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EFFECTS OF VARIED CULTURING AND EXPERIMENTAL TEMPERATURE ON ELECTRICAL MEMBRANE PROPERTIES IN *PARAMECIUM*

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SUMMARY

Effects of temperature on the electrical properties of the *Paramecium* membrane were investigated under constant current and voltage-clamp stimulation. With cells cultured at 18 °C, the resting potential was largely stable with *experimental temperatures* varied over the range of 10–25 °C, whereas action potential amplitude and membrane input resistance were inversely related to temperature increases. During voltage clamp, the early calcium current was increased, the time-to-peak decreased, and the early conductance increased with temperature. Similar modifications of the *culturing temperature* did not affect the resting potential, input resistance and stimulus-response relationship of the action potential, but the early conductance was reduced with increase in temperature. Possible effects of long- and short-term temperature changes upon intraciliary calcium concentration are discussed.

INTRODUCTION

Ciliate protozoa are sensitive to thermal shocks but they can eventually adapt within a comparatively wide range of temperatures (cf. Machemer & de Peyer, 1977; Nelson & Kung, 1978). Recent experiments have confirmed earlier behavioural tests showing that *Paramecium* detects gradients in temperature and congregates within preferred temperature zones (Mendelssohn, 1902; Nakaoka & Oozawa, 1977; Kawabuko & Tsuchiya, 1981). Raised temperature may produce avoidance responses similar to those elicited with depolarizing stimuli (Koehler, 1939; Hildebrand, 1978; Hennessey & Nelson, 1979). The threshold of thermal avoidance has been found to be dependent upon the culturing temperature (Hennessey & Nelson, 1979).

While behavioural effects of changes in temperature are obvious, the mechanisms by which temperature interferes with the membrane function and the cellular motor response are not well understood. Lowering of the experimental temperature produces very small depolarizations of the membrane (Yamaguchi, 1960) but depresses the rate of the action potential in *Paramecium* (Machemer, 1974) and *Stylonychia* (de Peyer & Machemer, 1977). Negative temperature shifts reduce the frequency and alter the direction of ciliary beating (Machemer, 1972) presumably due

Key words: Temperature, membrane potential, membrane currents, Ca^{2+}, *Paramecium*.

to an interference with active calcium extrusion (Browning & Nelson, 1976).

The present experiments were carried out to explore the effects of culturing and experimental temperatures on the passive and active membrane properties. Our emphasis has been to characterize the combined roles of short-term and long-term temperature in modifying basic membrane parameters.

METHODS

Paramecium caudatum (average length 220 μm) was reared in hay infusion at 10, 18 and 25 °C. For experimentation cells were harvested approximately 2 weeks after inoculation and washed in experimental solution containing 1 mM-$CaCl_2$ and 1 mM-KCl buffered with Tris-HCl at pH 7·4. They were collected at temperatures between 18 and 20 °C within 30 min prior to experimentation. An experimental cell was placed in a temperature-regulated 1-ml chamber. The actual temperature was monitored within 5 mm of the specimen using a calibrated NTC resistor.

The electrophysiological procedures have been described previously (Naitoh & Eckert, 1972). The membrane potential and input resistance of the cell were determined at the beginning of each experiment. The membrane potential was measured as the potential difference between an intracellular and an extracellular microelectrode, each filled with 1 M-KCl (resistance 40–60 MΩ). A third electrode, filled with 2 M-K-citrate, was inserted into the cell for current injection. The input resistance of the cell membrane was measured by injecting a 10^{-10} A hyperpolarizing constant current pulse.

The voltage clamp was performed by means of a conventional feedback system using a high-gain differential amplifier (AD 171K). The holding potential was kept equal to the resting potential. The membrane current was monitored through a current-voltage converter connected to the bath and held at virtual ground. Three types of experiments were performed: in the first section cells which had been cultured at 18 °C for at least 2 weeks were tested at short-term experimental temperatures (t_e) between 10 and 25 °C. Experiments in the second section were performed at t_e = 18 °C using cells grown at culturing temperatures (t_c) of 10, 18 and 25 °C. The third type of experiments served as controls, with the experimental temperature kept equal to the culturing temperature.

RESULTS

Short-term temperature effects

Resting potential and input resistance

The membrane resting potential of a cell cultured at 18 °C and bathed in an experimental solution of 1 mM-$CaCl_2$ + 1 mM-KCl was typically −36 mV at 25 °C and was not significantly affected by temperature (t-test, $P \leq 0{\cdot}05$) (Table 1, Fig. 1). There were rapid fluctuations in potential that appeared smaller during a slow decrease in temperature (Fig. 1A) than during an increase (Fig. 1B). The input resistance varied with short-term modifications of the experimental temperature from 67 MΩ at 10 °C to 41 MΩ at 25 °C corresponding to a temperature coefficient (Q_{10}) of 0·7 (Table 2).

Table 1. *Summary of electrical parameters of* Paramecium *at different culturing and experimental temperatures*

Temperature (°C)		Resting potential	Input resistance	N	Maximal early current		Late current (nA) at voltage step of		N
Culture	Experimental	(mV ± S.D.)	(MΩ ± S.D.)		Peak (nA)	Time-to-peak (ms)	− 60 mV	+ 35 mV	
10	18	−36·2 ± 2·6	52 ± 9	11	−13·4 ± 1·2	1·9 ± 0·1	−7·6 ± 1·2	5·2 ± 1·1	5
18	18	−36·4 ± 1·7	50 ± 13	13	−9·6 ± 3·1	2·0 ± 0·2	−6·2 ± 1·5	5·8 ± 2·5	5
25	18	−37·7 ± 2·8	50 ± 12	11	−8·9 ± 2·1	2·1 ± 0·4	−5·3 ± 2·0	11·0 ± 6·4	5
18	10	−33·1 ± 1·7	67 ± 13	10	−5·4 ± 0·7	4·3 ± 0·9	−4·9 ± 1·0	4·0 ± 0·9	6
18	15	−35·5 ± 2·2	59 ± 13	11	−8·3 ± 1·1	2·3 ± 0·3	−8·0 ± 1·6	6·2 ± 2·3	5
18	20	−35·4 ± 2·6	47 ± 11	9	−9·3 ± 1·4	1·7 ± 0·1	−9·0 ± 3·4	6·0 ± 1·9	5
18	25	−35·7 ± 1·9	41 ± 7	9	−9·6 ± 2·7	1·2 ± 0·2	−7·6 ± 1·8	5·8 ± 2·0	6
10	10	−36·0 ± 2·2	57 ± 7	5	−7·4 ± 0·9	3·7 ± 0·1	−4·4 ± 0·7	4·7 ± 1·1	5
25	25	−37·7 ± 1·0	41 ± 10	6	−9·5 ± 2·4	1·3 ± 0·0	−8·0 ± 0·9	8·5 ± 3·1	6

S.D., standard deviations; N, number of cells tested.

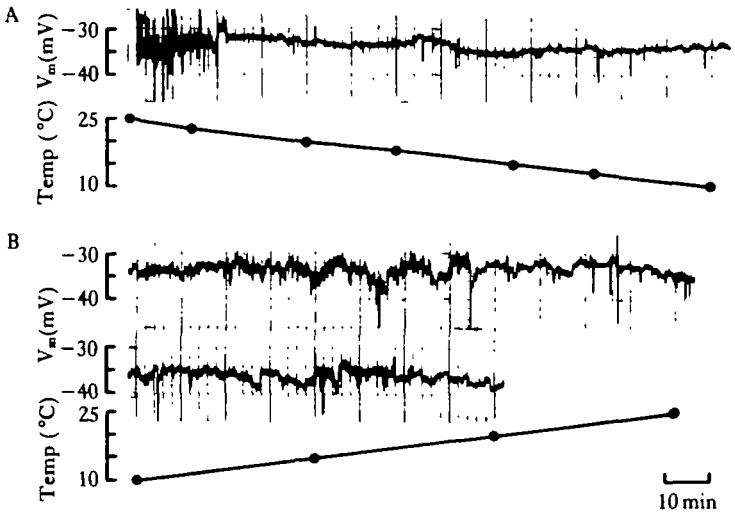

Fig. 1. Continuous records of the membrane resting potential during slow shifts in the bathing temperature (0·125 °C/min) between 10 and 25 °C. The sample records show fluctuations in the course of decreasing (A) and increasing (B) temperature which may mask a minor shift of the membrane potential toward more positive voltage during cooling and toward more negative voltage during warming up of the bath.

Responses to brief current pulses

The peak value of the action potential following brief square current pulses is sigmoidally related to the current strength (Naitoh, Eckert & Friedman, 1972). Fig. 2 shows that there was an increase in threshold of excitation from +3 mV to +5 mV when temperature was raised from 10 to 25 °C. The action potential amplitude was decreased whereas the rates of rise and decay were increased.

Responses under voltage clamp

Under voltage clamp, the effects of temperature upon the calcium conductance

Table 2. *Q_{10} values of electrical parameters corresponding to or derived from those as defined in Table 1.*

	t_c = constant t_e = 10–25 °C	t_c = 10–25 °C t_e = constant	$t_c = t_e$ 10–25 °C
Resting potential	1·0	1·0	1·0
Resting input resistance	0·7	1·0	0·8
Peak early current	1·5	0·8	1·2
Rate of activation (= reciprocal time-to-peak)	2·3	0·9	2·0
Late inward current (Δ−60 mV)	1·3	0·8	1·5
Late outward current (Δ+35 mV)	1·3	1·7	1·5
Resting input conductance	1·4	1·0	1·3
Early G_{max}	1·2	0·6	1·0

t_c, long-term culturing temperature.
t_e, short-term experimental temperature.

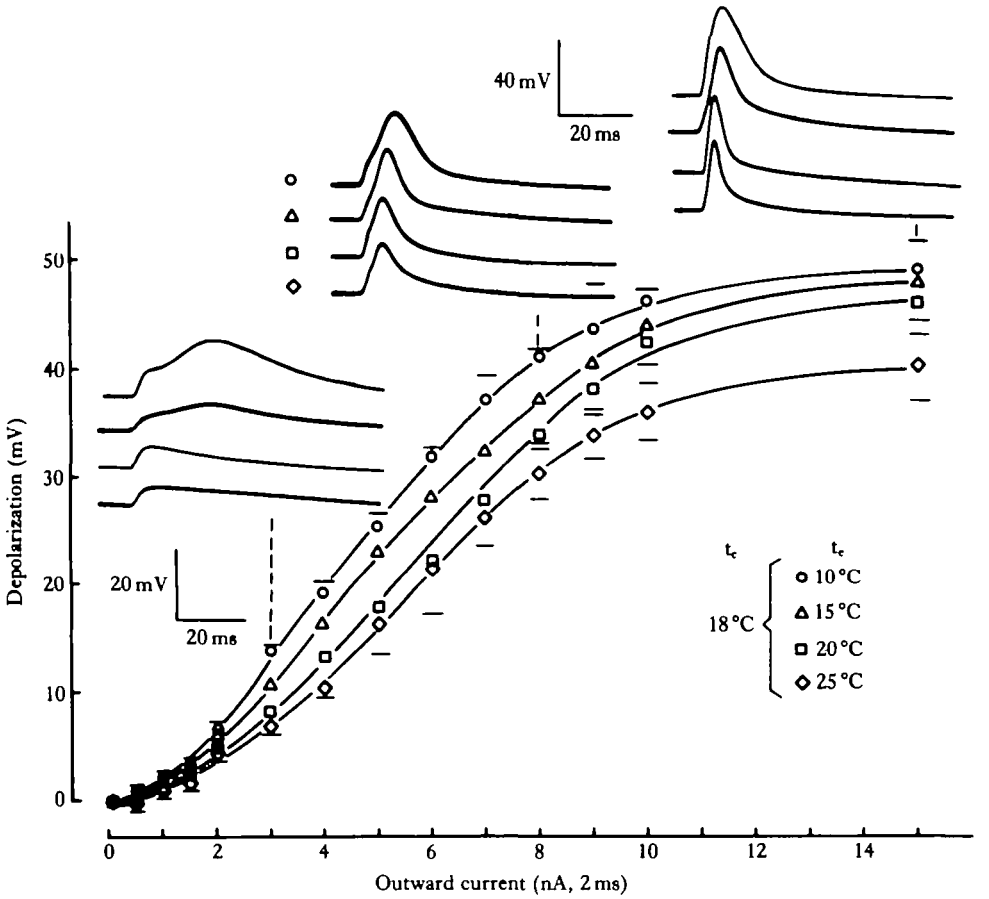

Fig. 2. Stimulus-response curves of action potentials generated from cells at different short-term experimental temperatures (t_e) between 10 and 25 °C after culturing at a temperature (t_c) of 18 °C. Insets: typical depolarizations corresponding to stimulus strength as indicated by dashed lines. The graph shows that rising t_e increased the threshold and rate of the action potential, while the amplitude was decreased. Each data point represents the mean of three cells with standard errors indicated; in cases of overlap only extremes of standard errors are given for clarity; curves fitted by eye (as are curves in Figs 3, 4, 7–10).

(G_{Ca}), which is rapidly activated, could be distinguished from the effects on the potassium conductance (G_K), which activates after some delay. Cells cultured at 18 °C were tested at four short-term experimental temperatures, 10, 15, 20 and 25 °C. At all temperatures tested the early current rose up to step amplitudes of 30 mV and declined beyond depolarizations of 35 mV. With increase in temperature, the early inward current showed an increase in rate of rise (Fig. 3A), and increase in maximum value (Fig. 3B) *from* −5·4 nA at 10 °C *to* −9·6 nA at 25 °C *corresponding to a* Q_{10} *of* 1·5 (Table 2). The steady-state I/V-relationships were essentially unchanged between 15 and 25 °C showing a familiar sigmoidal course. At 10 °C, however, the delayed outward and inward currents were reduced. Accordingly, Q_{10} values of the steady-state inward current (following −60 mV step) and outward current (+35 mV step) were close to 2·5 between 10 and 15 °C, but only 1·3 over the total range of temperatures tested (10–25 °C; Table 2).

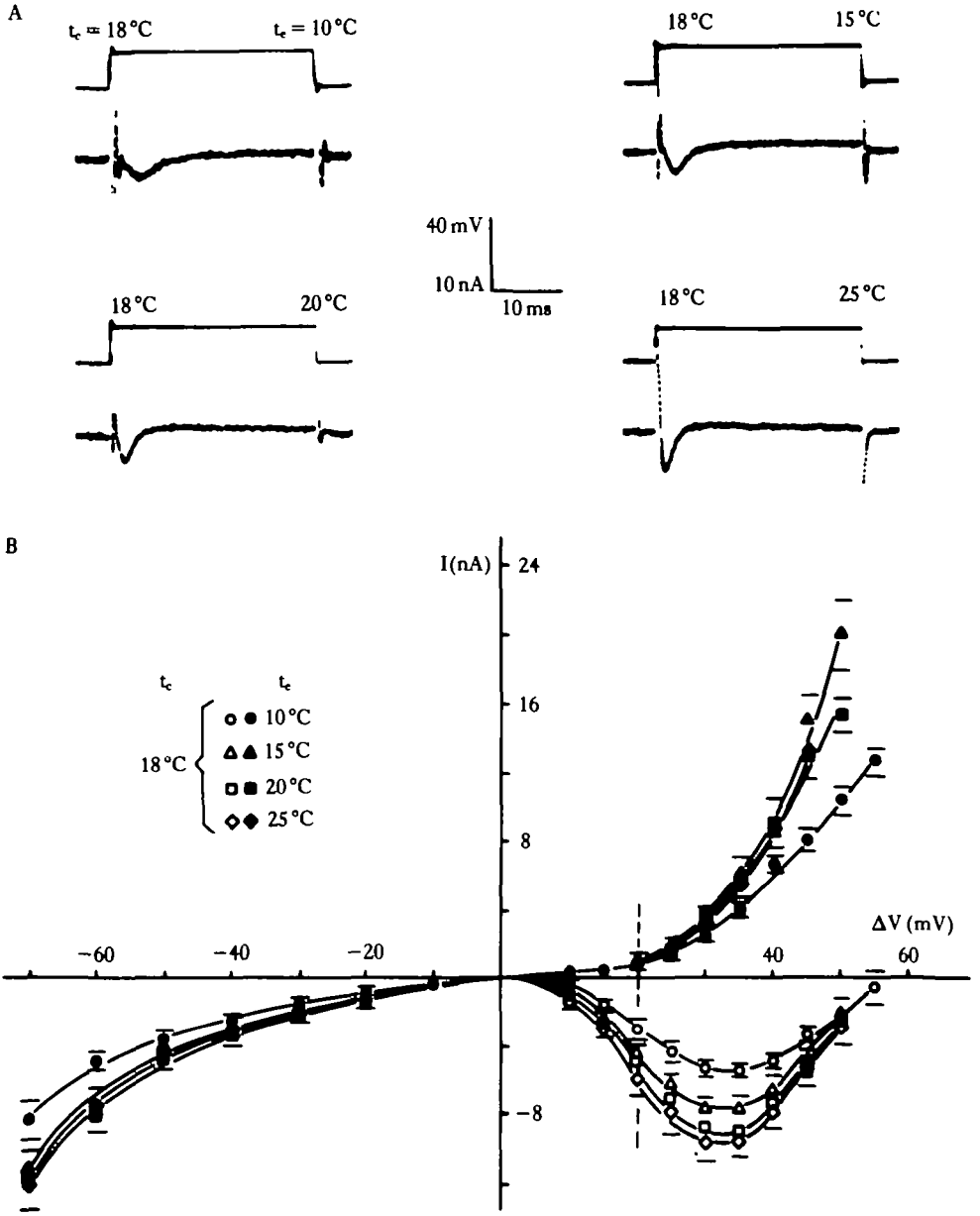

Fig. 3. Current/voltage relationships under voltage clamp of cells cultured at 18 °C, but tested at short-term temperatures between 10 and 25 °C. (A) Sample records of membrane current following a 20 mV, 30 ms step depolarization at four different experimental temperatures. The culturing temperature (t_c) is shown to the left, the short-term experimental temperature (t_e) to the right on top of oscilloscope traces (also in Figs 8 and 9). (B) I/V relation of early inward current (open symbols) and late current at the end of 30-ms step (filled symbols). The holding (=resting) potential is placed in the origin of the abscissa. Dashed line marks responses to +20-mV step as shown in (A). Means of five or six cells with standard errors (compare legend of Fig. 2). Note increase in average early current amplitude with increasing experimental temperature.

Early calcium conductance

In freshwater and marine *Paramecium* the early inward current is predominantly carried by Ca (Naitoh & Eckert, 1974; Deitmer & Machemer, 1982) according to the equation,

$$I_{Ca} = G_{Ca}(E'_{Ca} - V),$$

where G_{Ca} is the membrane conductance to Ca ions, E'_{Ca} the Ca equilibrium potential as effective during the early current, and V the membrane potential during step stimulation. The Ca equilibrium potential is thought to approximate to +116 mV while the membrane is at rest ($[Ca^{2+}]_a = 10^{-3}$ M; $[Ca^{2+}]_i = 10^{-7}$ M), but presumably drops significantly while Ca accumulates following activation of the ciliary Ca channels (Eckert, 1972). In *Stylonychia* and *Paramecium* the internal Ca concentration has been estimated to transiently increase to at least 10^{-4} M following voltage-activated (de Peyer & Machemer, 1978) and mechanically-activated Ca currents (Ogura & Machemer, 1980). Ca flux measurements during continued electric stimulation in *Paramecium* suggest a 5×10^{-4} M increment in intraciliary Ca (Martinac & Hildebrand, 1981).

For approximations of E'_{Ca} during maximal channel activation we assume a transient value for $[Ca^{2+}]_i$ of 10^{-4} M. This rough estimate does not take into account (1) differences in inward fluxes and (2) effects on transient $[Ca^{2+}]_i$ of temperature-dependent Ca pumping. The recorded early currents are sums of voltage-activated inward Ca and outward K currents. Short-circuiting of Ca current by K current is small with depolarizations up to 20 mV. Using the late outward current for reference the short-circuiting during the peak inward current was maximally 12–22 % (Figs 3, 8, 9) considering the difference in rates of activation of the Ca and K conductances. With these restrictions in mind we have determined the voltage/conductance relationship at different experimental temperatures (Fig. 4).

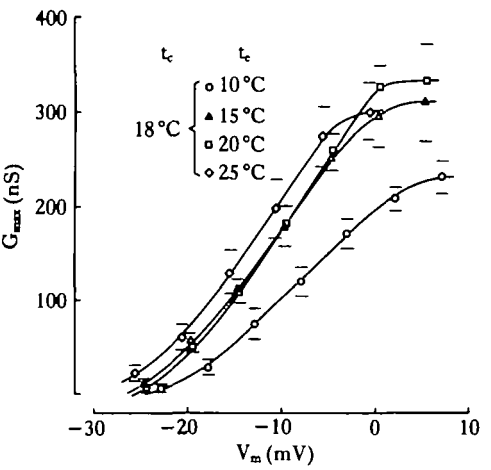

Fig. 4. Conductance/voltage relationships during early current peaks. Ordinate, maximal early conductance per cell (G_{max}); abscissa, absolute membrane voltage (V_m); data points calculated from means of five or six cells; see text for explanations. Cells were cultured at 18 °C and tested at short-term experimental temperatures (t_e) as indicated. Observe reduction of slope and peak conductance at $t_e = 10$ °C.

With cells cultured at 18 °C, average maximal conductances per cell (G_{max}) of 300 to 330 nS were found at temperatures between 15 and 25 °C. At 10 °C, G_{max} was reduced (230 nS) and the slope of the conductance/voltage curve was diminished.

Early current time course

The time interval between the onset of the depolarizing voltage step and the peak

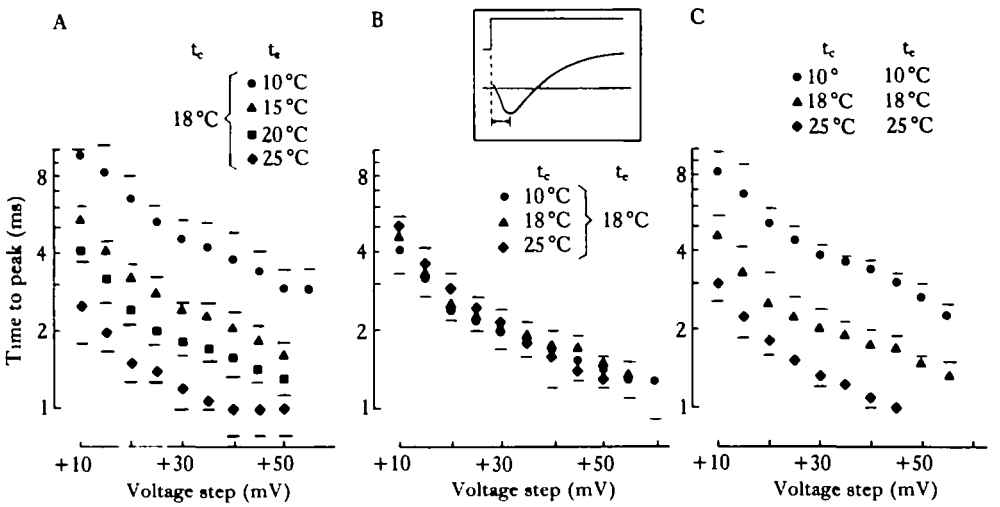

Fig. 5. Modifications of the time-to-peak of the early inward current as a function of depolarization and temperature. (A) Cells cultured at $t_c = 18$ °C, but tested at experimental temperatures (t_e) between 10 and 25 °C. (B) Cells cultured at different temperatures and tested at 18 °C. (C) Cells tested at the culturing temperatures of 10, 18 and 25 °C. Data points are means of five or six cells. Horizontal bars above or below data points are standard deviations. Observe that reductions in time-to-peak were coupled to increases in the experimental, not the culturing temperature.

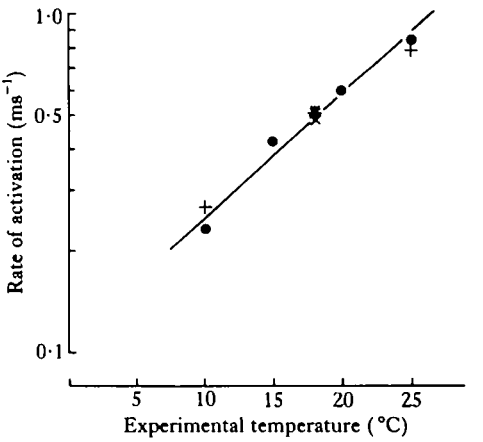

Fig. 6. Temperature-dependence of early current rate of activation (=reciprocal time-to-peak). Semilogarithmic plot shows exponential relationship of rate to experimental temperature. Straight line is least square regression fit (correlation coefficient = 0·99) of data points (circles) derived from time-to-peak following +35 mV steps (Fig. 5A). × and + symbols refer to data in Fig. 5B and C, respectively. Each data point corresponds to mean from five or six cells.

of the early inward current ('time-to-peak') is a measure of channel activation. For voltage-sensitive Ca channels and Na channels time-to-peak decreases with rising step amplitude. Change in the logarithm of time-to-peak with temperature (Fig. 5A) suggests that time-to-peak decayed exponentially with depolarizations of up to 30 mV; beyond this value the decay rate was reduced. When the experimental temperature was raised, time-to-peak decreased (Table 1) without major effects on its voltage-dependence. Semilogarithmic plots of the rate of current activation (=reciprocal time-to-peak) *versus* experimental temperature show that this relationship is exponential (Fig. 6) with a Q_{10} value of 2·3 (Table 2).

Long-term temperature effects

We have explored the long-term effects of environmental temperature upon the electrical properties of the *Paramecium* membrane by culturing cells for at least 2 weeks at preselected temperatures and then testing them at the temperature of 18 °C. In some cases the experimental temperature was kept equal to the culturing temperature.

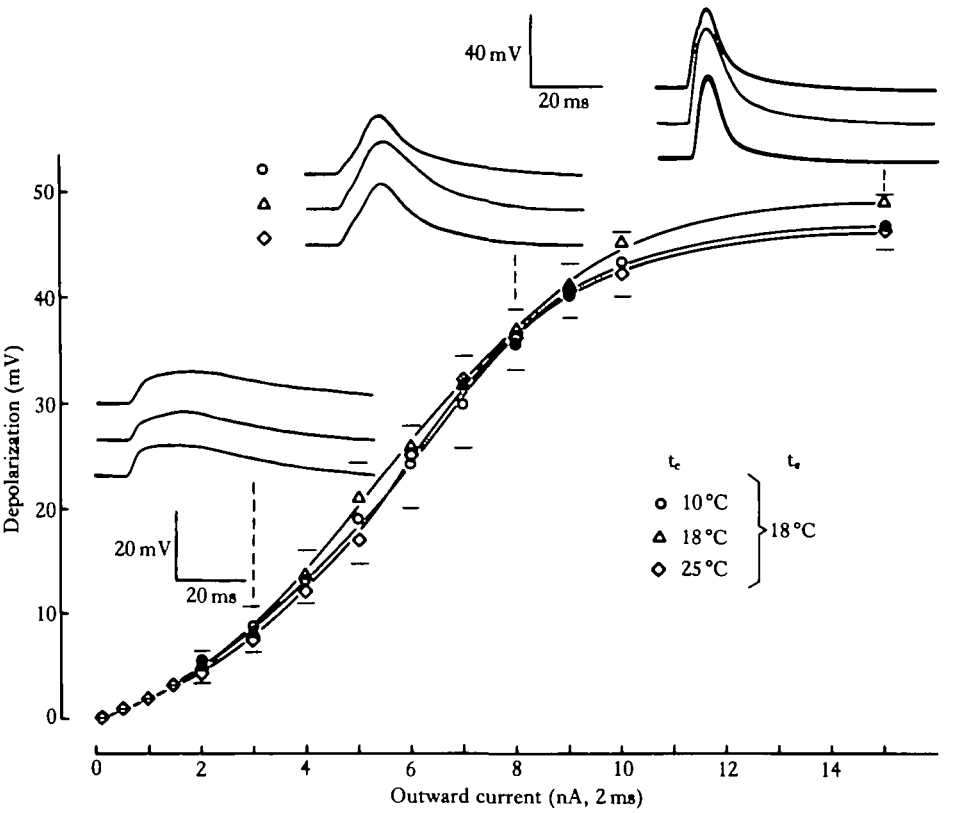

Fig. 7. Stimulus-response curve of action potentials of cells cultivated at different temperatures (t_c: 10, 18, 25 °C), but all tested at an experimental temperature (t_e) of 18 °C. The membrane responses were elicited with intracellular constant current pulses of 2 ms. Means of three cells with extremes of standard errors (see Fig. 2). Observe that the long-term culturing temperature did not affect the amplitude and time course of the graded action potential.

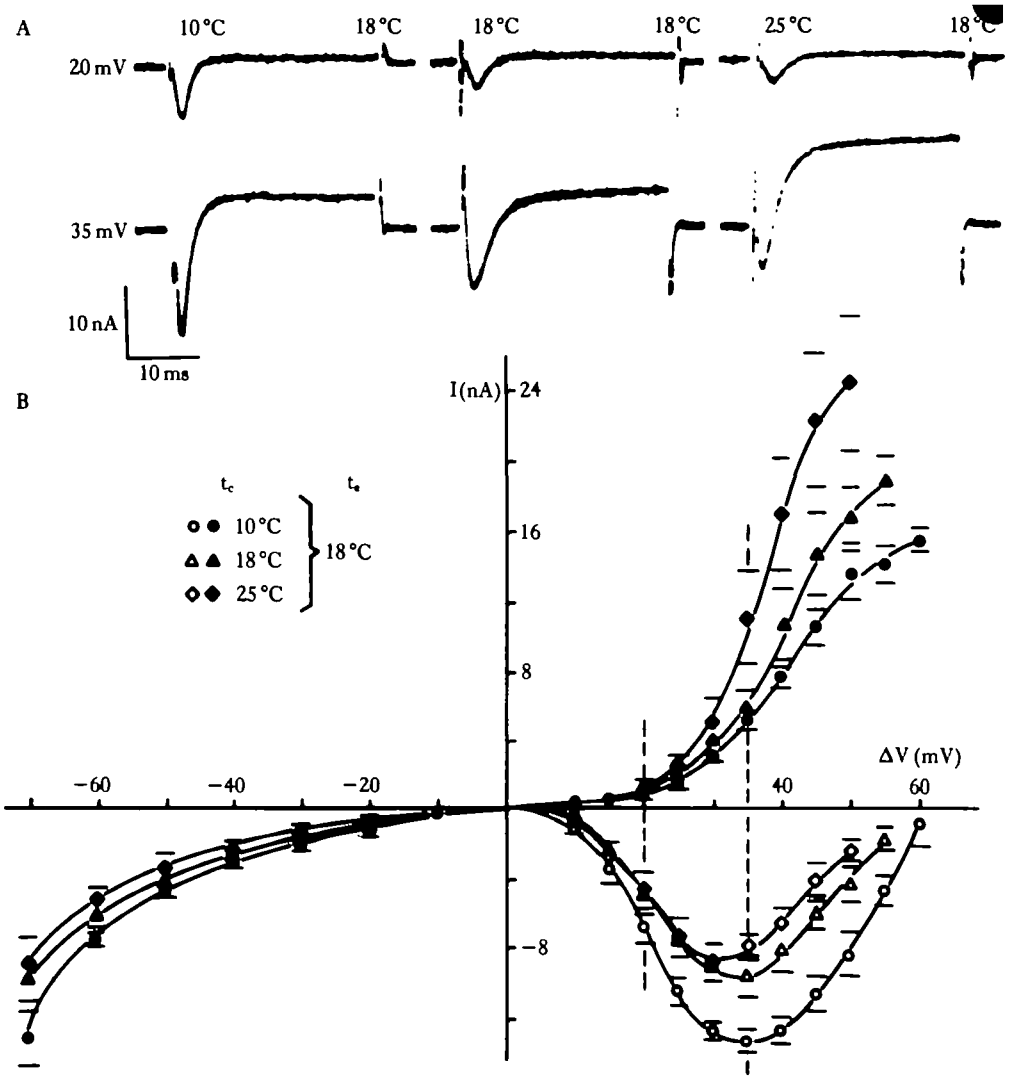

Fig. 8. Current/voltage relationships of cells cultured at 10, 18 or 25 °C and tested at 18 °C. (A) Sample records of membrane currents following voltage steps of +20 mV (top row) and +35 mV (below). (B) I/V relation of early current (open symbols) and late current (filled symbols). Dashed lines indicate responses to +20 mV and +35 mV steps shown in (A). Data points are means of measurements from five or six cells; standard errors according to Fig. 2. Observe that increased long-term temperature led to a decrease in mean early current peaks and to positive shifts of late inward *and* outward currents.

The resting potential and input resistance were not affected by the culturing temperature in the range 10–25 °C (Table 1).

The membrane responses to brief current pulses were also unaffected. For an experimental temperature of 18 °C the threshold of the regenerative response occurred uniformly between 4 and 5 mV. The stimulus/response relationship and the action potential amplitude (45–50 mV) were independent of the culturing temperature (Fig. 7).

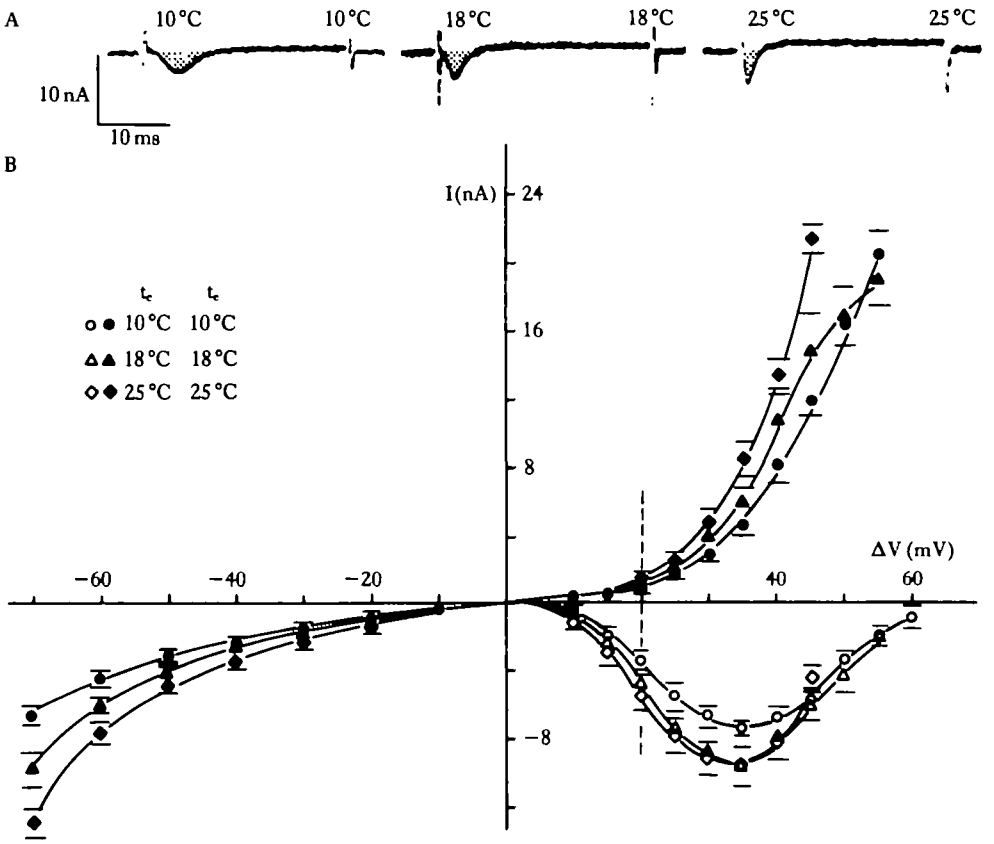

Fig. 9. Membrane currents of cells tested at their culturing temperatures ($t_e = t_c$). (A) Current traces following +20 mV step show that rate of rise and amplitude of the early current rose with the experimental temperature comparable to data in Fig. 3. Dotted areas indicate that the amounts of charges transferred during the early current are not constant at different temperatures. (B) I/V relationships. Data points are means from five or six cells; standard errors according to Fig. 2. The I/V relation resembles that seen with the culturing temperature kept constant (Fig. 3). The differences between the early current peaks at the extreme experimental temperatures (10, 25 °C) are less pronounced with $t_e = t_c$ (compare Fig. 3).

Responses under voltage clamp

The early inward current following a depolarizing voltage step decreased with increasing culturing temperature (Fig. 8). These temperature-dependent differences in current amplitude were most conspicuous when applying a step voltage of 35 mV or more. The early current maximum showed a negative shift along the voltage axis when the culturing temperature was increased. The late I/V-relationship suggests that this shift may be due, in part, to an increase in short-circuiting outward current during excitation. It is interesting to observe that a high culturing temperature increased the late outward current, but decreased the steady-state inward current during hyperpolarization; this suggests that a temperature-sensitive conductance may be common to both types of voltage-activated current. The Q_{10} value of the steady-state inward current equals that of the early inward current (0·8 for −60 mV step; Table 2) while

the steady-state outward current shows a pronounced inverse temperature relationship ($Q_{10} = 1{\cdot}7$ for +35 mV step).

Long-term and short-term temperature exposures affect the electrical parameters of the membrane in a complex manner. The early inward current increased as the experimental temperature was raised (Fig. 3), but decreased at higher culturing temperature (Fig. 8). As a result of such partial cancelling, the maximum inward current was less increased when tested at the culturing temperature (Fig. 9).

Comparison of Fig. 9 with Fig. 3 also indicates that the steady-state currents are primarily a function of the short-term experimental temperature. The Q_{10} values (1·5) resemble those calculated from data where the culturing temperature was held constant ($Q_{10} = 1{\cdot}3$; Table 2).

Stimulating cells at low culturing temperatures increased the duration of the early inward current (Fig. 9A). The amount of net charges passing the membrane during the early current was considerably reduced in 25 °C cells (+20 mV step: 6 pC) with respect to 10 °C cells (12 pC). We conclude that long-term temperature exposure does not help to stabilize the amount of net charges transferred across the membrane in the course of voltage-activated early current.

Early calcium conductance

Applying our premises on the Ca equilibrium potential (see above) modifications of the culturing temperature decreased G_{max} from an average of 540 nS at $t_c = 10$ °C to 250 nS at $t_c = 25$ °C (Fig. 10A). Testing cells at their culturing temperatures led to unaltered early conductances of about 300 nS. As temperature was raised the G/V relationship was shifted in the negative direction of the voltage axis (Fig. 10B).

Early current time course

Modifications of the culturing temperature had no effect upon the time-to-peak of the early inward current, which corresponded to values seen upon short-term modification of the experimental temperature (Fig. 5, compare B and C with A). Accordingly, the rate of activation of the early current was a function of the short-term experimental temperature, irrespective of whether or not the culturing temperature had been modified (Fig. 6).

DISCUSSION

The membrane resting potential in *Paramecium* is essentially independent of temperature within the ranges tested (Table 1). For a temperature increase of 10 °C estimates of E_{Ca} and E_K (Ogura & Machemer, 1980), together with a constant conductance ratio G_{Ca}/G_K, predict a negative shift of 1·2 mV of the membrane resting potential according to the Hodgkin-Horowicz equation. The observed average shift of 2 mV/10 °C approximates to the theoretical value. Our results confirm earlier measurements of the resting potential of *P. caudatum* in extremely diluted sea water (2 mV/10 °C; Yamaguchi, 1960) and in *Stylonychia mytilus* (1·5 mV/10 °C; de Peyer & Machemer, 1977). Thus, for ciliates so far tested, the ratio of resting conductance of Ca to K appears to be constant within a certain range of temperatures. For the

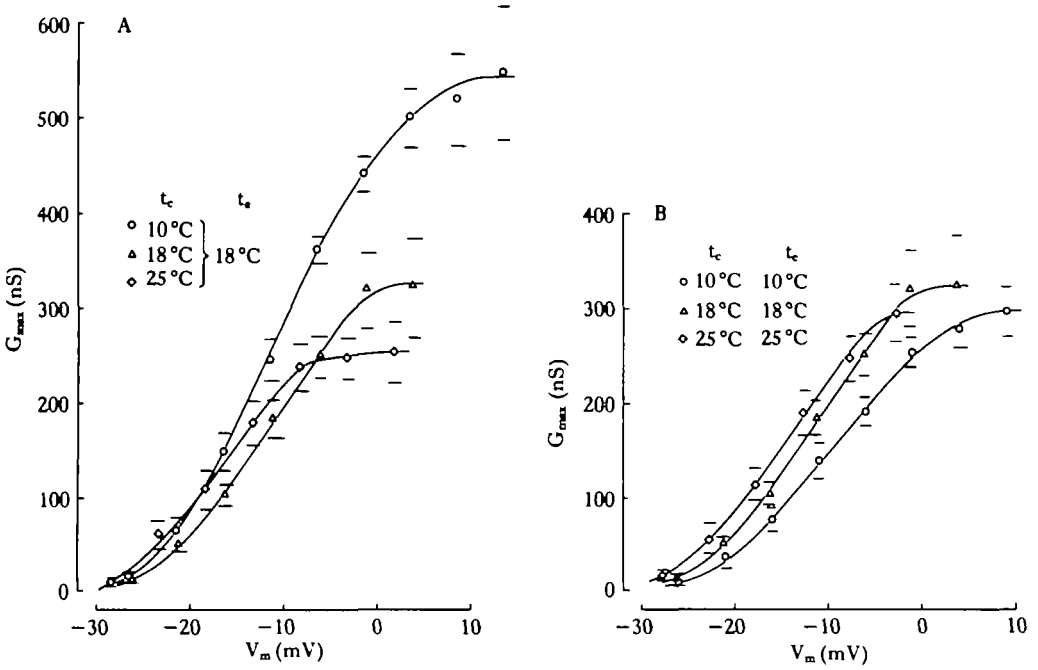

Fig. 10. Conductance/voltage relationships during early current peaks of cells cultured at different temperatures (t_c). Ordinate, maximal early conductance per cell (G_{max}); abscissa, absolute membrane voltage (V_m); data points were calculated from means of five or six cells. (A) cells tested at 18 °C. Increase in culturing temperature decreased the peak early conductance. (B) Testing cells at various culturing temperatures reduced differences in peak values of the conductance curve.

symbiotic flagellate *Opalina* a negative shift of the resting potential of 3·6 mV/10 °C has been observed (Ueda, 1961). Stability of the resting potential upon cooling has also been reported for squid giant axon (Hodgkin & Katz, 1949) and vertebrate myocardial fibres (Trautwein & Dudel, 1954).

Hennessey, Saimi & Kung (1982) have recently recorded a depolarization in *Paramecium* which occurred upon rapid increase of the environmental temperature (24 °C/min). No potential response was seen upon lowering the temperature. The heat-induced potential was graded with the temperature increase and related to a raised somatic Ca conductance. The time course of this very slow response to rapid changes in temperature shows some resemblance to fluctuations in membrane potential observed during slow heating, not cooling (compare Fig. 1B with A). Toyotama (1981) was able to record a depolarizing potential in *Paramecium* upon lowering the temperature at a rate of 1 °C/min between 25 and 20 °C. These so far heterogeneous data are not sufficient for discrimination between a 'thermoreceptor potential' and transient instabilities of the membrane resting potential seen during temperature changes. The available evidence suggests, however, that the membrane is differently sensitive to heating and cooling.

A raised threshold depolarization and reduction in amplitude of the action potential, observed with short-term increase in temperature (Fig. 2), is consistent with a raised membrane conductance, predominantly of K. Similar temperature effects on

threshold and action potential amplitude have been reported from squid axon (Hodgkin & Katz, 1949; Tasaki & Spyropoulos, 1957) and vertebrate heart tissue (Trautwein & Dudel, 1954; Marshall & Williams, 1956; Illanes, Carpentier & Reuss, 1970). Short-term increase in temperature increased voltage-activated early and late currents ($Q_{10} = 1{\cdot}5$ and $1{\cdot}3$, respectively; Table 2). The observed Q_{10} of rates of activation of the early current ($2{\cdot}3$) is in accordance with results on squid axon (Hodgkin & Huxley, 1952) and amphibian nerve and muscle (Schwarz, 1979). The absence of an inflection in the semilogarithmic plot of rate of activation *versus* experimental temperature (Fig. 6) suggests that no phase transitions in membrane lipids and/or channel proteins of *Paramecium* occur at temperatures between 10 and 25 °C.

Long-term exposure to different temperatures affected the maximal early conductance, which decreased with rising culturing temperatures ($Q_{10} = 0{\cdot}6$; Table 2). A comparison of the stimulus-response curves (Figs 2, 7), however, clearly indicates the leading role of the experimental temperature in determining the rate and amplitude of the action potential. The failure of the diverging curves of early and late current (Fig. 8), to modify the stimulus-response curve (Fig. 7), is puzzling at first sight. Inspections of the current time courses in Fig. 8A show, however, that the decay rate of the early inward current rose with the current size, not with depolarization. The size of the late outward current was not related to the rate of decay of the inward current, so that K current activation does not appear to limit the inward current decay. An inactivation of the voltage-sensitive early Ca channel and activation of the late K channel by intracellular ionic Ca has been recently demonstrated in ciliates (Brehm & Eckert, 1978; Deitmer, 1983) and in metazoan tissue (Meech, 1978; Tillotson, 1979). Our data are in line with the view of a Ca-dependent feedback control of the Ca conductance; such control can obviously help to stabilize the action potential at different temperatures.

We presume that the observed rise in early peak conductance following long-term cooling (Fig. 10B) rests on an increase of the number of voltage-activated Ca channels. Since the action potential does not benefit from this increase in conductance (Fig. 7), one may ask if it is functionally meaningful. It should be remembered that excitability in ciliates serves in the regulation of the internal Ca concentration (Eckert, 1972) and thereby the control of the ciliary motor response (Machemer & Eckert, 1973). Cooling has multiple effects on electric membrane properties, the chemomechanical transduction process of the ciliary axoneme, and Ca pumping from the ciliary space, so that the relationship between ciliary motility and temperature is complex. Short-term cooling of *Paramecium* predominantly depresses the reversed ciliary beating following an action potential (Machemer, 1974). Such altered motor responses can modify the phobic behaviour of cells, which contributes to their orientation in temperature gradients. Long-term cooling, on the other hand, increased the early peak conductance (Fig. 10A) and raised the amount of Ca influx (Figs 8A, 9A). It is conjectured that these long-term temperature effects serve to modify the time course of stimulus-induced increments in intraciliary Ca concentration with the effect that reduced frequencies of ciliary beating are associated with extended beating periods. As a result a similar amount of work is done by the cilia so that a specific behavioural response can be performed over a wide range of environmental temperatures.

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