Temperature Effects on Pacemaker Generation, Membrane Potential, and Critical Firing Threshold in *Aplysia* Neurons

DAVID O. CARPENTER

From the Laboratory of Neurophysiology, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

ABSTRACT Temperature increases cause a regular and reproducible increase in the frequency of generation of pacemaker potentials in most *Aplysia* neurons specialized for this type of activity which can only be explained as a direct stimulating effect of temperature upon the ionic mechanisms responsible for pacemaker potentials. At the same time all cells in the visceral ganglion undergo a membrane potential hyperpolarization of approximately 1–2 mv/°C warmed. In spite of the marked variation in resting membrane potential the critical firing threshold remains at a constant membrane potential level at all temperatures in the absence of accommodative changes. The temperature-frequency curves of all types of cells are interpreted as a result of the interaction between the effects of temperature on the pacemaker-generating mechanism and resting membrane potential. Previous observations on the effects of temperature on excitability of mammalian neurons suggest that other types of neurons may undergo similar marked shifts in resting membrane potential with temperature variation.

INTRODUCTION

The effect of temperature upon nerve cell activity is of obvious importance in poikilothermic animals which must perform essential functions over a range of temperatures. Two recent reports have described a striking variation of membrane potential in molluscan neurons (4, 14). There is an apparent paradox in the findings of these authors that there is a marked membrane hyperpolarization on warming, which implies a decreased excitability, and the fact that both report an increased spontaneous activity of most neurons at warm temperatures. For this reason the effect of temperature upon membrane potential, critical firing threshold, and frequency of spontaneous discharge was investigated in the neurons of *Aplysia*.

Nerve action potentials in *Aplysia* are of two types, depending upon whether the spike originates from a pacemaker mechanism endogenous to the cell or by synaptic excitation (26). Only certain cells are capable of initiating pacemaker spikes, and such spikes differ from synaptically generated spikes in mode and site of origin.[1] These experiments will demonstrate a stimulating effect of temperature upon the pacemaker-generating mechanism. The effects of this stimulation must interact in each neuron with the marked increase in membrane polarization that occurs when the temperature rises, since the frequency of pacemaker generation and the efficiency of synaptic excitation are dependent upon membrane potential (26).[1] In spite of the membrane potential shift and the fact to be documented that the critical firing threshold for spike initiation remains constant at all temperatures, the effect on the pacemaker-generating mechanism of an increase in temperature will be shown to be sufficient to raise the level of spontaneous firing of the majority of *Aplysia* neurons.

METHODS

Experiments were performed on 114 neurons in 83 isolated visceral ganglia of *Aplysia californica*, *A. dactylomela*, and *A. vaccaria*. These cells were not entirely unselected but are representative of the variety of large cells found on the dorsal surface of the ganglion.

The animal was pinned to a dissecting tray and the visceral ganglion exposed, dissected free with the five main nerve bundles, and removed to a Lucite chamber where it was fixed to a paraffin layer. Each of the five nerve bundles was mounted in a stimulating electrode which consisted of two glass pipettes, each containing a Ag-AgCl wire connected to a constant current stimulator. The nerves were drawn into the pipettes by gentle suction. The current flow through the tip of the pipette was sufficient to excite the nerve fibers with a minimal spread through the bath.

The ganglion was continuously bathed with artificial seawater (Marine Magic, Lambert-Kay, Inc., Los Angeles, Cal.). The temperature of the bathing solution was varied between 3 and 26°C by changing the length of the coiled polyethylene tubing through which the bathing solution flowed that was immersed in an ice bath. Temperature was measured through a small thermistor placed near the ganglion in the bath and was continuously monitored and recorded on both oscilloscope and penwriter. The temperature unit (Telethermometer, Yellow Springs Instrument Company, Inc., Yellow Springs, Ohio) gave a linear output over the temperature range used in these experiments.

Single neurons were recorded through intracellular glass pipette electrodes filled with 3 M KCl and having resistances between 0.5 and 2 megohms. The signal was fed into a Bak high impedance amplifier, then to a Tektronix 3A3 preamplifier, and was displayed on a Tektronix 565 oscilloscope (Tektronix, Inc., Beaverton, Ore.) and simultaneously recorded on a Massa penwriter (Cohu Electronics, Hingham, Mass.). The response time of the penwriter with no overshoot was 11 msec.

[1] B. O. Alving. Spontaneous activity of isolated somata of *Aplysia* neurons. Data to be published.

In early experiments, the signal was led from the microelectrode through a Ag-AgCl wire, and the indifferent electrode consisted of another Ag-AgCl wire inserted into the bath. This recording system did not allow accurate measurements of DC levels with varying temperature because of the temperature dependence of the junction potentials, and in later experiments was abandoned.

In the experiments necessitating accurate DC measurements the microelectrode was connected to a calomel cell (Radiometer Co., Copenhagen, Denmark) through a polyethylene tube filled with 3 M KCl. The indifferent electrode consisted of another polyethylene tube filled with seawater connecting the preparation bath to a second calomel cell, with the KCl-seawater junction outside of the bath. The DC shift in each electrode with a full range of temperature variation was recorded after each penetration. A shift of less than 1 mv in the base line when the electrode was in the bath was routinely obtained only when electrode resistances were less than 2 megohms and tip potentials were less than 3 mv. Recordings which did not meet these conditions were not included in studies necessitating accurate DC measurements. Intracellular electrode resistance was tested at each temperature and if greater than 2 megohms at any temperature, the results were discarded. The DC drift in the recording system was less than 1 mv in 4 hr.

TABLE I

THE EFFECT OF INCREASED TEMPERATURE ON FREQUENCY OF SPONTANEOUS DISCHARGE

Cell type	Total studied	Frequency increase	Frequency decrease	No frequency change
Pacemaker	33	27	3	3
Nonpacemaker	44	29	5	10
Pacemaker plus synaptic excitation	37	36	1	0
Totals	114	92	9	13

A grounded lead was displayed on the oscilloscope at all times at a fixed but arbitrary level to serve as a reference for temperature changes and shifts in the DC level of the intracellular recording.

RESULTS

A. *Temperature Effects on the Frequency of Spontaneous Discharge*

Table I shows the effect of temperature changes on the average frequency of spontaneous discharge in 114 neurons, which have been divided into three groups on the basis of whether the spikes were produced by pacemaker or synaptic mechanisms, or both. The majority of neurons in each of the three groups discharged more rapidly at warm temperatures.

Most of the pacemaker neurons studied discharged at a steady rate in the absence of synaptic activity, and these cells showed the most regular and reproducible changes in frequency with changes of temperature. A tempera-

ture-frequency curve from one such cell is shown in Fig. 1. Curves of this shape were always found for rhythmic pacemakers with positive temperature coefficients. These cells did not generate pacemaker potentials below a temperature threshold which in these experiments varied between 3–23°C. Three rhythmic pacemaker neurons were found which discharged transiently after penetration and after sudden temperature changes but had no main-

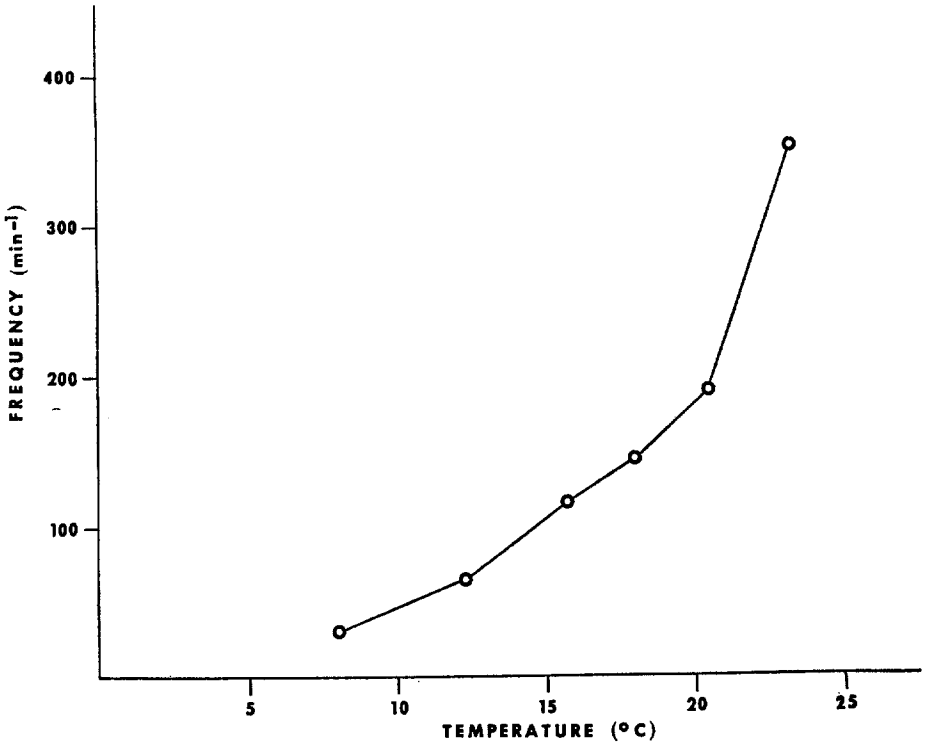

FIGURE 1. Temperature-frequency plot for a rhythmic pacemaker. All measurements were taken at no less than 10 min after a temperature change. Individual points were determined over a 4 hr period by alternately raising and lowering the temperature.

tained discharge. Also three rhythmic pacemakers were found which had their greatest frequency at cold temperatures, and tended to fire less rapidly as the temperature increased. These cells may represent specialized cold receptors.

Other pacemaker neurons in *Aplysia* alternate periods of discharge and silence. The effect of temperature change on one of these bursting pacemakers is shown in Fig. 2. All bursting pacemakers studied had positive temperature coefficients but because of variations in the bursting pattern with time and temperature the frequency curves for these cells were less regular and reproducible, perhaps in part because of cyclic variations in firing such as has been described in a cell of this type (24).

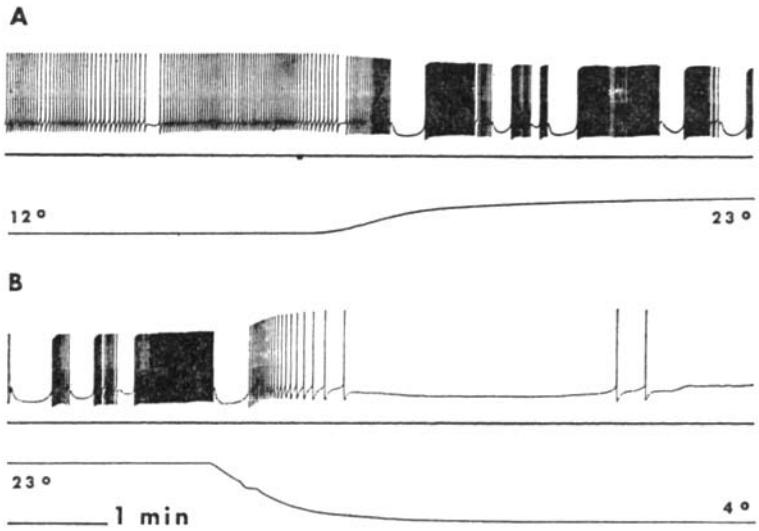

FIGURE 2. Penwriter records of the pattern of discharge of a bursting pacemaker, recorded with calomel electrodes. A and B are continuous records. The lower trace is a temperature record, and rises on warming. The middle trace is a grounded base line to serve as reference for the temperature and intracellular measurements. The positive portion of the action potential is 100 mv at 4°C. No voltage calibration is shown because of spike amplitude distortion produced as a result of the long response time of the penwriter.

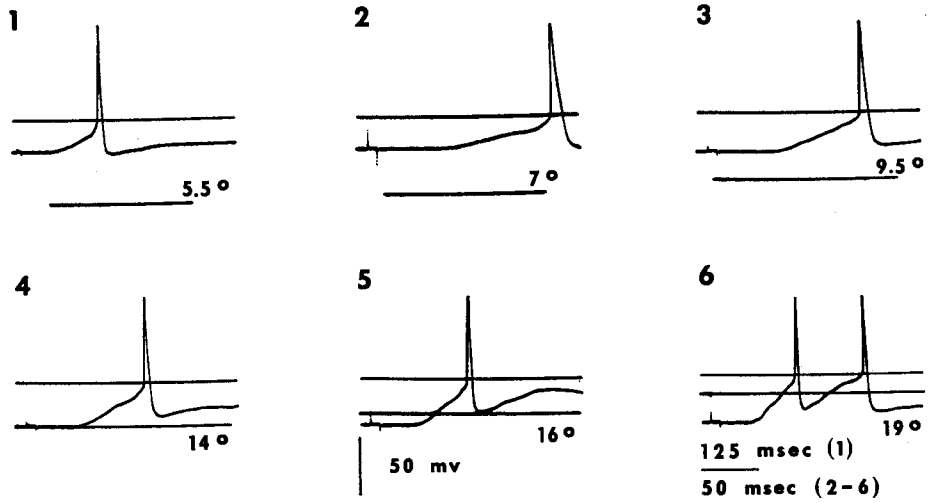

FIGURE 3. The effect of temperature change on resting membrane potential (RMP) and critical firing threshold (CFT) in the right upper quadrant giant cell, recorded with calomel electrodes. The upper trace is grounded base line for reference against RMP and temperature changes. The lower trace is the output of the thermometer unit, and rises with warming. Record 1 is of necessity taken at a slower sweep speed than 2–6 because of the greater latency of response at low temperatures.

In contrast to pacemaker neurons, all others in the ganglion were very erratic in their responses to temperature changes. Nonpacemakers showed particularly variable frequency responses. Temperature-frequency curves for nonpacemakers were usually not smooth, and frequently showed one or more sudden peaks of activity at intermediate temperatures. Although a majority of nonpacemakers showed an irregular increase in spontaneous firing with temperature, one-third had lower or no significant change in frequency when warmed, as is shown in Table I. The irregularities of the temperature-frequency curves and the unitary and negative temperature coefficients could in the great majority of cases not be correlated with the presence of inhibitory postsynaptic potentials.

The cells with both pacemaker and synaptic spikes were intermediate in behavior between the pure pacemakers and the nonpacemakers. Although as is shown in Table I the great majority had positive temperature coefficients, their temperature-frequency curves tended to be more irregular than for pacemakers.

B. *The Effects of Temperature on the Resting Membrane Potential (RMP) and Critical Firing Threshold (CFT)*

43 cells were studied with calomel electrodes in a recording system which allowed accurate measurement of DC levels over long periods of time and changes of temperature. Fig. 3 illustrates the effects of temperature changes on the resting membrane potential (RMP) and critical firing threshold (CFT) in the right upper quadrant giant cell, which is normally silent at all temperatures. The middle trace is a recording of the intracellular potential. The lower trace indicates the temperature and rises as the temperature increases, while the upper trace is a grounded base line which serves as a reference for changes in temperature and RMP. The action potential was elicited by stimulation of the left connective nerve which contains many fibers forming excitatory synaptic endings on the giant cell. Stimulation of the nerve bundle was not more frequent than 1 min intervals in order to avoid accommodative changes and to allow time for the multisynaptic activity elicited by the stimulation to cease. All records were taken at not less than 10 min after the temperature change.

Frame 1 of Fig. 3 shows the synaptically initiated spike in the giant cell at 5.5°C. The membrane potential at this temperature was 55 mv, as determined by comparison with the value obtained upon withdrawal of the electrode from the cell at the end of the experiment. As the temperature increased there was an increase in the membrane polarization which is indicated by the progressive separation between the upper base line and the intracellular response. In frame 6 at 19°C the RMP measures 72 mv.

The values of RMP in this cell are plotted against temperature in Fig. 4.

The numbers in the center represent the order in which the temperature measurements were made. No measurements were taken in the first 70 min after penetration, but there was a considerable increase in the RMP over the 2 hr taken for the first six measurements. This RMP development, which

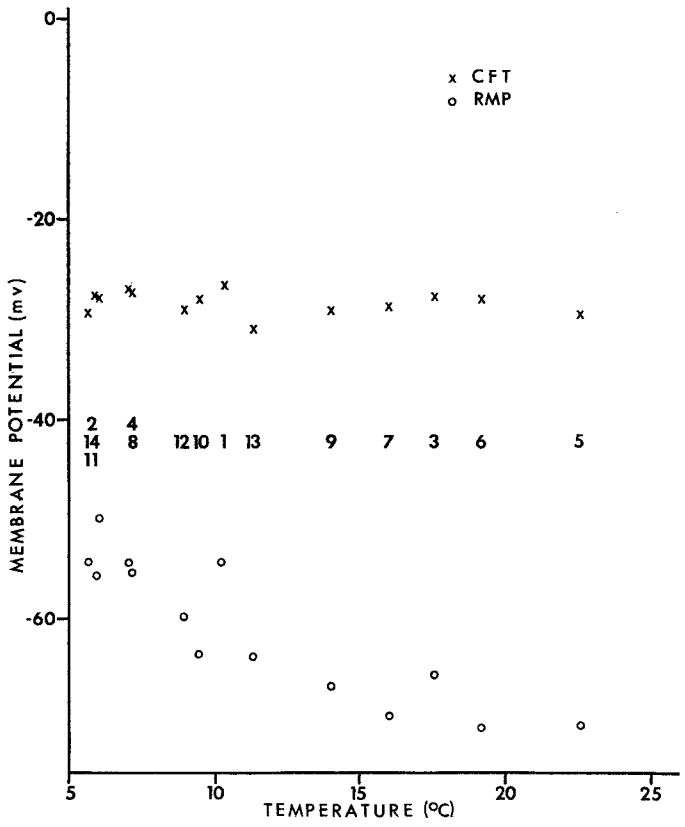

FIGURE 4. Plot of resting membrane potential (RMP) and critical firing threshold (CFT) variation with temperature in the right upper quadrant giant cell. Each point is an average of three measurements. The numbers in the center of the graph indicate the order in which the measurements were taken. The points corresponding to the first several measurements of RMP are raised from later RMP measurements, probably reflecting injury of penetration.

probably represents recovery from the injury of penetration, was complete only after nearly 3 hr. A similar time was required for complete RMP development in many cells.

The plot of RMP in Fig. 4 shows that there was a progressive increase in RMP with temperature, amounting to about 1.5 mv for each degree warmed. The points taken before complete development of the membrane potential are raised from the later measurements, but the direction and magnitude of

the RMP shift for these early points are consistent with measurements taken several hours later.

In Fig. 3 the size of excitatory postsynaptic potential (EPSP) necessary to initiate the spike increases with temperature. In frame 1 at 5.5°C the EPSP is 26 mv at the point of spike initiation, while in frame 6 at 19°C it is 40 mv. The critical firing threshold (CFT), as indicated by the final point of inflection before the rapidly rising spike, is very nearly identical at each of the six temperatures illustrated. In the upper part of Fig. 4 CFT is plotted as a function of temperature. There is no systematic variation of CFT with temperature and the points are all clustered about a value of approximately 29 mv, inside negative, at which the action potential originates. It is worthy of note that the CFT did not change during the period of recovery of RMP.

TABLE II

RMP RESPONSE TO INCREASED TEMPERATURE

Cell type	Total studied	Hyperpolarization	Hyperpolarization-Depolarization	Not determined
Pacemaker	11	8	0	3
Nonpacemaker	20	16	3	1
Pacemaker plus synaptic excitation	12	8	2	2
Totals	43	32	5	6

Consequently, the amplitude of EPSP necessary for spike initiation is considerably greater in the fully recovered condition than during injury depolarization, and similarly is greater at warm than cold temperatures.

Table II shows the effect of temperature changes on membrane potential in 43 cells. In cells which had no spontaneous pacemaker rhythms and little enough synaptic activity so that accurate membrane potential measurements were possible, RMP was always found to increase with increasing temperature. In 32 of 43 cells the hyperpolarization on warming was apparent at all temperatures where RMP measurements could be made. The magnitude of the shift in most cells was between 1–2 mv/°C, but occasional cells had shifts as small as 0.25 mv/°C. Occasionally there was some initial adaptation of the membrane potential shift, but in those cells which were studied for 4–8 hr and which presumably achieved complete recovery from the injury of penetration, no change in RMP was ever found at a constant temperature after the initial 5–10 min.

The five cells in the column of Table II labeled Hyperpolarization-Depolarization showed the usual increase in RMP to small temperature increases at relatively low temperatures. However, as the temperature was raised further, small EPSP's began to appear and increased in number until

the DC level of the base line appeared to rise with further increase in temperature. The base line in these cells was never flat at warm temperatures because of the superimposed synaptic activity, and thus it is reasonable to ascribe

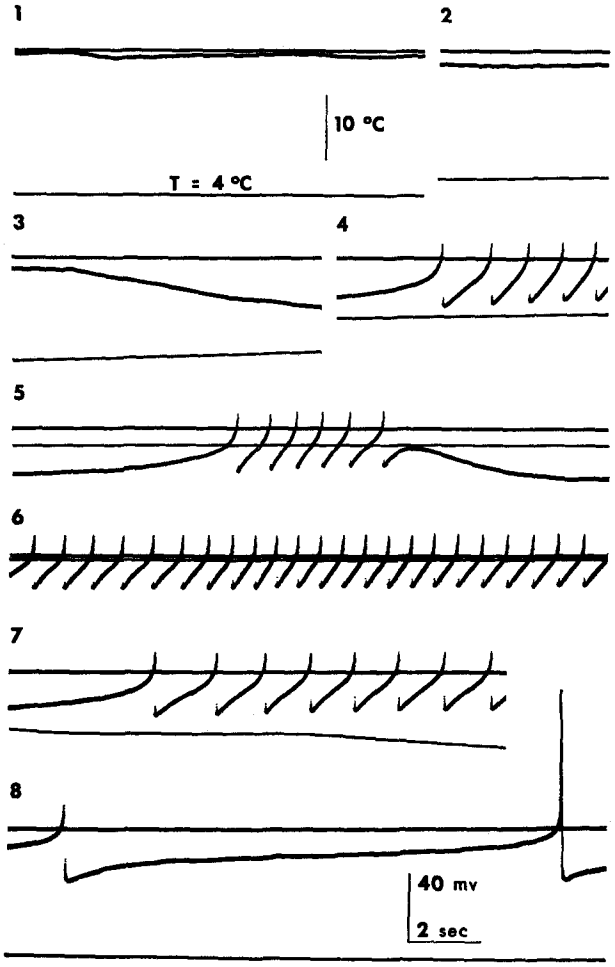

FIGURE 5. Oscilloscope records of activity of a bursting pacemaker, recorded with calomel electrodes. Lowest trace records temperature changes, and rises with warming. The initial temperature is 4°C. The temperature increases in 1–6 and decreases in 7–8. The upper trace is a grounded base line. Records 1–2 demonstrate the RMP shift seen in pacemakers at temperatures below threshold for firing. All spikes are not retouched except for the last one in 8 which shows the full spike at 8°C.

the apparent depolarization at warm temperatures to the effect of increased excitatory synaptic activity which was sufficient to obscure the RMP shift.

Fig. 5 shows the effect of temperature on RMP and CFT in a bursting pacemaker. The 2.5°C temperature change between 1 and 2, below threshold for pacemaker generation, resulted in a RMP hyperpolarization of nearly 5

mv. In 3, with warming, the cell begins its active state by undergoing a hyperpolarizing phase which develops into the firing seen in 4. At this and warmer temperatures the cell maintained alternating discharge and hyperpolarization phases. Records 4–8 show unretouched photographs of the pacemaker potentials leading to spike generation at various temperatures, while

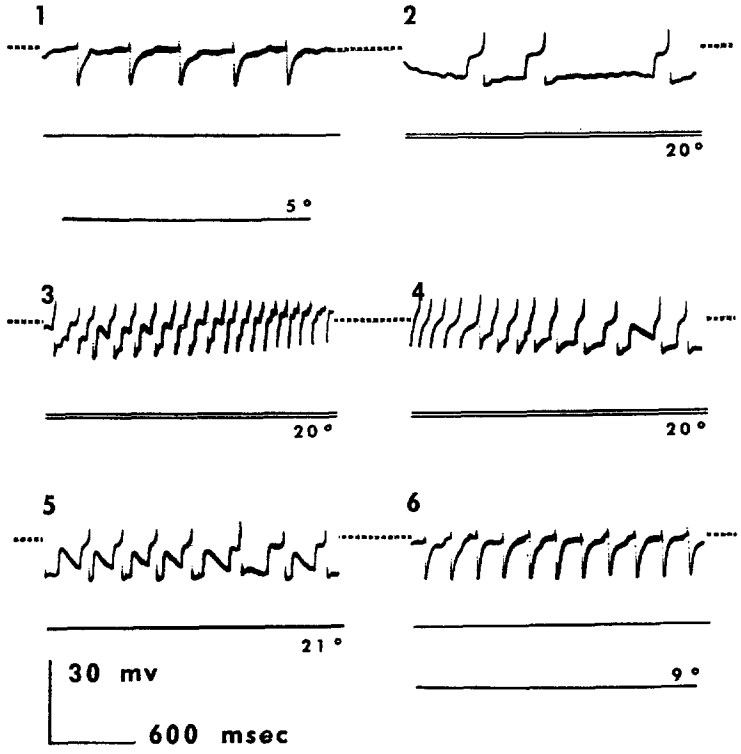

FIGURE 6. Accommodation during a synaptic burst in a nonpacemaker neuron, recorded with calomel electrodes. The lowest trace indicates the temperature and rises with warming. The middle trace is a grounded base line. Records are not retouched, and spikes are detected by the rapid rise which precedes the spike and the negative afterpotential. The dashed line drawn in between frames represents the CFT level at 5°C for comparison with other temperatures.

the retouched full spike is shown at the end of record 8, at 8°C. The approximate point at which the spike was initiated is indicated by the tips of the unretouched potentials where the rate of rise became too rapid for photographic duplication and shows no significant variation with temperature.

CFT remained constant at all temperatures in 16 of 21 cells. In the five remaining cells CFT was constant except during periods of rapid firing at which time successive spikes arose at greater levels of depolarization. This is illustrated in Fig. 6 in a synaptically activated cell where the CFT did not change with temperature in spite of a large variation in RMP (1–2, 5–6) but

increased markedly during a burst of synaptic activity (3–4). Accommodation was also seen in one bursting pacemaker where CFT was always constant for the first spike in the burst but increased for later spikes at high temperatures when the frequency during the burst increased.

C. *Transient Frequency Responses Evoked by Temperature Changes*

In approximately half of the 114 cells in which temperature-frequency relationships were determined a change in temperature caused a gradual assump-

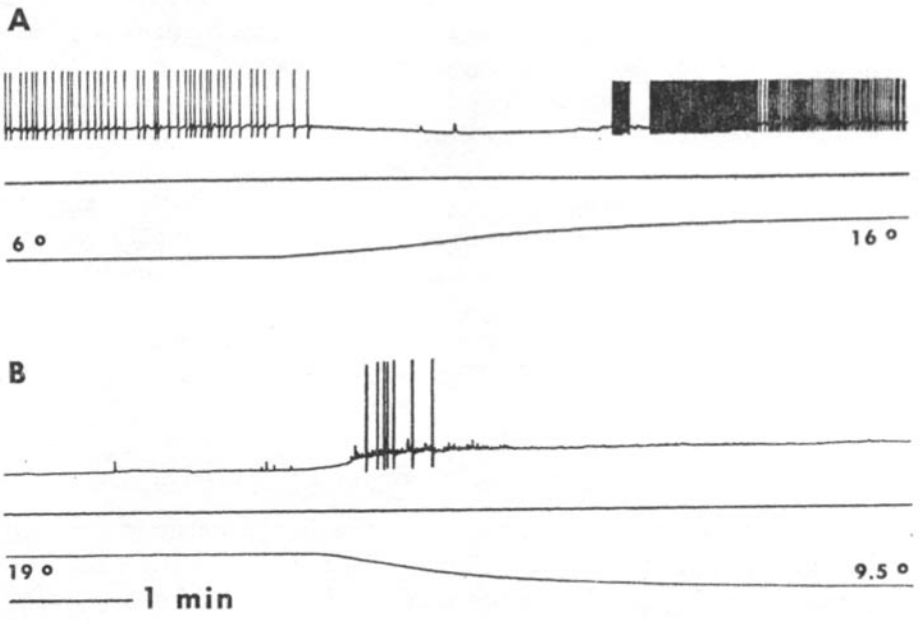

FIGURE 7. Penwriter records of the transient responses of two cells during temperature changes, recorded with calomel electrodes. Line A shows the response of one synaptically activated cell during warming, as indicated by the rise of the lowest trace. The action potential was 75 mv at 6°C. The middle trace is a grounded base line for reference. Line B is the response during cooling of another nonpacemaker which fired but rarely at all constant temperatures. This spike was 67 mv in amplitude at 9.5°C. No voltage calibrations are shown because of the failure of the penwriter to accurately record spike amplitude.

tion of the frequency of discharge characteristic of the new temperature. However, the other half showed anomalous transient frequencies during temperature changes which consisted of an increase in frequency on cooling and a decrease in frequency on warming lasting only during the actual temperature change, after which the discharge rate characteristic of the new temperature appeared. These anomalous transients were particularly frequent in nonpacemakers, but were found in one-third of the pacemakers also. Fig. 7 is a recording of the responses of two nonpacemaker neurons during

temperature changes and demonstrates the interaction between membrane potential shifts and the presence of synaptic potentials. In the upper record on warming the membrane hyperpolarizes with little or no delay and simultaneously the synaptic potentials disappear. As a result the firing ceases. As the temperature begins to stabilize at the new and higher value, synaptic potentials reappear and the membrane depolarizes. At 16°C the cell fires more frequently than it did at 6°C, but now the pattern is irregular and there is tendency for bursting.

Part B of Fig. 7 shows the response to cooling of another cell which showed only occasional discharges at all adapted temperatures. With cooling from the initial temperature of 19°C many EPSP's appear and the RMP simultaneously decreases. As a result the cell discharges several times during the temperature change. After the temperature change the synaptic potentials slowly cease and at the new temperature occur only occasionally.

When anomalous transient responses were found in pacemaker cells, they had the same general appearance as in Fig. 7, but RMP changes were much less easily followed as a result of the pacemaker activity. Some pacemakers showed a complete cessation of firing during transient warming, and in these a progressive hyperpolarization of the RMP was apparent during the silent period similar to that illustrated in Figure 7 A.

DISCUSSION

A. *The Significance of the Temperature Effects on Pacemaker Neurons*

These experiments have demonstrated two opposing influences on neuronal activity resulting from temperature changes. All types of cells in *Aplysia* show an increase in resting membrane potential (RMP) with rising temperature which tends to lower excitability by increasing the depolarization necessary to reach the critical firing threshold (CFT). At the same time the discharges of pacemaker neurons, which are the ultimate source of all spontaneous activity in the isolated ganglion, increase markedly with increasing temperature. The interaction between these two processes, with their opposite effects on excitability, is undoubtedly important in achieving a flexibility of the nervous system in an animal which must function over a range of ambient temperatures.

Pacemaker neurons show a membrane potential variation with temperature which is comparable in magnitude to the RMP shift of silent nonpacemakers when observed at temperatures below threshold for pacemaker generation. However, there is evidence that pacemaker generation is very sensitive to membrane potential and can be depressed or stopped completely by hyperpolarization produced by injected current (26).[1] It is apparent that not only does the membrane potential shift not explain the increased frequency of generation of pacemaker potentials at warm temperatures, but in itself this

membrane potential shift would tend to depress pacemaker activity. The increased frequency of pacemaker generation at warmer temperatures can only represent a temperature stimulation of the pacemaker-generating mechanism which is sufficient to obscure the effect of the increase in RMP in the great majority of the pacemaker neurons.

The mechanism of generation of pacemaker potentials in *Aplysia* is not known. In mammalian Purkinje fibers it has been suggested that the pacemaker potential is a result of a high resting sodium conductance (7). If a similar mechanism applies to pacemaker generation in *Aplysia*, the resting sodium conductance must have a high, positive temperature coefficient. It is of interest in this regard that the frequency of pacemaker potentials in mammalian Purkinje fibers is decreased with lowered temperatures (6).

B. *The RMP Shift and Its Effect on Cell Discharge*

On the basis of the temperature factor in the constant field equation the RMP should increase by about 3% for a 10°C increase in temperature provided that there are not concomitant changes in permeabilities (11). A RMP shift with temperature change of about this amount has been observed in muscle fibers (13, 18, 21) but not in squid axon (12). The present experiments have shown RMP shifts of greater than 15% for a 10°C temperature change in *Aplysia* neurons. A shift so large cannot be explained solely on the basis of a proportionality to absolute temperature and must result from a selective permeability change or an electrogenic pump (16). The mechanism is presently under investigation.

The unpredictable and erratic temperature-frequency relationships of nonpacemakers can be explained as an interaction between the effects of membrane hyperpolarization with increasing temperature at the same time that the cells are receiving a greatly increased frequency of synaptic potentials, both excitatory and inhibitory, as a result of the effect of the temperature change on pacemaker neurons. The cells which are pacemakers but have superimposed synaptic activity would be expected to demonstrate intermediate frequency relationships, as has been found to be the case in these experiments. It is known that synaptic potentials, both excitatory and inhibitory, can interact with pacemaker potentials to cause either a facilitation or an inhibition of the discharge, depending upon the time interval (22).

The anomalous transient responses can also be explained as an interaction between the effects of temperature change on RMP and pacemaker generation. Pacemaker generation is facilitated by membrane depolarization and is slowed by hyperpolarization achieved by injected current.[1] The RMP shifts occurring during temperature changes are in the direction which would tend to produce frequency changes similar to those observed during the anomalous transient responses. It is reasonable to conclude that these responses reflect the

effect of membrane polarization on pacemaker generation, and occur when the RMP shift precedes the full development of the temperature effect on the pacemaker mechanism. In nonpacemakers the RMP shift and the variation in synaptic activity produced by the anomalous responses of pacemaker neurons interact to produce a similar transient response.

C. *On the Constancy of the Critical Firing Threshold*

These experiments suggest that in the absence of excessive synaptic excitation CFT is very much a constant, since it did not change with time or over a wide range of membrane potentials resulting from temperature changes and during recovery from the injury of penetration. This conclusion is in agreement with the observations of Kolmodin and Skoglund (17) who observed CFT to remain constant in cat motoneurons except during periods of rapid discharge, at which time it increased. In addition, Frank and Fuortes (9) determined CFT during discharge produced by intracellularly applied currents and found the CFT did not change regardless of the slope of the applied currents.

Many of the results and conclusions of this study differ from those reached in an investigation of some of the same properties of *Aplysia* neurons by Murray (20), who reported a general tendency for the spike to be initiated at a lesser membrane potential at lower temperatures. Similar findings were reported by Burkhardt (3) for the crayfish stretch receptor. Both of these authors used electrodes with resistances of 10–30 megohms, whereas accurate DC recordings could not be obtained in the present experiments with electrodes with resistances of greater than 2 megohms because of the temperature dependence of the tip potentials which are much greater in high-resistance electrodes. It is possible that the high-resistance electrodes used by Burkhardt and Murray led to systematic errors in DC measurements with temperature changes.

D. *Possible Relevance of These Findings to Other Electrically Excitable Tissues*

Anomalous transient frequency responses to temperature change have been described in a number of invertebrate neurons (3, 5, 15, 23) and in mammalian smooth muscle fibers (1). Since each of these preparations in known to have pacemaker properties, and in light of the present results, it is reasonable to believe that all of these anomalous transient responses result from temperature effects on RMP and pacemaker generation similar to those observed in *Aplysia* neurons.

In mammalian preparations, cooling lowers the threshold of muscle spindle (19) and tongue mechanoreceptor (10) afferent fibers and greatly augments spinal reflexes (25), dorsal root reflexes (2), and presynaptic inhibition (8). Because of the difficulties in accurately recording DC potentials there is little information in these preparations on RMP variation with temperature. How-

ever, in view of the fact that mammals apparently do not have the large number of pacemaker neurons found in *Aplysia*, the increased excitability of mammalian neurons under hypothermia suggests that here also RMP varies with temperature and that excitability is greater in the cold because less depolarization is necessary to reach CFT.

The author is particularly grateful to Dr. Wade H. Marshall for his constant advice and criticism as well as to Drs. B. Alving, Deborah Carpenter, W. Freygang, K. Frank, E. Henneman, P. Nelson, E. Wolpow, and R. Wurtz for helpful discussions and criticisms of the manuscript. A. Ziminsky provided invaluable technical assistance.

Received for publication 11 August 1966.

BIBLIOGRAPHY

1. AXELSSON, J., and E. BÜLBRING. 1961. Metabolic factors affecting the electrical activity of intestinal smooth muscle. *J. Physiol.*, (*London*). **156**:344.
2. BARRON, D. H., and B. H. C. MATTHEWS. 1939. Dorsal root reflexes. *J. Physiol.*, (*London*). **94**:26P.
3. BURKHARDT, D. 1959. Die Erregungsvorgänge sensibler Ganglienzellen in Abhängigkeit von der Temperatur. *Biol. Zentr.* **78**:23.
4. CHALAZONITIS, N. 1961. Inhibition thermique des ondes électriques lentes d'un neurone géant identifiable (Neurone Br d'Aplysia fasciata). *J. Physiol.*, (*Paris*). **53**:289.
5. CHALAZONITIS, N. 1962. Évolutions différenciées en fonction de la température des caractéristiques de l'activité electrique de quelques neurones identifiables, (Neurons d'Aplysia). *Compt. Rend. Acad. Sci.* **255**:1962.
6. CORABOEUF, E., and S. WEIDMANN. 1954. Temperature effects on the electrical activity of Purkinje fibers. *Helv. Physiol. Pharmacol. Acta.* **12**:32.
7. DUDEL, J., and W. TRAUTWEIN. 1958. Der Mechanismus der automatischen rhythmischen Impulsbildung der Herzmuskelfaser. *Arch. Ges. Physiol.* **267**:553.
8. ECCLES, J. C., F. MAGNI, and W. D. WILLIS. 1962. Depolarization of central terminals of group I afferent fibers from muscle. *J. Physiol.*, (*London*). **160**:62.
9. FRANK, K., and M. G. F. FOURTES. 1960. Accommodation of spinal motoneurons of cats. *Arch. Ital. Biol.* **98**:165.
10. HENSEL, H., and Y. ZOTTERMAN. 1951. The response of mechanoreceptors to thermal stimulation. *J. Physiol.*, (*London*). **115**:16.
11. HODGKIN, A. L. 1951. The ionic basis of electrical activity in nerve and muscle. *Biol. Rev. Cambridge Phil. Soc.* **26**:339.
12. HODGKIN, A. L., and B. KATZ. 1949. The effect of temperature on the electrical activity of the giant axon of the squid. *J. Physiol.*, (*London*). **109**:240.
13. KERKUT, G. A., and R. M. G. P. RIDGE. 1961. The effect of temperature changes on the resting potential of crab, insect and frog muscle. *Comp. Biochem. Physiol.* **3**:64.
14. KERKUT, G. A., and R. M. G. P. RIDGE. 1962. The effect of temperature changes on the activity of the neurons of the snail Helix aspersa. *Comp. Biochem. Physiol.* **5**:283.

15. KERKUT, G. A., and B. J. R. TAYLOR. 1956. Effect of temperature on the spontaneous activity from the isolated ganglia of the slug, cockroach and crayfish. *Nature.* **178**:426.
16. KERKUT, G. A., and R. C. THOMAS. 1965. An electrogenic sodium pump in snail nerve cells. *Comp. Biochem. Physiol.* **14**:167.
17. KOLMODIN, G. M., and C. R. SKOGLUND. 1958. Slow membrane potential changes accompanying excitation and inhibition in spinal moto and interneurons in the cat during natural activation. *Acta Physiol. Scand.* **44**:11.
18. LING, G., and J. W. WOODBURY. 1949. Effect of temperature on the membrane potential of frog muscle fibers. *J. Cellular Comp. Physiol.* **34**:407.
19. LIPPOLD, O. C. J., J. G. NICHOLLS, and J. W. T. REDFEARN. 1960. A study of the afferent discharge produced by cooling a mammalian muscle spindle. *J. Physiol., (London).* **153**:218.
20. MURRAY, R. W. 1966. The effect of temperature on the membrane properties of neurons in the visceral ganglion of Aplysia. *Comp. Biochem. Physiol.* **18**:291.
21. NASTUK, W. L., and A. L. HODGKIN. 1950. The electrical activity of single muscle fibers. *J. Cellular Comp. Physiol.* **35**:39.
22. PERKEL, D. H., J. H. SCHULMAN, T. H. BULLOCK, G. P. MOORE, and J. P. SEGUNDO. 1963. Pacemaker neurons: Effect of regularly spaced synaptic input. *Science.* **45**:61.
23. SAND, A. 1938. The function of the ampullae of Lorenzini, with some observations on the effect of temperature on sensory rhythms. *Proc. Roy. Soc. (London), Ser. B.* **125**:524.
24. STRUMWASSER, F. 1965. The demonstration and manipulation of a circadian rhythm in a single neuron. *In* Circadian Clocks. J. Aschoff, editor. North Holland Publishing Co., Amsterdam. 442.
25. SUDA, I., K. KOIZUMI, and C. McC. BROOKS. 1957. Analysis of effects of hypothermia on central nervous system responses. *Am. J. Physiol.* **189**:373.
26. TAUC, L. 1960. Diversité des modes d'activité des cellules nerveuses du ganglion deconnecté de l'Aplysia. *Compt. Rend. Soc. Biol.* **154**:17.