B and C; Nakajima and Onodera, 1969b). In both receptor neurons, the generator potential declines from an initial peak to a lower plateau. Experiments employing length-clamping and tension-clamping techniques suggested that about three-fourths of the observed generator adaptation could be accounted for by the viscoelastic properties of the muscle, rather than by changes in the dendritic membrane. Thus, as far as membrane specialization is concerned, the major focus of difference lies in the spike-initiating region of the axon and not in the stretch-sensitive dendrites.

It should be noted, however, that the overall performance of RM_1 and RM_2 are influenced by *both* types of adaptation. In the slowly adapting neuron, generator adaptation plays a key role, since the generator potential declines before the spike-initiating zone shows any adaptation. By contrast, spike adaptation is relatively more significant in limiting the discharge of the rapidly adapting neuron.

A number of possible factors which could be involved in spike adaptation were considered by Nakajima and Onodera (1969a). Among these were slow changes in K^+ and/or Na^+ gradients across the membrane, slow changes in Na^+ permeability, slow changes in K^+ permeability, and contribution of an electrogenic Na^+ pump to membrane potential. The first factor was ruled out by the observation that full-sized action potentials could be obtained very soon after an intense discharge initiated by a strong depolarizing stimulus. Thus, ionic gradients had not changed sufficiently during spike adaptation to alter the action potentials.

Slow *increases* in K^+ permeability, seen during moderate stimuli under voltage clamp conditions, may play a role in spike adaptation by raising the threshold for spike initiation. Very strong stimulation leads to slow *decreases* in K^+ permeability which could contribute to spike adaptation by suppressing the repolarizing phase of the action potential, thereby contributing to Na^+ inactivation.

Still another factor which could be important is the "posttetanic hyperpolarization" observed after an impulse train (Nakajima and Takahashi, 1966). This hyperpolarization was shown to be due to activation of an electrogenic Na^+ pump; it was not associated with a membrane conductance change, and it could be suppressed either by removal of Na^+ from the extracellular solution or by addition of 2,4-dinitrophenol. Also, it could be counterfeited by injection of Na^+ into the neuron. The hyperpolarizing current passed by the electrogenic

pump would act to increase the current threshold for spike initiation and could, therefore, contribute to spike adaptation during a prolonged discharge.

Nakajima and Onodera (1969a) concluded that different membrane mechanisms could contribute to spike adaptation, depending on the strength of the depolarizing stimulus. The different effects of depolarization on K^+ permeability, noted above, show one way in which spike adapting factors alter with membrane potential.

The ionic mechanisms of the generator potential have been studied over many years by several groups of investigators. A conductance increase of the dendritic membranes of both slowly adapting and rapidly adapting neurons to small cations was demonstrated by experiments involving ionic substitutions in the extracellular solution (Terzuolo and Washizu, 1962; Edwards et al., 1963; Obara and Grundfest, 1968; Obara, 1967,1968). The stretch-activated generator membrane appeared to be less selective for cations than certain other receptor membranes; many monovalent cations could be admitted, but anions (in particular, Cl^-) were excluded (Obara, 1968).

More recently, voltage clamp techniques applied to the soma (Klie and Wellhoner, 1973) and to the dendrites (Chaplain, 1975) have provided additional information. An inward current, induced by stretch, can be measured under voltage clamp when the membrane potential is clamped near its resting level (Klie and Wellhoner, 1973). A reversal potential of +25 mV was determined for this inward current. It showed adaptation during maintained stretch. It is reduced in low external Na^+ and is not much affected by tetrodotoxin. Reduction of external Ca^{2+} leads to an *increase* in the inward current coupled with a decrease in membrane resistance.

The latter observation has been extended by Chaplain (1975), using more sophisticated voltage clamping of the dendritic membrane. This author measured a reversal potential of +18 mV for the stretch-induced inward current; the more positive value obtained in the previous study (Klie and Wellhoner, 1973) can be attributed to the attenuation of clamp voltage from soma to dendrites. At rest (i.e., without stretch), a small inward current exists at clamp potentials between -50 and -80 mV, and an outward current exists at clamp potentials of -40 to +30 mV. Lowering the Ca^{2+} level in the extracellular solution enhanced the stretch-induced inward current, whereas increasing Ca^{2+} to 75-150 mM reduced it. Although Na^+ appeared to be the main ion carrying this current, a small contribution of Ca^{2+} was also detected (this is in contradiction of Obara's (1968) earlier conclusions in which a role for Ca^{2+} was denied).

A role for Na^+ pump activation in the decline of the generator potential was supported by experiments employing ouabain, Li^+, or

zero K^+ solutions. These treatments inhibited hyperpolarization, especially upon release of tension.

A role for Ca^{2+} in transduction was postulated on the basis of the observations relating to the Ca^{2+} effect on the stretch-induced inward current. Initially, the mechanical deformation of the membrane may lead to Ca^{2+} binding to proteolipids, and the initial Ca^{2+} uptake may then lead to alteration of ionic channels in the membrane, producing the inward current underlying the generator potential. Thus, Ca^{2+} could play an important part in controlling conductance channels for other ions. This proposal is in line with other work relating to the role of Ca^{2+} in controlling membrane permeability (Frankenhaeuser and Hodgkin, 1957; Moore, 1971; Inoué et al., 1973).

Among other recent findings of interest, studies by Sjolin and Grampp (1975) on "membrane noise" in the slowly adapting stretch receptor neuron are particularly noteworthy. Membrane noise can be detected as current noise under voltage calmp. It increases with membrane depolarization and appears to be generated by the Na^+ channels of the membrane, since it is blocked by tetrodotoxin or by removal of extracellular Na^+ and is unaffected by the K^+ channel blocker, tetraethylammonium chloride. Analysis of the spectral density function and the covariance of the noise indicated that a rather low degree of Na^+ inactivation occurred at membrane potentials close to firing threshold. The soma and the adjacent axon both contribute to the subthreshold membrane current (Grampp and Sjolin, 1975).

In the rapidly adapting neuron, the membrane noise was absent. Therefore, a fundamental difference between the two cell types may be the degree or rate of inactivation of the sodium conductance system. The low degree of inactivation in the slowly adapting neuron provides for a subthreshold steady-state Na^+ conductance and subthreshold membrane current (Grampp and Sjolin, 1975) which can act to maintain repetitive firing by providing continuous inward current during depolarization.

These observations indicate approaches to comparative studies of membrane performance in particularly favorable crustacean neurons.

B. Coxal Muscle Receptors

Recently, another crustacean mechanoreceptor system, the coxal muscle receptor of crabs, has appeared as an important preparation for the study of receptor membrane properties (Ripley et al., 1968; Bush and Roberts, 1968,1970,1971; Roberts and Bush, 1971; Bush, 1977).

Two neurons (S and T neurons) are associated with a small receptor muscle in this system (Figure 1E). The T neuron has dendrites

embedded in the tendon of the receptor muscle and is activated either by stretch of the muscle or by tension developed in the tendon; it occurs essentially in series with the muscle. The S neuron is attached to elastic strands flanking the proximal region of the receptor muscle and, thus, is located in parallel with the proximal region of the muscle.

Both neurons travel to the thoracic nervous system where they elicit motor reflexes when activated (Bush, 1977). Unlike the abdominal stretch receptors, neither is directly innervated by efferent neurons, but efferent control of receptor response is achieved through the motor innervation of the receptor muscle itself.

Stretching the dendrites of these neurons elicits a receptor potential rather similar in shape to those seen in many other receptors, including the abdominal muscle receptors (Figure 5B and C). A large initial "dynamic" component, and a subsequent maintained "static" component, appear. Unlike the neurons of the abdominal muscle receptors, neither the S nor the T neuron generates conventional action potentials. Instead, the receptor potential itself is conducted passively along the neuron into the ganglion, where it is effective in recruiting motoneurons, probably monosynaptically. The passive conduction is facilitated by the relatively large diameters (50-70 μm) and long length constants (2-15 mm) of these neurons (Roberts and Bush, 1971; Bush, 1977). The length constants in various crab species are roughly equivalent to the total conduction distances.

Differences in the receptor potentials of S and T neurons to an applied stimulus have been analyzed by Bush and Roberts (1971) and attributed to the different mechanical couplings of the stretch-sensitive dendrites to the receptor muscle and associated elastic strands.

In some preparations, an abortive spike appears during, or shortly after, the dynamic phase of the receptor potential (Roberts and Bush, 1971), but such responses are not actively conducted along the neuron and do not appear to contribute significantly to the reflexes (Bush, 1977).

The ionic mechanisms of the receptor potentials and abortive spikes have been analyzed in experiments involving ionic substitutions, applications of drugs, and voltage clamping. Tetrodotoxin abolishes the abortive spikes, if present, but has little effect on the receptor potentials. However, in sodium-free solutions, the receptor potentials are reduced to about 30% of their initial values. In addition, voltage clamp experiments show a reversal potential of about +30 mV for the receptor potential. Taken together, the observations suggest that the receptor potential is generated by a membrane conductance increase for Na^+ and, to a lesser extent, Ca^{2+}, and that the conductance channel is distinct from that involved in the action potential mechanism.

The resting membrane potential is K^+-sensitive but shows a dependence of only 30 mV per decade change in external K^+, indicating that other ions are involved in this potential (Roberts and Bush, 1971). Part of the resting potential is contributed by an electrogenic pump (Bush, 1977).

It is likely that some of the variation in membrane properties of these neurons encountered in different preparations can be attributed to hormonal conditions within the animal, or to environmental effects (Roberts and Bush, 1971). This problem awaits further investigation.

C. Photoreceptors

Recently, sophisticated experiments on photoreceptor neurons in the lateral eyes of barnacles, *Balanus amphitrite* and *Balanus eburneus*, have provided detailed information on membrane processes involved in coupling between activated receptor photopigment and neuronal electrical events. Parallel studies on photoreceptor neurons in another simple arthropod system, the "ventral eye" of *Limulus*, have also been very productive and will be mentioned here for comparative purposes.

The barnacle's lateral eye consists of three neurons, each of which possesses numerous short dendrites and a single long axon projecting from the soma (in the eye) to the supraoesophageal ganglion. The dendrites are the site of the light-absorbing part of the system; numerous microvilli, presumably well-endowed with photopigment molecules, occur on them. The three cells are known to be coupled together through electrical junctions (Brown et al., 1971; Shaw, 1972).

An interesting feature of these neurons is that, as in the coxal muscle receptors of crabs, the receptor signals are conducted decrementally along their axons to the supraoesophageal ganglion, where reflexes are set up. This situation has been well analyzed by Shaw (1972), who was able to record from, and to mark with fluorescent dyes, all of the receptors. During illumination, a depolarization is set up in the receptors, but no impulses can be recorded either in their somas or in their intraganglionic axonal terminations. The nonimpulsive depolarization is recorded decrementally along the axon, and the event recorded intracellularly from the axon terminal is attenuated (over a distance of about 1 cm) to about one third the value seen in the soma. It appears that the axons have an unusually high membrane resistance (about 10^5 Ω cm^2, see Table 1), which permits efficient cable conduction of the visual signal along the nonspiking axon.

Normally, the visual signal from either the lateral eye (Shaw, 1972) or the median eye (Millecchia and Gwilliam, 1972) evokes impulses in second-order or higher order cells of the ganglion upon

termination of the depolarizing receptor potential, or upon hyperpolarization of the receptor from a depolarized condition. In the intact animal, this "off-response" impulse activity generates a "shadow reflex" in which the barnacle withdraws its cirri and closes its opercular plates (Millecchia and Gwilliam, 1972). It has been proposed that the illuminated and depolarized receptors release gamma-aminobutyric acid (GABA) from their terminals, thereby inhibiting the ganglionic interneurons. The darkened and hyperpolarized receptors stop releasing GABA, whereupon the ganglionic neurons depolarize and fire impulses, initiating the "shadow reflex" in lower motor centers.

Shaw (1972) showed that membrane potential changes applied to the receptor soma by means of an intracellular microelectrode were as effective as the illumination-induced visual signal in setting up impulse activity in the postsynaptic neurons. Thus, the conducted electrical change-induced light, and not some other light-induced process, is the effective visual signal.

The electrical properties of the receptor's soma have been examined in studied employing voltage clamp (Brown et al., 1969, 1970) and measurements of intracellular ionic activities with ion-sensitive microelectrodes (Brown, 1976). The latter studies, though more recently performed, are helpful for interpretation of the former.

In the resting condition, the average intracellular ionic activities of K^+, Na^+, and Cl^- in photoreceptors of *Balanus eberneus* were 120, 28, and 65mM, respectively. The corresponding Nernst equilibrium potentials were E_K, -77 mV; E_{Na}, +64 mV; and E_{Cl}, -42 mV. The average resting potential of these cells was -41 mV. From this it is apparent that the resting potential at normal external K^+ levels is more positive than E_K and coincides with E_{Cl}.

Ionic dependence of the resting potential (E_m) was studied by subjecting the cells to solutions of altered ionic composition. When K^+ was increased at the expense of Na^+, the photoreceptors depolarized, and the membrane potential changed 43 mV for a tenfold change in external K^+. The ratio of membrane sodium permeability (P_{Na}) to membrane potassium permeability (P_K) was calculated from a version of the Goldman equation

$$\exp \frac{E_m F}{RT} = \frac{\alpha M}{a_k^i} + \left(\frac{1 - \alpha}{a_k^i} \right) a_K^o$$

where α is the ratio $P_{Na}:P_K$, a^i and a^o are internal and external ionic activities, $M = a_{Na}^o + a_K^o$, and F, R, and T are as defined previously (Section IV).

This equation is based on the assumptions that a_k^i remains constant for all concentrations of external K^+, that a_k^i can be determined from the relation between E_m and a_K^o, and that P_{Cl} is very small relative to P_K and P_{Na}. The use of ion-selective microelectrodes to measure a_K^i, a_{Na}^i, and a_{Cl}^i directly showed that these assumptions were not valid; a_K^i increased as external K^+ was increased, values of a_K^i obtained from the membrane potential versus log a_K^o curve were overestimates of the true a_K^i, and P_{Cl} was found to be greater than P_{Na}. Thus, estimates of α based on the above equation were on the order of 0.1, but estimates of α based on the measurements obtained with ion-selective electrodes were on the order of 0.04 at normal resting potential. Cl^- permeability appeared to be about one-third of K^+ permeability, and the distribution of Cl^- appeared to be passive, so that $E_{Cl} = E_m$, as in frog muscle fibers (Hodgkin and Horowicz, 1959). The experiments indicate that the Goldman equation must be used with caution for estimates of P_{Na}, and that better agreement between E_m and E_K than expected from the E_m versus log a_K^o relation obtains when the directly measured values of a_K^i are used to calculate E_K.

The above measurements were made in dark-adapted photoreceptors. The illumination-induced receptor potential attains inside-positive membrane potentials with bright flashes of light, and a significant increase in a_{Na}^i can be demonstrated with prolonged exposure. From this data it is apparent that the receptor potential involves an increase in membrane Na^+ conductance.

More detailed information on the kinetics and reversal level of the receptor potential has been provided by voltage clamp studies (Brown et al., 1967,1970). The receptor potential consists of a depolarizing phase which has a pronounced transient peak at high light intensities, followed by a slow hyperpolarization (Figure 5D and E). The hyperpolarizing phase is produced by an outward current of low density unaccompanied by any significant change in membrane conductance (Koike et al., 1971). This hyperpolarization is abolished by ouabain (10^{-5} M), is reduced by removal of external Na^+ or K^+, and can be duplicated by an intracellular injection of Na^+. All of these features indicate rather strongly that the hyperpolarizing phase of the receptor potential (which, as noted previously, is important in regulating synaptic transmission to second-order ganglionic neurons) represents

an increase in activity of an electrogenic Na^+ pump, activated by Na^+ influx during the depolarizing phase of the receptor potential. This conclusion is reinforced by the observation that postillumination hyperpolarization can sometimes attain a more negative potential than either E_K or E_{Cl} (Brown, 1976).

Voltage clamp studies on the depolarizing phase of the receptor potential showed a well-defined inward current which reversed when the membrane potential was clamped at an inside-positive value of about +25 mV (Brown et al., 1969,1970). From the current-voltage relationships seen under voltage clamp, it was found that membrane conductance was increased with illumination and declined with time after illumination. These changes in membrane conductance can account for the time-dependent and illumination-dependent changes in the shape of the receptor potential.

The above results showed that the depolarizing phase of the receptor potential was satisfactorily explained by light-induced membrane conductance changes, and not, as suggested earlier for the *Limulus* ventral photoreceptors (Smith et al., 1968a,b), by conductance-independent changes in pumping activity of an electrogenic Na^+ pump. Indeed, more recent work on the *Limulus* photoreceptor (Millecchia and Mauro, 1969a,b) has shown that it also produces a receptor potential generated by a membrane conductance change.

As to the ionic mechanism of the depolarization, experiments in altered external Na^+ indicated that the reversal potential for light-induced current was shifted to more negative values with Na^+ reduction. Thus, a major part of the depolarizing current is carried by Na^+. However, in very low external [Na^+], alterations of external [Ca^{2+}] had a significant effect on the reversal potential, indicating some contribution of Ca^{2+} to the light-induced depolarizing current. Comparison of the normal reversal potential (+25 to +27 mV) with E_{Na} (+64 mV) shows considerable disparity and indicates participation of another ion with a more negative equilibrium potential.

Reducing external Ca^{2+} when external Na^+ was present gave rise to a larger light-induced current with little change in reversal potential (Brown et al., 1970). From this observation it appeared that Ca^{2+} is normally effective in suppressing part of the light-induced Na^+ current. The relationship between membrane current and external Ca^{2+} concentration is a rectangular hyperbola, suggesting that the Ca^{2+} mechanism of action could involve competitive binding to a membrane site or carrier responsible for the conductance change (Brown et al., 1970). More recently, Brown and Ottoson (1976) have provided evidence for competition between Ca^{2+} and K^+ at membrane sites involved in light-induced current; when K^+ is absent, the effect of Ca^{2+} in suppressing this current is much stronger.

A further study on barnacle photoreceptors is concerned with membrane potential changes induced by light of different wavelengths (Brown and Cornwall, 1975a,b). Red light at a high intensity induces a depolarization which persists in darkness; this "quasistable" depolarization is termed "latch-up" and occurs in *Limulus* photoreceptors as well as in barnacles (Nolte et al., 1968). It can be terminated by green light. Voltage clamp methods and ionic substitution experiments showed that the membrane mechanism underlying the effect involved a membrane conductance increase similar to that normally appearing with white-light illumination. The major difference between latch-up and normal light-induced depolarization was in persistance of the former.

Studies on absorbance of single cells at different wavelengths, and on the action spectrum for the latch-up phenomenon, suggested that two photopigments may be involved in the induction and termination of latch-up, one absorbing light with wavelengths of 540 nm or greater (induction), and the other absorbing at 480 nm (termination). There was no evidence for conversion of one pigment to the other during illumination as proposed in one model of the latch-up phenomenon in *Limulus* photoreceptors (Hochstein et al., 1973). Work on these lines represents an interesting approach to investigation of the role of photopigments in production of membrane conductance changes.

Receptor potentials of another large arthropod photoreceptor, the "ventral eye" of *Limulus*, are similar in many respects to those of the barnacle's eye. If we temporarily allow honorary crustacean status for *Limulus*, some of the recent results obtained with its photoreceptor can profitably be used to supplement the discussion of the barnacle photoreceptor.

There have been two hypotheses advanced to explain the depolarizing receptor potential (Brown et al., 1967) of the *Limulus* photoreceptor: (1) a light-induced increase in membrane conductance, primarily for Na^+ (Millecchia and Mauro, 1969a,b), and (2) a light-induced change in activity of a Na^+ pump (Smith et al., 1968a,b). The latter hypothesis has now been abandoned and it appears that the mechanism of the *Limulus* receptor potential is essentially the same as in the barnacle.

A hyperpolarizing after-potential occurs in the *Limulus* photoreceptor, and its mechanism appears to be the same as in the barnacle—the increased activity of an electrogenic sodium pump which transports outward current across the membrane (Brown and Lisman, 1972).

Of particular interest are studies on the *adaptation* of the photoreceptor to repeated or continuous illumination. A prolonged adapting light stimulus leads to marked reduction in sensitivity of the receptor to a test flash (Figure 6). Since the receptor potential involves an influx of Na^+ (and to a lesser extent, Ca^{2+}), one possible mechanism for

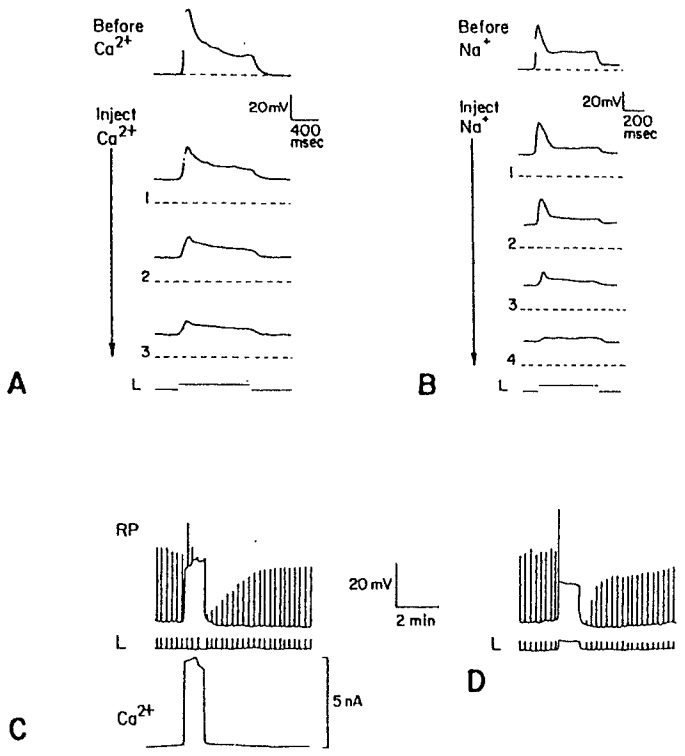


Figure 6 Sensory neurons. A and B. Intracellular injections of Ca^{2+} (A) and Na^+ (B) depress the receptor potentials of *Limulus* ventral photoreceptor evoked by a flash of light (L). The records show a progressive change with time after injection in each case; the changes were reversible when injection was stopped. The dotted line represents the resting potential. (From Lisman and Brown, 1972.) C and D. Comparison of injection of Ca^{2+} (C) and steady illumination (D) on receptor potentials of a *Limulus* ventral photoreceptor evoked by brief flashes of light. Light stimuli are indicated by L; receptor potentials by RP; and Ca^{2+} injection current by Ca^{2+}. (From Brown and Lisman, 1975a.)

the adaptation could be an accumulation of intracellular Na^+ and/or Ca^{2+}. This possibility has been explored by injecting various ions into the receptor iontophoretically and observing the resulting changes in sensitivity to test flashes of light. These experiments have shown that injection of Ca^{2+} mimics light adaptation; that is, the receptor potential generated by a test flash declines in amplitude (Figure 6). Furthermore, injection of Na^+ also leads to a decline in sensitivity, provided extracellular Ca^{2+} is present (Lisman and Brown, 1972). Injections of K^+ and Li^+ are ineffective, as is injection of Na^+ when the photoreceptor is bathed in a medium containing very low $[Ca^{2+}]$. The higher the external $[Ca^{2+}]$, the more rapid and pronounced the desensitization of the receptor potential becomes. Injection of Ca^{2+} chelators decreases the light-induced changes in sensitivity (Lisman and Brown, 1975b). Inhibition of the Na^+ pump (which leads to a buildup of Na^+) also leads to desensitization, provided external Ca^{2+} is available (Brown and Lisman, 1972).

These results, taken together, lead to an hypothesis for light adaptation based on intracellular Ca^{2+} levels. Increased intracellular $[Ca^{2+}]$, whether produced directly by iontophoretic injection, indirectly by injection of Na^+, or by illumination, leads to a decreased membrane conductance change for subsequent test flashes of light. The membrane current induced by a given amount of light declines with light adaptation or injection of Ca^{2+} (Lisman and Brown, 1975a, b). During illumination, some of the increase in the desensitizing intracellular Ca^{2+} may come from Ca^{2+} entry through light-activated membrane conductance channels, and some may gradually accumulate as a result of decreased transmembrane Na^+ gradient, owing to higher intracellular $[Na^+]$. The latter effect is known in squid axons, where it has been shown that increased intracellular Na^+ leads to a rise of intracellular Ca^{2+} (Baker et al., 1969) through an effect on the Na^+/Ca^{2+} exchange mechanism, or via the "Ca^{2+} pump" (reviewed by Baker, 1972; Blaustein, 1974).

Most recently, additional experiments on an even finer scale have been reported which support the hypothesis. Local illumination of the photoreceptor membrane with an adapting light stimulus leads to adaptation of the membrane response to a test flash in that region, but not at unilluminated regions of the membrane. Local injection of small amounts of Ca^{2+} similarly leads to membrane desensitization at the site of injection, but not at other regions of the same cell. Thus, intracellular gradients of Ca^{2+} can occur over small (50-60 μm) distances and may be important as an intracellular signal (Fein and Lisman, 1975).

Injection of Ca^{2+} also decreases the latency of response to test flashes, as does an adapting illumination. Thus, the time scale of

response appears to be modulated by intracellular Ca^{2+}, as is the sensitivity (Brown and Lisman, 1975).

Parenthetically, it can be noted that photopigment bleaching may not be involved in these adaptation effects in *Limulus* photoreceptors, although this mechanism operates in vertebrate photoreceptors.

The calcium mechanism for modulation of sensitivity and time scale of response in *Limulus* photoreceptors appears well founded and is of interest in connection with other physiological processes such as synaptic transmission (Section IX). Recent work on other photoreceptors, such as those in eyes of insects (Bader et al., 1976), has also shown similar features. Further work may reveal more details of the way in which calcium produces its effects on the photoreceptor membrane.

IX. SYNAPTIC MEMBRANES

Most neurons make synaptic connections with other neurons, or with muscle cells (Figure 1). Some are neurosecretory, releasing chemicals into the haemolymph at nonsynaptic locations (Evans et al., 1976; Kravitz and Battelle, 1976). An important feature of chemically transmitting synapses is specialization of the neuronal presynaptic membrane for transmitter release. In addition, the postsynaptic membrane is specialized for reception of the chemical transmitter and possesses appropriate receptor proteins. In crustaceans, work on neuronal postsynaptic membranes has been very limited, but observations on presynaptic mechanisms have advanced through the study of the peripheral synapses of motoneurons. Some of the main features of neuronal presynaptic membranes will be reviewed here.

Currently, it is believed that some of the synaptic membrane specializations, particularly those involved in electrically mediated changes in membrane calcium conductance, occur at most, and perhaps at all, chemically transmitting synapses. It is worth noting that most of the crucial evidence for this belief comes from study of relatively few neurons; the frog neuromuscular synapse and the squid giant fiber synapses have received the most attention. The fact that most chemical synapses show similar physiological responses to certain experimental manipulations, e.g., reduction of external Ca^{2+}, is evidence to support the belief in communality of mechanism.

Certain crustacean synapses, particularly those of the neuromuscular systems, have been quite well studied and were the subject of a recent review (Atwood, 1976). Some of the important features of these synapses will be recounted briefly here.

Some crustacean neruons are thought to release transmitter substance when an action potential arrives at the terminal, whereas others

provide transmission in response to nonspiking membrane potential changes (Maynard and Walton, 1975). Although details of the latter type of transmission have not been completely elucidated, voltage and calcium dependence of transmitter release are similar to that of spike-induced transmission (Graubard, 1975), and it is likely that presynaptic membrane mechanisms are similar in the two types of neuron.

For many years it was thought that crustacean motor neurons did not conduct a propagated action potential into the synapse-bearing terminals (Dudel, 1963). More recently, Zucker (1974a,c) has shown that antidromic action potentials can be elicited at synapses by direct stimulation through a microelectrode, and that excitability changes indicative of a depolarizing after-potential can be demonstrated immediately after a motor impulse. It now appears that a "hybrid" situation of the type envisioned by Dudel, namely, spike-conducting axon and nonconducting terminal, probably does not occur in crustacean motor axons. However, as mentioned previously (Section VI.A), spike-conducting membrane of terminals may differ from that of the main axon.

The recent work of Llinas et al. (1976) on the giant synapse of the squid has provided a model for membrane mechanisms of transmitter release. In this recent model, it is proposed that voltage-controlled membrane Ca^{2+} channels are composed of five singly charged subunits, all of which have to undergo deformation in an electrical field, changing noncooperatively from an inactive to an active form. The model is based on curves obtained by voltage-clamping the presynaptic terminal. These curves showed an S-shaped relationship between presynaptic potential and transmembrane Ca^{2+} current and showed a linear relationship between Ca^{2+} current and transmitter release. The implication of this model is that only one Ca^{2+} has to cross the membrane for each quantum of transmitter substance released by a nerve impulse. This idea is at variance with earlier models proposed by Dodge and Rahaminoff (1967), and by Katz and Miledi (1968), in which three or four Ca^{2+} were thought to act cooperatively in release of a single transmitter quantum.

In crustaceans, variable results for Ca^{2+} dependence of transmitter release have been obtained. A linear relationship between external Ca^{2+} and transmitter release has been described in the crayfish opener muscle (Bracho and Orkand, 1970); however, there is a possibility that the presence of presynaptic inhibition, and of tonic release of inhibitory transmitter substance, could modify Ca^{2+} dependence at this synapse, as it appears to at certain synapses (Parnas et al., 1975). This point requires clarification before any definite model for crustacean synaptic release mechanisms can be formulated.

Closely related to the problem of transmitter release is that of facilitation. Short-term facilitation, seen after one of a few impulses and resulting in augmented transmitter release, decays with a time course of a few seconds (in one case, facilitation after a single impulse can still be detected after 10 seconds: Atwood et al., 1977). The idea that facilitation is due to a residue of Ca^{2+} crossing the membrane after a single impulse is not sustainable if the relationship between Ca^{2+} and transmitter release is linear. Another idea, that membrane Ca^{2+} conductance is augmented by preceding impulse (Zucker, 1974b), remains viable but requires supporting evidence. Facilitation of Ca^{2+} entry has in fact been observed in molluskan neurons (Stinnakre and Tauc, 1973).

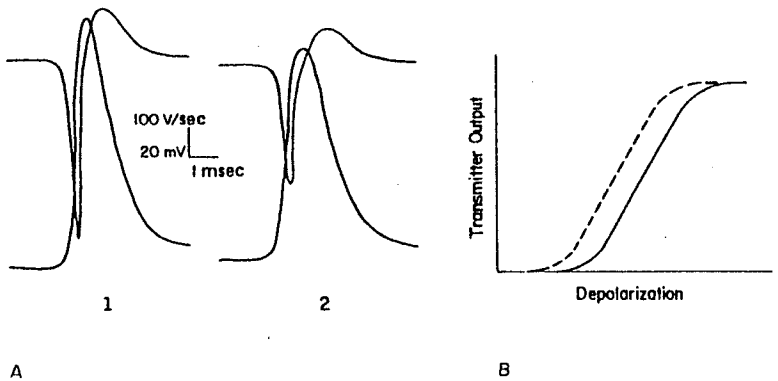
Long-term facilitation, which builds up over several minutes of stimulation and persists for minutes to hours (Sherman and Atwood, 1971), has been related to gradual accumulation of Na^+ in the nerve terminal (Atwood et al., 1975). Kinetically, long term facilitation in crustaceans has much in common with "potentiation" and "augmentation" seen in frog neuromuscular synapses (Magelby, 1973a,b). Accumulation of Na^+ in nerve terminals has an effect on the shape of the nerve action potential, making it broader and of smaller amplitude (Figure 7A). However, it is not likely that this effect by itself would lead to more transmitter output (Atwood, 1976). Accumulation of Na^+ could lead secondarily to elevated Ca^{2+} through an effect on membrane Na^+-Ca^{2+} exchange mechanisms (Blaustein, 1974). This possibility is illustrated in Figure 7C and is reminiscent of the mechanism described previously for light adaptation in the *Limulus* photoreceptor (Section VIII.C).

Recent experiments on squid synapses have indicated that injection of Na^+ directly into the presynaptic terminal shifts the depolarization versus transmitter release curve toward lower depolarizations (Fig. 7B; Charlton and Atwood, unpublished results). It has not yet been established whether Na^+ injection has an effect on kinetics of membrane Ca^{2+} channels or only on the Na^+-Ca^{2+} exchange mechanism.

It seems likely, from the differences in facilitation and long-term facilitation seen at different synapses, that subtle variations in synaptic membranes are involved. Crustacean synapses provide excellent material for investigation of these differences.

X. CONCLUSION

In this comparative survey of crustacean neuron membranes, one of the main themes has been neuronal diversity. A large number of different neuronal types has evolved in crustaceans, leading to membrane specializations appropriate for particular functions. Of equal interest



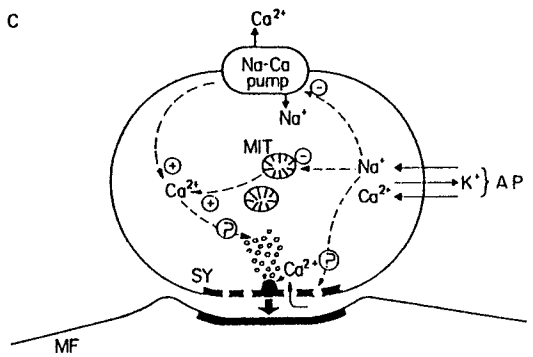


Figure 7 Possible mechanisms of long-term synaptic facilitation. A. Changes in action potential of a crab motor axon brought about by repetitive stimulation. Lower traces, action potential; upper traces, the derivative of the action potential. Record 1 was taken at the beginning of stimulation, and record 2 was taken after 10 min stimulation at 15 Hz. The action potential has declined in amplitude and broadened; the maximum derivative is reduced. The changes are due in part to Na^+ loading. Postsynaptic potentials of crustacean neuromuscular synapses are markedly enhanced (long-term facilitation) by this type of stimulation. (After Takeda, 1976.) B. Release of transmitter as a function of membrane potential in a squid giant synapse before (solid line) and after (dashed line) injection of Na^+. More transmitter is released by an equivalent depolarization after the injection, indicating that Na^+ buildup is a mechanism for long-term facilitation. (Charlton and Atwood, unpublished experiment.) C. Model for long-term facilitation at a crustacean nerve terminal. Entry of Na^+ and Ca^{2+}, and exit of K^+, occurs during the action potential (AP); a high level of intracellular Na^+ may act to prevent uptake or cause release of Ca^{2+} by mitochondria (MIT), interfere with extrusion of Ca^{2+} by the Na^+ -Ca^{2+} membrane pump, or alter the kinetics of synaptic (SY) calcium channels. Increased intracellular Ca^{2+} could bind to synaptic vesicles or to the synaptic membrane, increasing the probability of transmitter release onto the muscle fiber (MF).

is that different membrane regions of individual neurons are specialized in having different assortments of ionic channels and, hence, different electrical properties. In addition, it appears that membrane properties can rapidly change, as evidence by hormonal effects and thermal acclimation (Section VI.D).

Many of the general features found in crustacean neuronal membranes can be found also in neuronal membranes of other animals. For example, membrane transport systems for Na^+ and for Ca^{2+} appear to be similar in many organisms (Blaustein, 1974). The electrically operated membrane channels for Na^+ and for Ca^{2+} also appear generally similar in many organisms, although the relative numbers of the different channels vary considerably from one neuron to another and at different places in a single neuron. The receptor potentials of crustacean sensory neurons (Section VIII) may be similar to those in many other animals.

It is the subtle variations in membrane performance from one neuron to the next, and from one location to another within a neuron, that attracts particular interest in crustaceans. The classic studies of Wiersma (1961) clearly showed the individuality and uniqueness of crustacean neurons. This concept has been extended to other invertebrate groups (Hoyle, 1975) and may also apply to vertebrates (Bullock, 1975).

What determines the membrane properties of different neurons and of different neuronal regions? One important factor appears to be interactions with neighboring cells. In vertebrates, it is well known that motor nerves exert an important influence on the muscle fibers they supply. This acts to localize the membrane acetylcholine receptors manufactured by the muscle genome at the end plate region (Guth, 1968; Fambrough, 1970). In crustaceans, motor axons severed from the cell body can survive for many months, partly because of an influence of the neighboring glial cells which can supply them with maintenance materials (Bittner and Mann, 1976). The motor neurons, in turn, appear to be able to maintain the integrity of the muscle fibers (Atwood et al., 1973). Also, neurons connected by electrical synapses can transfer materials to each other (Herman et al., 1975) and promote mutual survival (G. Bittner, unpublished results). Thus, there are many cases known in which membrane properties and other cellular functions are influenced by adjacent cells. Interactions of some sort at the cellular level probably play an important role in membrane differentiation and in maintenance or alteration of the established membrane properties.

It is conceivable also that the ionic fluxes across the membrane may have an interactive influence on protein synthesis, known to be locally active in crustacean nerves (Sarne et al., 1976), or on genetic expression which is responsive to ion levels in at least some animals (Lezzi, 1970). Feedback loops could thereby be established which would serve to maintain or to produce alterations in steady state of membrane composition. For example, perturbation of Na^+ transport by lowering the temperature would lead initially to a higher than normal intracellular Na^+ level, and this could lead in turn to alterations in protein synthesis, or in genetic translation, which would readjust the situation by supplying the membrane with more of the machinery for Na^+ transport.

Such speculations are presently a long way from being tested, but they serve to show that a fertile field for future exploration is provided by membrane diversity as exemplified in crustacean neurons.

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