ELECTROPHYSIOLOGICAL STUDIES ON THERMORECEPTION IN *PARAMECIUM CAUDATUM*

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CONTENTS

INTRODUCTION

Thermoreception in Paramecium

Thermotactic behavior

Since Mendelssohn's pioneering works (Mendelssohn, 1895, 1902) it has been known that specimens of *Paramecium* accumulate in a region with a temperature close to their culture temperature (the optimum temperature region) (Jennings, 1906). More recently it has been shown that specimens increased their forward swimming velocity when moving towards their optimum temperature region (Tawada and Oosawa, 1972). In contrast, they exhibited frequent changes in swimming direction when move away from this region (Nakaoka and Oosawa, 1977). These motile responses are the major causes of their accumulation in the optimum temperature region.

A specimen swimming along a temperature gradient is subjected to a difference in temperature between the anterior and posterior ends and to a change in temperature with time. By examining the accumulation of *P. caudatum* in solutions of differing viscosity and temperature gradients, Tawada and Miyamoto (1973) demonstrated that the rate of change in temperature around the specimens was an important aspect of thermal stimulation involved in causing the accumulation.

On the other hand, Hennessey and Nelson (1979) reported that a specimen of *P. tetraurelia* exhibited a thermal avoidance behavior to higher temperature region, and threshold temperature causing this thermal avoidance behavior was much higher in the specimen cultured in higher temperature. In addition, they also reported that the lipid composition of the surface membrane was changed with change in culture temperature (Hennessey and Nelson, 1983). Nakaoka et al. (1982) reported that the accumulation of the specimens in the optimum temperature was affected by the density of specimens in an

assay chamber. Ugawa (1984) developed a novel system for examining thermotactic behavior of *Paramecium*. The system consisted of two vessels with different temperatures. He examined the effects of the external ionic environment on the distribution of the specimens in the assay chamber. Tawada and Nakaoka (1979) reported ATP-reactivated specimens of triton-extracted specimen of *P. caudatum* showed a transient increase in the swimming velocity when the external temperature was changed toward its culture temperature, and they swam at their maximum velocity when they were at the culture temperature.

Membrane potential correlates

It has long been known that ciliary movement-mediated locomotion of the specimens of *Paramecium* is under the control of membrane electrogenesis. A membrane hyperpolarization leads to an increase in forward swimming velocity (escape response) caused by an increase in the ciliary beat frequency, while a membrane depolarization leads to a slowing down of the forward swimming rate, a change in swimming direction, or backward swimming (avoidance response) caused by inactivation of ciliary beating and/or to a reversal of the ciliary beating direction (Eckert, 1972; Naitoh, 1974, 1982; Machemer, 1975, 1988; Kung and Saimi, 1982; Machemer and Sugino, 1989).

It is, therefore, presumed that a specimen of *Paramecium* ascending a temperature gradient produces a membrane hyperpolarization when it approaches towards the optimum temperature region, while it produces a membrane depolarization when it leaves the optimum temperature zone. In other words, a rise in temperature causes a membrane hyperpolarization, if the surrounding temperature is lower than the optimal temperature, while similar rise in temperature causes a membrane depolarization when the surrounding temperature is higher than the optimal temperature.

Membrane potential responses to thermal stimulus

Membrane potential responses of *Paramecium* to a thermal stimulus (a sudden rise or fall in temperature) was first reported by Toyotama (1981), i.e. a rise in ambient temperature evoked a membrane depolarization. His results were confirmed by Hennessey et al. (1983). By employing deciliation technique (Machemer and Ogura, 1979), Hennessey et al. (1983) found that ion channels responsible for the thermostimulation-evoked membrane potential response (thermoreceptor potential) were present on the somatic membrane and not on the ciliary membrane. Nakaoka et al. (1987) reported that upon thermal stimulation, an anterior fragment of a specimen of *P.multimicronucleatum* produced a membrane hyperpolarization, while a posterior fragment produced a membrane depolarization (see also Inoue and Nakaoka, 1990).

Thus the primary objectives of the first portion of the present works are (1) to examine whether the polarity reversal in the thermoreceptor potential takes place depending on the ambient temperature relative to the optimum temperature and (2) if this is the case, what is the underlying mechanism.

Comparison of the thermoreceptors to the mechanoreceptors

As will be described in the results section, it was demonstrated that a specimen of *P. caudatum* exhibited a depolarizing membrane potential response to thermal stimulation of the anterior region of the cell, while it exhibited a hyperpolarizing response to thermal stimulation of the posterior region. These findings suggest topographical differentiation of two different kinds of thermoreceptor channels on the surface membrane of *Paramecium*.

Topographical distribution differences of two kinds of mechanoreceptor potentials on the cell surface of *Paramecium* was first demonstrated by Naitoh and Eckert (1969b). *Paramecium* showed a depolarizing membrane potential response when a mechanical stimulus was applied to its anterior region, while it showed hyperpolarizing membrane potential response when similar stimulus was applied to the posterior region. Similar

mechanoreceptor potential distribution patterns on the cell surface had been reported in some other ciliate protozoan, such as *Euplotes* (Naitoh and Eckert, 1969a), *Stylonychia* (De Peyer and Machemer, 1978) and *Tetrahymena* (Takahashi *et al.*, 1980) (see review articles by Naitoh, 1982, 1984, by Machemer, 1988 and by Deitmer, 1992).

Similarity in the distribution on the cell surface of *Paramecium* between thermoreceptor potential and mechanoreceptor potential suggests the kinship of the thermoreceptor mechanism with the mechanoreceptor mechanism. As a matter of fact, receptors which respond to both thermal and mechanical stimulation have been reported in mammals (Hensel, 1973, 1974a; Burgess and Perl, 1973), birds (Gottschaldt, 1985; Gentle, 1989), fish (Hensel, 1974b), crustaceans (Burkhardt, 1959) and insects (Altner and Loftus, 1985). These facts strongly suggest that both receptor systems have a common developmental and evolutionary origin.

Thus the primary objectives of the second portion of the present works are to examine and compare the electrophysiological characteristics and mechanisms of the thermoreceptor and mechanoreceptor currents in voltage-clamped specimens of *P. caudatum*.

MATERIALS AND METHODS

Specimens and Media

Specimens of *Paramecium caudatum* (Strain G3, mating type V) were cultured in a bacterized (*Klebsiella pneumoniae*) lettuce infusion at 25 ± 2 °C. After their final feeding, the culture specimens were kept immersed in a constant temperature water bath (± 0.1 °C) for more than a day. The temperature of the water bath was regarded as the "culture temperature", T_c. Prior to experimentation the specimens were washed with a standard saline solution (4mmol l^{-1} KCl, 1mmol l^{-1} $CaCl_2$ and 1mmol l^{-1} Tris-HCl or Mops-KOH buffer: pH 7.2), and then maintained in this solution for more than 30 min at T_c.

Behavioral experiments

To examine the locomotor activity of specimens in the boundary between two regions with different temperatures, a thin-walled (0.1 mm) glass capillary (40 mm length, 1 mm diameter) containing about 50 specimens suspended in the standard saline solution was placed on two copper blocks (20 mm x 20 mm x 5 mm) 0.5 mm apart (Fig. 1). In less than 1 min, the temperature of the suspension in the capillary tube reached an equilibrium level dependent on the temperature of the copper block beneath it. When the temperature of the two blocks differed, a temperature gradient was established in the capillary immediately above the gap between the two copper blocks. This gradient was stable over the experimental time-frame. The temperature of each block was stabilized (± 0.1 °C) with the aid of an electronically controlled Peltier module. The lower temperature was regarded as the "experimental temperature", T_e. The difference in temperature between the two regions was always 10 °C, irrespective of T_e. Fig. 2 shows a typical temperature profile at the boundary. The maximum temperature gradient was 2.5 ° mm^{-1}. The temperature profile did not change significantly for different values of T_e.

To examine locomotor responses of specimens to a sudden rise in temperature, about 50 individuals suspended in the standard saline solution (45 μl) were introduced into a thin-walled (0.1 mm) rectangular glass vessel (15 mm x 15 mm x 0.2 mm) shown in Fig. 3. The vessel was then put on a laminated nichrome heater with a copper block (15 mm x 15 mm x 5 mm) beneath it. When the temperature of the suspension in the vessel had been kept constant at a particular T_e (± 0.1 °C) for more than 2 min with the aid of an electronically controlled Peltier module placed beneath the block, the temperature was suddenly and temporarily raised to a certain level by applying an electric current pulse to the nichrome heater.

The behavior of the specimens in the experimental chamber was recorded on videotape, and their locomotor activities were determined by analyzing the tapes frame-by-frame with the aid of an ultrasonic digitizer and a microcomputer. The locomotor activities determined were: (1) swimming velocity, which is the distance along the swimming path accomplished by a specimen in a unit time; (2) the linearity of swimming, which is the ratio of the linear distance accomplished by a specimen in a unit time to its corresponding distance along the swimming path; and (3) the turning frequency (the frequency of the avoidance response per unit time).

Electrophysiological experiments

The responses of a specimen's membrane potential and membrane current to thermal stimulation were examined using conventional electrophysiological techniques (Naitoh and Eckert, 1972a). The temperature of the experimental vessel for electrophysiology was kept constant at the appropriate T_e (± 0.2 °C) with the aid of an electronically controlled Peltier module placed beneath the vessel (Fig. 4).

Thermal stimulus

A microheater of the Nicklas (1973) type was made for thermal stimulation of a specimen impaled by microelectrodes (Fig. 5). The middle portion of a 0.1 mm thick, U-shaped piece of tungsten wire was electrolytically polished to 0.05 mm in diameter in the central region. This thin region of the wire was covered with solder glass, while the rest of the wire was covered with silicon paste and cashew nutshell liquid for its electrical insulation. The insulated tungsten wire was glued on to a glass capillary mounted on a acrylic holder for placement on a micromanipulator.

When a current pulse (50 ms duration) was applied to the microheater, heat was generated primarily in this region of the wire. This caused a transient rise in the temperature of the surrounding solution. Time courses of the rise in temperature at different distances from the tip of the heater were monitored by a small thermocouple (0.15 mm tip diam, 50 ms time constant) and are shown in Fig. 6A. The rise was faster and larger in the region closer to the tip of the heater. By the time that the temperature in a region 50μm from the tip had reached its maximum, there had still been little rise in the temperature of a region 250μm from the tip. Using this procedure, one end of an animal can be subjected to a substantial thermal shock while the other end is relatively unaffected by the stimulus.

The relationship between the peak rise in temperature and the square of the intensity of the current applied to the heater was almost linear. The square is proportional to the heat generated at the heater tip. An example of this relationship in a region 50μm from the heater tip is shown in Fig. 6B.

Mechanical stimulus

Mechanical stimulus was given with an aid of a microstylus (5 μm in tip diameter) driven by a phono-cartridge (Naitoh and Eckert, 1969a). The stimulus was made by tapping surface membrane of a specimen by the tip of the microstylus attached to the surface membrane before stimulation. Stimulus strength was given by the voltage of a square electric pulse applied to an piezoelectric element for driving the microneedle against

the specimen. Excursion of the microneedle was proportional to the voltage in a range employed (Katoh and Naitoh, 1992). Duration of mechanical stimulation was kept constant at 5 ms throughout the experiments. Detailed movement of the tip of the microneedle was examined precisely by De Peyer and Machemer (1978). All the experiments were performed at a controlled room temperature of 23 ± 3 °C.

RESULTS

Behavioral response to a thermal stimulus

Behavioral response at the boundary between different temperature zones.

When specimens swimming in the lower-temperature (T_e) region of the assay capillary encountered the boundary with the higher-temperature region, they used an avoidance response to change their swimming direction. This avoidance response was more common when T_e was closer to T_c (25 °C). This is well illustrated in Fig. 7, where the strength of the avoidance response (P_a) is plotted against T_e. P_a is defined as the ratio of the number of specimens that failed to enter the boundary because of the avoidance response to the total number of specimens encountering the boundary.

Locomotor responses to a sudden rise in temperature

The temperature of the experimental chamber containing the specimens was slowly changed from 25 °C (T_c) to T_e at a rate of 2.5 °min^{-1} and then kept at T_e for 1 min. The specimens were then subjected to a sudden transient rise in their surrounding temperature at a maximum rate of 2.5 °s^{-1} by a magnitude of the transient of 7.1 °C. This rate is comparable to that experienced by a specimen in the assay capillary when it enters the temperature boundary from the lower-temperature region at a swimming velocity of 1 mm s^{-1} (the mean swimming velocity of specimens in standard saline solution at 25 °C).

The specimens responded to this thermal stimulus with an avoidance response, which appears in Fig. 8 as an increase in turning frequency (F_t) and a decrease in the linearity of swimming (L). The avoidance response was less conspicuous when T_e was below T_c, and no avoiding response was observed at a T_e of 15 °C (Fig. 8A). The strength of the avoidance response was slightly reduced when T_e was above T_c (compare Fig. 8C with Fig. 8D).

The swimming velocity of the specimens (V_S) decreased concomitantly with the avoidance response, then increased as the response ceased (Fig. 8C). The increase in V_S was clearly observed when the avoidance response was infrequent (e.g. when T_e was lower than T_c: Fig. 8A, B).

Membrane potential response to a thermal stimulus.

Membrane potential responses to an overall thermal stimulus

The response of a specimen's membrane potential to a sudden rise in the surrounding temperature was examined in two groups; one cultured at 25 °C, and the other at 15 °C. The microheater was placed about 100 μm from both ends of a specimen impaled by microelectrodes, so that the whole surface of the specimen was subjected to a virtually simultaneous rise in temperature when an electric current pulse (0.8A, 1s) was applied to the heater. The time course of the rise in temperature is shown in Fig. 9A. The maximum rate of rise was 4.7 $°s^{-1}$.

When T_e was equal to or greater than T_c, specimens responded with a membrane depolarization upon which spikes were sometimes superimposed (Fig. 9A, 25 , 30 °C; Fig. 9B, 15 , 25 °C). When T_e was lower than T_c, the membrane was transiently depolarized, then hyperpolarized for a sustained period (Fig. 9A, 15 °C; Fig. 9B, 25 °C). Because the specimens deteriorated when kept at a T_e higher than T_c, their responses became less conspicuous with time (e.g. Fig. 9A, 30 °C and Fig. 9B, 25 °C).

Membrane potential responses to a localized thermal stimulus

A localized thermal stimulus was applied to the anterior or posterior region of a specimen using a microheater placed 50μm from the appropriate end (Fig. 6A). Representative examples of membrane potential responses are shown in the right-hand side of Fig. 10. The upper set of traces shows the responses of specimens tested at 25 °C, while lower set is for specimens tested at 15 °C.

When T_e was equal to T_c (25 °C), responses of both the anterior and posterior ends were depolarizing (Fig. 10A). The amplitude of each response (peak voltage with reference to the resting membrane potential) increased with an increase in the stimulus intensity. The anterior response was always larger and faster than the posterior response (traces to right of Fig. 10A). The relationship between response amplitude and stimulus intensity is shown on the left-hand side of Fig. 10A.

When T_e was 15 °C, the posterior response became hyperpolarizing, while the anterior response remained depolarizing (traces to the right of Fig. 10B). The amplitude of both the hyperpolarizing and depolarizing responses increased with an increase in the stimulus intensity before saturating. The relationship between response amplitude and stimulus intensity is shown in the left-hand side of Fig. 10B.

In the next series of experiments, the membrane potential response to a localized thermal stimulus strong enough to evoke the maximal response was examined at various values of T_e while T_c was kept constant at 25 °C. The relationship between the response and T_e is shown in Fig. 11 together with examples of some individual responses.

The anterior response was always depolarizing, irrespective of T_e. The amplitude of this depolarization increased at lower values of T_e (Fig. 11A). The depolarizing response was followed by a small hyperpolarization when T_e approached 15 °C.

In contrast, the posterior response was depolarizing when T_e was equal to or greater than T_c (Fig. 11, see also Fig. 10A). The depolarizing response decreased and was followed by a hyperpolarization when T_e was lower than T_c. When T_e approached 15 °C only a hyperpolarizing response occurred (see also Fig. 10B). The relationships between the amplitude of each component of the response and T_e are shown in Fig. 11B.

The effects of varying T_c on the dependence on T_e of the membrane potential response to a localized thermal stimulus.

In this series of experiments, the relationship between the amplitude of the membrane potential response to a localized thermal stimulus and T_e was examined using three groups of specimens, each cultured at a different T_c (15, 20 and 25 °C).

The anterior responses were only slightly affected by varying T_c (Fig. 12A), whereas the posterior responses were strongly affected (Fig. 12B). The peak depolarization for each posterior response shifted in parallel with the shift of T_c. In other words, the amplitude of the posterior depolarizing response was always maximal when T_e was equal to T_c. Furthermore, the plot of the posterior hyperpolarizing response also shifted in parallel with the shift of T_c. In other words, the posterior hyperpolarizing response only occurred when T_e was lower than T_c, regardless of the value of T_c.

Effect of the external ion concentration

In this series of experiments, effect of the external ion concentration on the membrane potential response to a localized thermal stimulus was examined. As shown in Fig. 13A, amplitude of the anterior depolarizing membrane potential response shifted in accordance with change in the extracellular Ca^{2+} concentration, while the amplitude of the posterior hyperpolarizing response did not changed much. On the other hand, the amplitude of the posterior hyperpolarizing response shifted in accordance with a rise in the extracellular K^+ concentration, while the amplitude of the anterior depolarizing response did not change much (Fig. 13B). When Co^{2+} was added to standard saline solution by 16 mmol l^{-1}, the posterior membrane potential response to a localized thermal stimulus was not changed even when the ambient temperature was decreased from 25 °C to 15 °C.

Comparison between thermoreceptor and mechanoreceptor currents

Membrane electric responses to localized thermal or mechanical stimulation

A specimen of *P. caudatum* exhibited a depolarizing membrane potential response upon thermal stimulation of its anterior end, while it exhibited a hyperpolarizing response upon thermal stimulation of its posterior end. When the membrane potential level was

clamped at its resting level, an inward membrane current was evoked by anterior stimulation, while an outward membrane current was evoked by posterior stimulation. Representative traces of the membrane electric responses to thermal stimulation are shown in Fig. 14T.

Similarly to thermal stimulation, a specimen exhibited a depolarizing membrane potential response upon mechanical stimulation of its anterior end, while it exhibited a hyperpolarizing membrane potential response upon mechanical stimulation of the posterior end. When the membrane potential level was clamped at the resting level, an inward membrane current was evoked by anterior stimulation, while an outward membrane current was evoked by posterior stimulation. Representative traces of the membrane electric responses to mechanical stimulation are shown in Fig. 14M. Hereafter, these receptor currents will be abbreviated to the following: The anterior thermoreceptor current, ATC; the posterior thermoreceptor current, PTC; the anterior mechanoreceptor current, AMC; the posterior mechanoreceptor current, PMC.

Deciliated specimen was used to examine whether the thermoreceptor channels are existing on somatic membrane or cilia. Deciliation was done with a conventional 5 % ethanol (Ogura and Takahashi, 1976; Machemer and Ogura, 1979). As shown in Fig. 15, a regenerative membrane potential response to injected outward current was decreased when the specimen was incubated and shacked in standard saline solution and 5 % ethanol mixture for 2 min. While a membrane potential response to an injected inward current was not much changed. Under the voltage-clamped condition, transient inward current was decreased with deciliated specimen. The thermoreceptor current was kept intact with a deciliated specimen.

The intensity of each receptor current increased with increasing stimulus strength. The relationships between the current intensity and the stimulus strength at two different

experimental temperatures, 15 °C and 25 °C, are shown in Fig. 16. The threshold for the thermoreceptor currents were lower at 15 °C than at 25 °C (0.9 ± 0.08 A^2 at 15 °C and 1.7 ± 0.09 A^2 at 25 °C for ATC, 1.0 ± 0.07 A^2 at 15 °C and 2.6± 0.19 A^2 at 25 °C for PTC; mean ± S.E. in four to five measurements with different specimens). In contrast, the threshold for mechanoreceptor currents were higher at 15 °C than at 25 °C (24.5 ± 1.79 V at 15 °C and 16.0 ± 2.40 V at 25 °C for AMC, 5.3 ± 0.55 V at 15°C and 1.4 ± 0.15 V at 25 °C for PMC).

ATC saturated (ca. -10 nA per cell) when stimulus strength was as high as about 2 A^2 at 15 °C. The receptor current, however, showed an abrupt increase when the stimulus intensity was higher than 3 A^2. Saturation was not seen at 25 °C in a range of stimulus strength employed. PTC did not saturate even when the stimulus intensity was so high as the specimen lysed upon stimulation at 15 °C and the receptor current was very small at 25 °C.

AMC did not saturate in a range of stimulus strength employed, while the PMC tended to show saturation when the stimulus strength was as high as 10 V. The quasisaturated receptor current was larger at 25 °C (ca. 10 nA per cell) than that at 15 °C (ca. 6 nA per cell). When the stimulus strength was higher than 20 V, the receptor current tended to increase again. This tendency was more remarkable at 25 °C than at 15 °C.

Effect of T_e on the receptor currents.

As described in the previous section, the experimental temperature affected the receptor currents. To examine more precisely the effects of experimental temperature on the receptor currents the intensity of each receptor current evoked by a stimulus at a definite strength was determined at experimental temperatures ranging from 15 to 30°C. The stimulus strength was 2.0 A^2 for thermal stimulation, 25.0 V for anterior mechanical stimulation and 7.5 V for posterior mechanical stimulation, respectively.

As shown in Fig. 17, ATC decreased to its smallest value, while AMC increased to its largest value, by raising the experimental temperature from 15 °C to 27 °C. On the other hand, PTC decreased to 0, while PMC increased to a more-or-less fixed value as the experimental temperature rose from 15 °C to 25 °C.

Effects of the membrane potential level on the receptor currents

To determine effects of the membrane potential level on the receptor currents, each receptor current evoked by a stimulus of a definite intensity (2 A^2 for thermal stimulation, 25 V for anterior mechanical stimulation and 7.5 V for posterior mechanical stimulation) was determined while the membrane potential level was held at various levels in a range from -100 mV to 70 mV. A stimulus was applied to the specimen 300 ms (for thermal stimulation) or 800 ms (for mechanical stimulation) after the membrane potential level was held at a potential level. The experimental temperature was 15 °C for thermal stimulation and 25 °C for mechanical stimulation.

A representative series of traces for each receptor current is shown in Fig. 18 together with each corresponding plot of the peak value for the receptor current against the membrane potential level (the I-V relationship). The peak value was determined as a difference between a value for the membrane current measured immediately before stimulation (the steady membrane current) and a peak value for the membrane current during subsequent stimulation.

ATC decreased as the membrane potential level was made more positive than the resting potential level (-26.3 ± 1.3 mV; mean ± S.E., n = 15). Sign-reversal of the receptor current took place at a membrane potential level of about -5 mV (-3.7 ± 3.8 mV; n=7; reversal potential). The sign-reversed (outward) current increased as the membrane potential level was made more positive than the reversal potential level. On the other hand,

the inward ATC increased as the membrane potential level was made more negative than the resting potential level. However, it tended to decrease when the membrane potential level was more negative than about -40 mV.

PTC decreased as the membrane potential level was made more negative than the resting potential level. Sign-reversal of the receptor current took place at a membrane potential level of about -60 mV (-52.1± 5.9 mV; n=5). The sign-reversed (inward) current increased as the membrane potential level was made more negative than the reversal potential level. On the other hand, the outward PTC increased as the membrane potential level was made more positive than the resting potential level. However, it abruptly decreased when the membrane potential level was made more positive than about 10 mV, and only a very small outward current was observed at a membrane potential level of 70 mV.

AMC decreased as the membrane potential level was made more positive than the resting potential level (-4.4 ± 4.0 mV; n= 9). Sign-reversal of the current took place at a membrane potential level of about 20 mV (9.9 ± 11.5 mV: n=5). The sign-reversed (outward) current increased as the membrane potential level was made more positive than the reversal potential. The inward AMC increased as the membrane potential level was made more negative than the resting potential level. However, it tended to decrease as the membrane potential level was made more negative than -40 mV.

PMC decreased as the membrane potential level was made more negative than the resting potential level. Sign-reversal of the receptor current took place at a membrane potential level of -50 mV (-58.1±5.1 mV: n=5). The sign-reversed (inward) current increased as the membrane potential level was made more negative than the reversal potential. However, it tended to decrease as the membrane potential level was made more

negative than about -60 mV. The outward PMC increased as the membrane potential level was made more positive than the resting potential level. Whereas, it abruptly disappeared as the membrane potential level was more positive than about -5 mV.

Concentration effects of some cations on the receptor currents

To identify species of ions which carry the receptor currents, effects of external concentration of several common cations such as, Na^+, K^+, Rb^+, Mg^{2+}, Ca^{2+} and Mn^{2+} on the reversal potential level for each receptor current were examined. Stimulus strength was 2 A^2 for thermal stimulation, 25 V for anterior mechanical stimulation and 7.5 V for posterior mechanical stimulation. The experimental temperature was 15 °C for thermal stimulation, and 25 °C for mechanical stimulation.

As shown in Fig. 19A, an increase in the external Ca^{2+} concentration, $[Ca^{2+}]_o$, at a constant $[K^+]_o$ (4 mmol l^{-1}) brought about a marked shift of the reversal potential level towards the positive direction in both ATC and AMC (22.1 mV/log$[Ca^{2+}]_o$ for ATC and 20.7 mV/log$[Ca^{2+}]_o$ for AMC; Table 1). In contrast, the reversal potential level shifted slightly towards the negative direction as $[Ca^{2+}]_o$ increased in both PTC and PMC (-0.4 mV/log$[Ca^{2+}]_o$ for PTC and 0.7 mV/log$[Ca^{2+}]_o$ for PTC; Table 1).

On the other hand, as shown in Fig. 19B, an increase in the external K^+ concentration, $[K^+]_o$, at a constant $[Ca^{2+}]_o$ (1 mmol l^{-1}), brought about a marked shift of the reversal potential level towards the positive direction in both PTC and PMC, while it brought about only a slight shift in ATC and AMC (39.0 mV/log$[K^+]_o$ for PTC; 53.0 mV/log$[K^+]_o$ for PMC; 3.4 mV/log$[K^+]_o$ for ATC; 17.7 mV/log$[K^+]_o$ for AMC; Table 1).

When Mg^{2+} or Mn^{2+} was added to the external standard saline solution by 8 mmol l^{-1}, the reversal potential level shifted towards the positive direction for both ATC and AMC (13.8 mV/log$[Mg^{2+}]_o$, 15.7 mV/log$[Mn^{2+}]_o$ for ATC and 7.9 mV/log$[Mg^{2+}]_o$, 8.6 mV/log$[Mn^{2+}]_o$ for AMC; Table 1) . The reversal potential level for both PTC and PMC also shifted towards the positive direction with increasing $[Mg^{2+}]_o$ or $[Mn^{2+}]_o$, but to a

lesser degree than ATC and AMC (5.1 mV/log$[Mg^{2+}]_o$, 3.7 mV/log$[Mn^{2+}]_o$ for PTC and 8.7 mV/log$[Mg^{2+}]$, 5.9 mV/log$[Mn^{2+}]_o$ for PMC; Table 1) .

When external Rb^+ concentration, $[Rb^+]_o$ was increased while $[Ca^{2+}]_o$ was kept constant at 1 mmol l^{-1}, the reversal potential level for both PTC and PMC shifted towards the positive direction similarly to an increase in $[K^+]_o$ (37.9 mV/log$[Rb^+]_o$ for PTC and 35.5 mV/log$[Rb^+]_o$ for PMC; Table 1). The reversal potential level for both ATC and AMC also shifted towards the positive direction with increasing $[Rb+]_o$, but to a lesser degree than PTC and PMC (13.7 mV/log$[Rb^+]_o$ for PTC and 6.3 mV/log$[Rb^+]_o$ for PMC; Table 1).

An increase in the external concentration of Na^+, $[Na^+]_o$ at a constant $[Ca^{2+}]_o$ (1 mmol l^{-1}) showed little effect on the reversal potential levels for both ATC and PTC (0.2 mV/log$[Na^+]_o$ for ATC and 7.7 mV/log$[Na^+]_o$ for PTC; Table 1). The reversal potential level for AMC shifted towards the positive direction, while that for PMC shifted in the negative direction with increasing $[Na^+]_o$ (25.7 mV/log$[Na^+]_o$ for AMC and -13.0 mV/log$[Na^+]_o$ for PMC; Table 1).

Effects of TEA^+ on the posterior receptor currents

The presence of TEA^+ in the external solution brought about reduction of both PTC and PMC, but did not affect ATC and AMC. Concentration effects of TEA^+ on the receptor currents are shown in Fig. 20. In this figure, the receptor current intensity in the presence of TEA^+ is expressed as a value relative to the current intensity in the absence of TEA^+, P, and plotted against the logarithm of external TEA^+ concentration, $[TEA^+]_o$. A small difference in the plots is found between PTC and PMC. The 'Hill-plot' for the $[TEA^+]_o$-P relationship is shown as an inset of Fig. 20, and will be considered in the discussion section. The receptor currents resumed their respective original values as TEA^+ was removed from the external solution.

Successive application of thermal and mechanical stimulation

In this series of experiments a thermal and a mechanical stimuli were applied to the cell so as both thermo- and mechanoreceptor currents to reach their respective peak levels simultaneously, and summation of the peak values for both currents was examined. The experimental temperature was 25 °C for anterior stimulation experiments and 20 °C for posterior stimulation experiments, because the receptor current intensity was almost the same between thermo- and mechanoreceptor currents at these temperatures, and, therefore, examination of the summation was reliable (Fig. 16). Strength of a stimulus applied to the anterior end of the specimen was 2.6 A^2 for thermal stimulation and 25 V for mechanical stimulation, and that applied to the posterior end of the specimen was 2.3 A^2 for thermal stimulation and 15 V for mechanical stimulation.

Two representative series of traces for the anterior receptor currents (A) and for the posterior receptor currents (P) are shown in Fig. 21. Each series consists of a trace for a current evoked by a thermal stimulus (T), a mechanical stimulus (M), both thermal and mechanical stimuli (T+M), and that for the difference between T and T+M signals ((T+M)-T). As is clear from the T+M traces, the mechanoreceptor current was seen superimposed on the thermoreceptor current evoked by a preceding thermal stimulus. Each (T+M)-T trace was almost identical with each corresponding M trace.

Effect of hyperpolarizing pulse on PTC

In this series of experiments, effects of a membrane hyperpolarization preceding a thermal or mechanical stimulus on PTC and PMC were examined. The membrane potential level of a voltage-clamped specimen was first shifted to a level 25 mV more negative than the resting potential level for 100 ms, then a thermal stimulus or a mechanical stimulus was applied to the posterior region of the cell after various intervals from the end of the preceding hyperpolarization.

The hyperpolarizing step evoked a conspicuous cellular contraction. As shown in Fig. 22A, PMC was not evoked by a mechanical stimulus when the interval was less than about 120 ms, and its amplitude became identical with that of PMC evoked without preceding hyperpolarization when the interval was more than 400 ms.

In contrast to PMC, the magnitude of PTC was not decreased by the preceding hyperpolarization to a level 25 mV more negative than the resting membrane potential (Fig. 22B). The magnitude decreased when the hyperpolarization level was 80 mV more negative than the resting potential level. However, the magnitude became almost the same with that of PTC without preceding hyperpolarization when the interval was more than about 100 ms.

Effect of Ca^{2+} concentration on voltage-dependence of PTC

In this series of experiments, effect of the extracellular concentration of Ca^{2+} on the voltage-dependence for PTC was examined.

As shown in Fig. 23, potentials where PTC was began to decrease in positive potential region were not much changed when the extracellular Ca^{2+} concentration was changed from 0.063 mM to 16 mM. But the rate of decrease in PTC with a change in holding potential was decreased when the extracellular Ca^{2+} concentration was increased.

DISCUSSION

Membrane potential responses and the control of thermoaccumulation

T_e-dependent polarity reversal of the membrane potential response to an overall thermal stimulus and its relationship to thermoaccumulation

Specimens of *P. caudatum* exhibited an avoidance response to a rise in their surrounding temperature (an overall thermal stimulus) when they were in a region with a temperature (T_e) equal to or higher than their culture temperature (T_c). The response was less conspicuous when T_e was lower than T_c (Figs 7 and 8).

Specimens responded to an overall thermal stimulus with a membrane depolarization when T_e was equal to or higher than T_c, but with a membrane hyperpolarization when T_e was lower than T_c (Fig. 9). The suppression of the avoidance response at lower values of T_e is attributable to this polarity reversal of the membrane potential response.

As shown in Figs 10 and 11, a localized thermal stimulus applied to the anterior region of a specimen always produced a membrane depolarization. However, similar stimuli applied to the posterior region produced a membrane depolarization when T_e was equal to or higher than T_c, but a membrane hyperpolarization when T_e was lower than T_c.

Since the cytoplasm of *Paramecium* is virtually isopotential (Eckert and Naitoh, 1970), the membrane potential response of a specimen to an overall thermal stimulus is determined by the algebraic sum of the membrane conductance changes occurring in both its anterior and posterior regions. Therefore, when T_e is lower than T_c, a membrane depolarization generated in the anterior region is reduced in amplitude or overcome (reversed) by a membrane hyperpolarization generated in the posterior region. The overall

response is therefore less depolarizing or even hyperpolarizing. In contrast, when T_e is higher than T_c, the overall change is always depolarizing (anterior and posterior responses are both depolarizing). Therefore, it can be said that the T_e-dependent polarity reversal of the membrane potential response to an overall thermal stimulus is caused by the T_e-dependent polarity reversal of the posterior membrane potential response, although the mechanism for the reversal remains unclear.

The temperature difference between the ends of a specimen ascending a temperature gradient is so small (less than 0.5 °C even in a temperature gradient as sharp as that shown in Fig. 6A) that both ends are subjected to a virtually simultaneous rise in temperature (overall thermal stimulation). It is therefore presumed that a specimen ascending such a gradient produces a membrane hyperpolarization before it reaches the region having a temperature equal to T_c, since the temperature around the specimen (T_e) is lower than T_c. Thus, the specimen continues (or even accelerates) its forward swimming towards the region of higher temperature. In contrast, the membrane of the specimen is depolarized after it has passed over the region, because T_e then exceeds T_c. The specimen consequently makes an avoidance response which returns it to the region closer to T_c. The T_e-dependent polarity reversal must be a major cause of thermoaccumulation of specimens in a region with a temperature equal (or close) to T_c.

It should be noted that the threshold rate of rise in temperature for evoking a membrane potential response was lower for overall stimulation than for localized stimulation (Figs 9 and 10). Overall thermal stimulation affects a wider membrane area for a longer period than dose a localized stimulation. This might be a possible cause of the lower response threshold. Other aspects of thermal stimulation that affect the membrane potential response should be examined in detail.

Change in the optimum temperature for thermoaccumulation caused by a change in the culture temperature

Mendelssohn (1895, 1902) and Jennings (1906) reported that the temperature of the region where specimens of *Paramecium* accumulated differed depending on the temperature at which the specimens had previously been kept equilibrated for several hours. This implies that the temperature at which the polarity reversal of the membrane potential response takes places (the reversal temperature) changes in accordance with the culture temperature. Hennessey and Nelson (1979) reported that the threshold temperature for thermal avoidance in *Paramecium* changed in accordance with the culture temperature.

It was found that a hyperpolarizing response to an overall thermal stimulus was seen whenever T_e was lower than T_c, irrespective of the value of T_c (Fig. 9). This indicates that the reversal temperature dose shift according to T_c as predicted by the behavioral observations.

It was also demonstrated that a plot of the amplitude of the posterior hyperpolarizing response to a localized thermal stimulus against T_e shifted in conjunction with T_c (Fig. 12B), while a corresponding plot of the anterior depolarizing response shifted only slightly (Fig. 12A). It is therefore concluded that the T_c-dependent shift of the reversal temperature is attributable to the T_c-dependent shift of the posterior hyperpolarizing response. The mechanism underlying this shift remains unclear.

The peak of the plot of the amplitude of the posterior depolarizing response against T_e also shifted in conjunction with T_c. The peak always occurred at the value of T_e that was equal to T_c (Fig. 12B). The anterior and posterior depolarizing responses seem to combine to cause the specimen to exhibit an avoidance response to a thermal stimulus. The specimen thus remains in the area with a temperature equal or close to T_c.

Martinac and Machemer (1984) found that the input resistance of a specimen of *Paramecium* was higher when T_e was lower than T_c, whereas the voltage-activated maximum calcium conductance was not much affected by lowering T_e (see also Inoue and

Nakaoka, 1990). We found that the amplitude of the anterior depolarizing response increased as T_e decreased (Figs 11A and 12A). The increased amplitude of this response might be attributable to an increased input resistance at low values of T_e if the heat-activated conductance change is not greatly affected by lowering T_e.

Nakaoka et al.(1987) found that in dissected fragments of *P. multimicronucleatum*, the membrane potential response to a thermal stimulus was hyperpolarizing in anterior fragments, but depolarizing in posterior fragments. Recently Matsuoka et al. (1991) reported that the dissected fragments of *Blepharisma* elicit the ciliary reversal to the rise in temperature in posterior fragment and repression of the spontaneous ciliary reversal in anterior fragment. Our results for the posterior region are consistent with their observations, while those for the anterior region are inconsistent. Although the causes of the discrepancy have not been determined, it is highly probable that regenerated membrane at the cut end of a cell fragment will have thermoreceptive properties different from those of normal membrane. This problem should be further examined. It should be noted that many pioneering workers, such as Jennings and Jamieson (1902), Alverdes (1923), Koehler (1939) and Kamada and Kinosita (1940), examined thermal and/or chemical sensitivity in fragmented *Paramecium*.

A thermal stimulus or a mechanical stimulus

It is well known that mechanical stimulation of the anterior region of *Paramecium* produces a depolarizing mechanoreceptor potential, while mechanical stimulation of the posterior region results in a hyperpolarizing one (Naitoh and Eckert, 1969a). Application of current pulse to the microheater caused an elongation of the heater, as well as expansion and convection of the solution around the heater. This heat-mediated mechanical turbulence might activate the mechanoreceptor channels. However, tapping the microheater, or squirting the bath solution against the specimen did not evoke mechanoreceptor potentials. It is, therefore, highly probable that the membrane potential responses caused by

application of an electric current to the microheater are caused by heat and not by mechanical turbulences.

Frequent mechanical stimulus applied by vibrating bathing solution or constant membrane deformation by osmotic pressure did not cause adaptation in mechanosensitivity in a specimen. In some specimens, however, mechanosensitivity was decreased while thermosensitivity was kept unaffected by these procedures, or vice versa. This implies the deterioration of the cell can affect mechanosensitive mechanism and thermosensitive mechanism differently. Therefore, the mechanisms responsible to mechanosensitivity could be different from that to thermosensitivity.

Ionic mechanism for membrane potential responses to thermal stimulus

As shown in Fig. 13, peak value of the anterior depolarizing membrane potential response increased with increase in extracellular Ca^{2+} concentration, while that of the posterior hyperpolarizing membrane potential response was unchanged. On the other hand, peak value of the posterior hyperpolarizing membrane potential response increased with increase in extracellular K^+ concentration, while that of the anterior depolarizing membrane potential response was not much changed. This implies the anterior depolarizing membrane potential response was caused by currents carried by Ca^{2+}, while posterior hyperpolarizing membrane potential response was by K^+, though the effects of voltage dependent channels were not negligible.

It should be note here that 16 $mmol^{-1}$ of Co^{2+} affects T_e-dependence of the posterior response to a thermal stimulus, whereas Ca^{2+} and K^+ did not affect. The posterior membrane potential response was not changed when T_e was lowered below T_c. The meanings of this change in T_e-dependence is not clear.

The thermoreceptor current responses and its comparison to the mechanoreceptor currents.

Localized thermal stimulation of *P. caudatum* with a microheater (Tominaga and Naitoh, 1992a) revealed that ion channels responsible for the inward thermoreceptor current are present predominantly in the anterior region of the cell, while those responsible for the outward thermoreceptor current are present predominantly in the posterior region of the cell (Fig. 14T). The distribution of these thermoreceptor channels resembles that of the mechanoreceptor channels, i.e. ion channels responsible for the inward mechanoreceptor current are present predominantly in the anterior region, while those for the outward mechanoreceptor current are present predominantly in the posterior region of the cell (Fig. 14M) (Naitoh and Eckert, 1969a; Ogura and Machemer, 1980). As shown in Fig. 15, the anterior and posterior thermoreceptor current was kept intact in a deciliated specimen. Hence, the thermoreceptor channels are present on a somatic membrane. The mechanoreceptor channels is known to present in a somatic membrane (Ogura and Machemer, 1980).

The receptor currents increased with increasing the stimulus strength, tending to reach a saturated level, but they increased further with further increase in the stimulus strength (Fig. 16). This unorthodox shape of the stimulus strength-response curve is attributable most probably to combined effects of an increase in the stimulus strength and the concomitant spread of the stimulated area along the membrane. The absence of saturation in AMC is attributable to lower mechanical sensitivity of the anterior region of the cell (Naitoh and Eckert, 1969a).

It can be assumed that the receptor currents described in this paper are carried by Ca^{2+} and/or K^{+} in the mixtures of KCl and $CaCl_2$ employed, since membrane conductance to anions is negligible in the cell of *Paramecium* (Kamada, 1934; Tominaga and Naitoh,

1992a). Therefore, the reversal potential levels for the receptor currents are determined by membrane conductances to both Ca^{2+} and K^+ (g_{Ca} and g_K) and concentrations of these cations.

The fractional conductance to Ca^{2+}, T_{Ca} and that to K^+, T_K during thermal or mechanical stimulation were estimated by introducing the value for the rate of shift in the reversal potential level per ten-fold change in $[Ca^{2+}]_o$ and that in $[K^+]_o$ (see Appendix section B and Table 1) respectively to equation 6 in Appendix section B, and these are shown in Table 2.

The higher T_{Ca} value (and therefore lower T_K value) during anterior stimulation than during posterior stimulation supports the idea that thermal or mechanical stimulation of the anterior region of the cell predominantly activates Ca^{2+} receptor channels. On the other hand, higher T_K value (and therefore lower T_{Ca} value) during posterior stimulation than during anterior stimulation supports the idea that thermal or mechanical stimulation of the posterior region of the cell activates predominantly K^+ receptor channels.

The fractional conductances of thermally or mechanically stimulated membrane to various cations other than Ca^{2+} and K^+, such as Mg^{2+}, Mn^{2+}, Rb^+ and Na^+, were also estimated from the rate of shift in the reversal potential level per ten-fold change in concentration of corresponding cation (Table 1)(see Appendix section B), and are also shown in Table 2. T_{Mg} and T_{Mn} for thermally or mechanically stimulated anterior region of the cell were higher than those for the posterior region. These facts suggest that the stimulus-activated Ca^{2+} receptor channels are permeable also to Mg^{2+} and to Mn^{2+}. The degree of permeation of these cations, however, is smaller than that of Ca^{2+}, as shown by the values for T_{Mg} and for T_{Mn} smaller than that for T_{Ca}.

Naitoh and Eckert (1972b) reported that Mn^{2+} did not reduce the amplitude of the depolarizing membrane potential response of *P. caudatum* to mechanical stimulation of its anterior end. De Peyer and Deitmer (1980) reported that the mechanosensitive Ca^{2+} channels in *Stylonychia*, a relative of *Paramecium*, were permeable to Mg^{2+} in a degree similar to that to Ca^{2+}, while they were blocked to some extent by Mn^{2+}.

The fractional conductance of the membrane to Rb^+, T_{Rb}, during thermal or mechanical stimulation was always higher than that of the anterior membrane. This indicates that thermally or mechanically activated K^+ channels are permeable also to Rb^+. The degree of permeation of Rb^+ is almost identical with that of K^+ when thermally activated, while it was a little lower when mechanically activated (compare the values for T_{Rb} with those for T_K shown in Table 2).

In contrast to Rb^+, the fractional conductance of the membrane to Na^+, T_{Na}, was very low in most cases. This indicates that thermally or mechanically activated receptor channels are almost impermeable to Na^+. The exceptionally high value for T_{Na} during anterior mechanical stimulation is presently unexplained. Naitoh and Eckert (1973) reported that $[Na^+]_o$ did not affect the membrane potential responses to mechanical stimulation in *P. caudatum*.

Externally applied TEA^+ blocked the posterior receptor currents, PTC and PMC (Fig. 20). The inset of Fig. 20 is the plots of the ratio of the number of blocked channels to that of non-blocked channels against $[TEA^+]_o$ both in logarithmic scale (Hill-plot) from which the number of binding sites of the ion channel to TEA^+ (corresponding to the slope of the plot) and the binding constant (K_d) of the binding site to TEA^+ (calculatable from the intersection of the plot with the X-axis, which corresponds to $\log K_d^{-1}$) can be estimated. No significant ($P<0.05$) difference in the number of binding sites was found between the

posterior thermoreceptor channel and the posterior mechanoreceptor channel (0.95 ± 0.10 for the thermoreceptor channel; 1.02 ± 0.07 for the mechanoreceptor channel, mean ± S.D. n=5). However, the binding constant (K_d) was slightly, but significantly ($P<0.05$), different between these two kinds of receptor channels (0.19 mmol l^{-1} for thermoreceptor channel, 0.12 mmol l^{-1} for the mechanoreceptor channel).

Effects of the membrane potential level on the thermoreceptor currents were essentially identical with those on the mechanoreceptor currents (Fig. 18). The similarity in the membrane potential-dependence between ATC and AMC and that between PTC and PMC imply that the charge properties of the ionic pores responsible for the receptor currents are similar between thermoreceptor channels and mechanoreceptor channels.

In spite of many similarities in their characteristics, effects of the experimental temperature on the thermoreceptor currents were dramatically different from those on the mechanoreceptor currents (Figs 16 and 17). These facts suggest that the thermoreceptor currents are dependent on ion channels different from those responsible for the mechanoreceptor currents.

In this connection, it should be noted that when a mechanical stimulus was applied to the membrane where a thermoreceptor current was being produced by a preceding thermal stimulus, a mechanoreceptor current appeared superimposed on a preceding thermoreceptor current (see T+M traces). The intensity of ATC evoked by only a thermal stimulus (trace T) was about 50% of its saturated value (ca. 10 nA, Fig. 16). It is therefore presumed that the intensity of AMC evoked by a subsequent mechanical stimulus should be 50% of the intensity of AMC evoked by only a mechanical stimulus (trace M), if AMC is dependent on the same ion channels responsible for ATC. Subtraction of the trace T from the trace T+M gives an AMC evoked by a subsequent mechanical stimulus. The time course and the peak value of the AMC was almost identical with AMC evoked by only a mechanical stimulus. Similarly to the cases of anterior stimulation, PMC evoked by a mechanical stimulus during

PTC was almost identical with that evoked by only a mechanical stimulus (compare (T+M)-M trace with M trace in Fig. 21). These facts strongly support the idea that the thermoreceptor currents are dependent on ion channels different from those responsible for the mechanoreceptor currents.

It should be noted that the thermoreceptor current decreased, while the mechanoreceptor current increased with raising the experimental temperature (Fig. 17). This fact together with similarities between effects of the external cations and the membrane potential level on the thermoreceptor currents and those on the mechanoreceptor currents suggests a possibility that a thermoreceptor mechanism exclusively shares an ionic pore (Ca^{2+} pore or K^+ pore) with a mechanoreceptor mechanism. Some environmental factors might make one of these two receptor mechanisms apparent. For instance, lowering the experimental temperature makes the thermoreceptor mechanism dominant, while rising the experimental temperature makes the mechanoreceptor mechanism dominant.

In this connection it is interesting to mention that a single-gene mutant of *P. caudatum*, *tsb*(temperature-sensitive behavior; Takahashi, 1979) shows vigorous avoidance response to mechanical agitation as well as a long-lasting backward swimming in response to a raising temperature. These behavioural responses imply that the mutant can not produce hyperpolarizing receptor potential in response to mechanical or thermal stimulus. A membrane hyperpolarization has been known to inhibit avoiding response and backward swimming, and accelerates forward movement (Eckert and Naitoh, 1972; Naitoh, 1974). The abnormal behaviors exhibited by the mutant, therefore, can be well understood if we assume that the mutant has a malfunction with K^+-selective pores which the hypothetical thermoreceptor mechanism shares with the hypothetical mechanoreceptor mechanism.

Effect of Ca^{2+} on the voltage dependence for PTC

Effect of Ca^{2+} concentration on the voltage-dependence of the posterior thermoreceptor currents.

As shown in Fig. 18, PTC decreased when the membrane potential was raised above certain level, which was close to the reversal potential of the ATC. Hence, it can be thought that the PTC was modulated by Ca^{2+}. To examine this possibility, effect of the Ca^{2+} concentration on the current-voltage relation of PTC. As shown in Fig. 23, the level at which PTC began to decrease when the membrane potential was raised was not much changed with change in Ca^{2+} concentration, though the rate of decrease in PTC was raised with decease in Ca^{2+} concentration. Hence, the effect of Ca^{2+} on voltage dependence for PTC seems to be complicated.

Further problems

Recently other adaptation mechanism in relation to the temperature factor was reported by Malvin and Wood (1992). They showed that the hypoxic *Paramecium caudatum* accumulated at lower temperature in temperature gradient and the survival of them was increased at lower temperature. Though the underlying mechanism of this adaptation is unknown, there might be a some changes in membrane potential response as seen in this thesis work. It should be further examined.

The behavioral and membrane potential responses to the fall in temperature was reported by several authors (Tawada and Oosawa, 1972; Nakaoka and Oosawa, 1977; Tawada and Nakaoka, 1979; Nakaoka et al., 1987; Inoue and Nakaoka, 1990; Matsuoka et al., 1991). Though the distribution of the receptors to fall in temperature was examined with a dissected specimens (Nakaoka et al., 1987; Inoue and Nakaoka, 1990; Matsuoka et al., 1991), there might be a difference of the regenerated membrane and the normal

membrane as shown in this thesis work. The local application of the cold stimulus should be examined.

The second aim of this thesis work was to know detailed electrophysiological characteristics of the thermoreception in *Paramecium caudatum* and its comparison to the mechanoreception. The possibility shown in this thesis work that the thermoreceptor mechanism exclusively shares the ionic pore with the mechanoreceptor mechanism is interesting to understand the evolution and the differentiation of receptor channels in a single cell. Mechanoreceptor mechanism is known to be distributed widely among organisms from bacteria to mammals (Morris, 1990). The mechanoreceptor mechanism of the cell is presumed to originate from a fundamental necessity of the cell to detect its osmotic distortion (Hille, 1992). Hence it is interesting to think all sensory channels are evolved from primitive mechanoreceptor channel. There are several papers showing close relationship between thermoreceptor and mechanoreceptor mechanism (Burkhardt, 1959; Hensel, 1973, 1974a, b; Burgess and Perl, 1973; Altner and Loftus, 1985; Gottschaldt, 1985; Gentle, 1989; Gödde and Haug, 1990).

Gödde and Haug (1990) discussed possible physical mechanisms to detect a thermal stimulus at a surface membrane of the cell. They propose an importance of the membrane lipid of the surface membrane on the basis of the energetic consideration of the signal transformation from thermal stimulus to the membrane potential change. On the other hand, Hennessey and Nelson (1979 and 1983) reported that lipid composition of the surface membrane of *Paramecium* changed dependent on its culture temperature. Moreover, the change in lipid composition is in parallel with a change in thermal avoidance behavior. They also propose the importance of membrane lipid in thermoreception mechanisms. It should be further examined.

APPENDIX

A. *Estimations of the fractional conductance of the membrane to various cations and of the membrane conductance to a cation other than Ca^{2+} and K^+ to that to Ca^{2+} or K^+ during activation of a receptor current*

If we assume that a receptor current I_m is carried by n different kinds of ion species, I_m can be written as:

$$I_m = \sum_i^n I_i \qquad (1)$$

where I_i is a component of the receptor current carried by the ion i. Equation 1 can be rewritten by introducing definition of the membrane conductance as:

$$I_m = \sum_i^n g_i(V_m - E_i) \qquad (2)$$

where V_m is a membrane potential, g_i is a membrane conductance to the ion i, and E_i is an equilibrium potential for the ion i. Since the reversal potential for the receptor current, V_r is the membrane potential level at which the net receptor current is 0, equation 2 can be written as:

$$I_m = \sum_i^n g_i(V_r - E_i) = 0 \qquad (3)$$

By rearranging equation 3, V_r can be written as:

$$V_r = \sum_i^n T_i E_i \qquad (4)$$

where T_i is the fractional conductance of the membrane to the ion i, which is defined as the ratio of g_i to the total membrane conductance G ($\sum g_i$). Equation 4 corresponds to the classical Hodgkin-Horowicz's equation (Hodgkin and Horowicz, 1959).

When extracellular concentration of a permeant cation with valency z, X^{z+} ($[X^{z+}]_o$) is varied while extracellular concentration of other permeant ions are kept constant, the relationship between V_r and $[X^{z+}]_o$ can be formulated as:

$$V_r = T_X \frac{RT}{zF} \ln \frac{[X^{z+}]_o}{[X^{z+}]_i} + \sum_j^{n-1} T_j E_j \qquad (5)$$

where R is the gas constant, F is Faraday's constant, T is the absolute temperature and $[X^{z+}]_i$ is X^{z+} concentration in the cytoplasm. This equation indicates that V_r is proportional to the logarithm of $[X^{z+}]_o$. By rearranging equation 5, T_X can be formulated as:

$$T_X = \frac{a_X}{58.2/z} \qquad (6)$$

where a_X is the constant of proportionality between V_r an $\log[X^{z+}]_o$ and 58.2 is a constant at the ambient temperature of 20°C and the voltage is presented in mV.

B. Estimations of intracellular Ca^{2+} and K^+ concentration.

Each line corresponding to the equation 5 with each different T_{Ca} (or T_K) converges a point satisfying the equation,

$$E'_{Ca} = E'_K \qquad (9)$$

$[Ca^{2+}]_o$ (or $[K^+]_i$) can be estimated from the membrane potential value and the values for $[Ca^{2+}]_o$ (or $[K^+]_o$) corresponding to the point satisfying equation 9 according to Nernst's equation.

The lines corresponding to 5 was shown in Fig. 24. When the external Ca^{2+} concentration was changed, the posterior receptor currents was shown that it did not include the Ca^{2+} current. Hence the apparent K^+ equilibrium potential (E'_K) can estimated from an average of posterior thermo- and mechano- receptor current as -54.4 mV. The intracellular K^+ concentration ($[K^+]_i$) was calculated from this E'_K as 34 mmol l^{-1}, the value consistent with that reported by Ogura and Machemer (1980). The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was calculated from the this E'_K and regression lines of reversal potentials for anterior thermo- and mechano-receptor currents, as 0.2 mmol l^{-1} and 0.02 mmol l^{-1} respectively (Fig. 24A). The value 0.2 mmol l^{-1} was identical with that reported for mechanoreceptor current by Ogura and Machemer (1980). This $[Ca^{2+}]_i$ was

far greater than the predicted $[Ca^{2+}]_i$ (10^{-7} mol l^{-1} ; Eckert et al., 1976), Ogura and Machemer (1980) discussed this high $[Ca^{2+}]_i$ is due to a localized increase in $[Ca^{2+}]_i$ following a Ca^{2+} influx. Difference in $[Ca^{2+}]_i$ between thermo- and mechano- receptor current might due to a effect of CsCl loaded when the response of the anterior mechanoreceptor current was measured. The apparent Ca^{2+} equilibrium potential was predicted from the cross point of the regression lines for reversal potentials for anterior and posterior receptor current as 5.1 mV for thermoreceptor current and 48.51 mV for mechanoreceptor current (Fig. 24B). From these values $[Ca^{2+}]_i$ was predicted as 0.5 mmol l^{-1} and 0.02 mmol l^{-1} for thermoreceptor current and mechanoreceptor current. This value is identical with the value calculated from a reversal potentials when $[Ca^{2+}]_o$ was changed.

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ABSTRACTS

1. The overall membrane potential response of the ciliate *Paramecium caudatum* to a rise in the temperature of its environment was depolarizing when the ambient temperature before stimulation (T_e) was equal to or higher than the culture temperature (T_c), but hyperpolarizing when T_e was lower than T_c.

2. The anterior region of the cell responded to a rise in temperature with a localized membrane depolarization. The posterior region was depolarized when T_e was equal to or higher than T_c, but hyperpolarized when T_e was lower than T_c. The T_e-dependent polarity reversal of the posterior response was responsible for the comparable reversal of the overall response.

3. The temperature at which the polarity reversal of the posterior response took place shifted according to T_c. This shift caused a comparable shift in the temperature at which polarity reversal occurred for the overall response.

4. The T_e-dependent polarity reversal of the posterior response and its T_c-dependence re major causes of thermoaccumulation mediated by ciliary activity of *Paramecium* in regions with temperatures close to T_c.

5. A voltage-clamped *Paramecium* produced an inward membrane current upon thermal or mechanical stimulation of its anterior region, while it produced an outward membrane current upon similar stimulation of its posterior region.

6. Anterior thermo- and mechanoreceptor currents decreased when the membrane potential level was shifted in a positive direction, showing sign-reversal at a positive membrane potential level, while posterior thermo- and mechanoreceptor currents decreased when the membrane potential level was shifted in a negative direction, showing sign-reversal at a membrane potential level more negative than the resting potential level.

7. The reversal potential levels for both anterior receptor currents shifted in a positive direction when $[Ca^{2+}]_o$ was increased, while those for both posterior receptor currents shifted in a positive direction when $[K^+]_o$ was increased.

8. Similar $[Mg^{2+}]_o$, $[Mn^{2+}]_o$, $[Na^+]_o$, $[Rb^+]_o$ and $[TEA^+]_o$ effects were observed on the thermo- and mechanoreceptor currents.

9. Thermoreceptor currents decreased whereas mechanoreceptor current increased as the ambient temperature was raised.

10. When a mechanical stimulus was applied to the membrane where a thermoreceptor current was being produced, an algebraic summation of these receptor currents occurred.

11. It is concluded that thermoreceptor currents are dependent on ion channels different from those responsible for the mechanoreceptor currents, though ionic pores for the channels are similar with each other in various respects.

12. A possibility that a thermoreceptor mechanism exclusively shares a Ca^{2+} pore in the anterior membrane, or a K^+ pore in the posterior membrane, with a mechanoreceptor mechanism was discussed.

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FIGURES AND TABLES

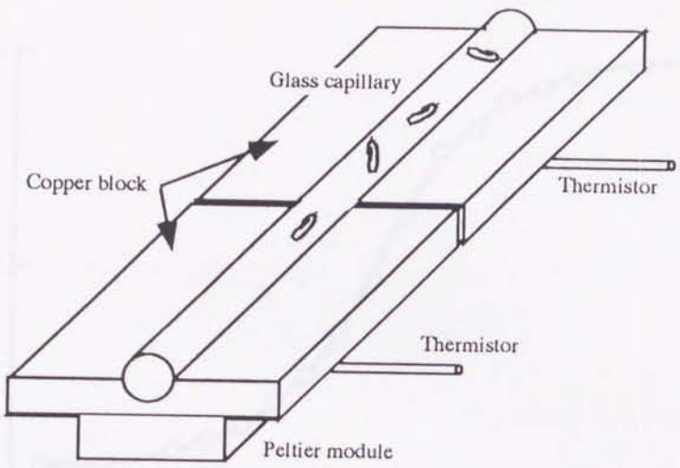


Fig. 1. Diagrammatic illustration of a experimental vessel to examine the locomotor activity of specimens of *Paramecium* in the boundary between two regions with different temperatures. See the text for detail.

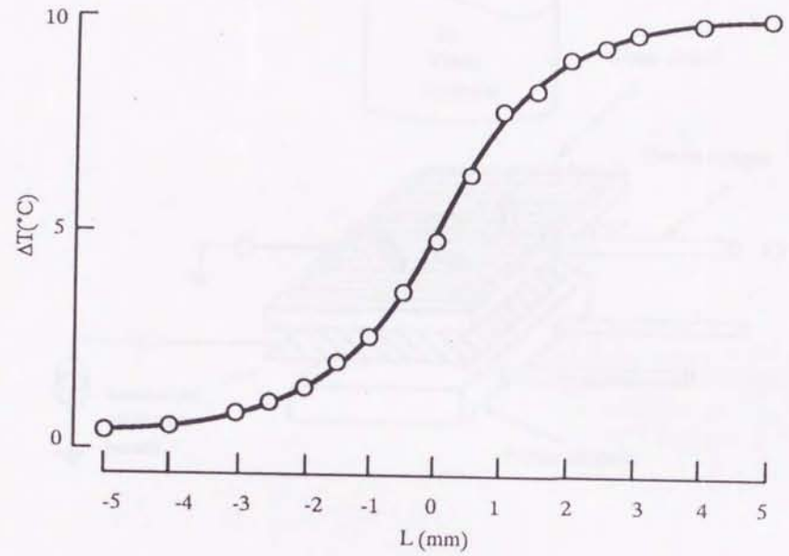


Fig. 2. A typical temperature profile of saline in a glass capillary at the boundary between two regions with different temperatures. The temperature at each position along the capillary measured with reference to the lower temperature (ΔT) is plotted against the distance (L) from the center of the boundary (positive towards the higher temperature region, negative towards the lower temperature region).

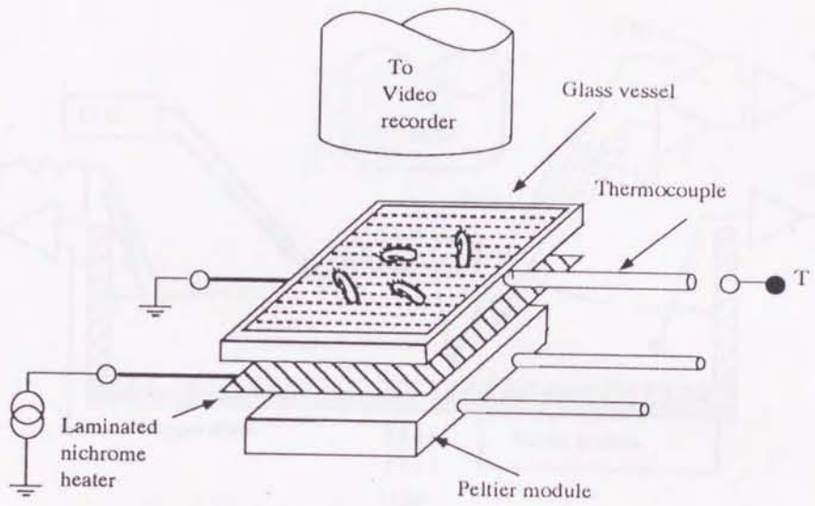


Fig. 3. A diagrammatic illustration of the experimental vessel to assay a locomotor response to an sudden rise in temperature in *Paramecium caudatum.* A thin-walled glass (0.1 mm) rectangular glass vessel (15 mm x 15 mm x 0.2 mm) which temperature was monitored thin thermocouple (0.2 mm tip diameter) was put on a laminated nichrome heater, the heat produced by this heater caused a sudden rise in vessel temperature by giving a constant current pulse. The temperature of the vessel and the heater was kept constant by the copper block (15mm x 15 mm x 5 mm) put beneath it. The temperature of the copper block was controlled electronically controlled feed back system consisted of the thermistor to monitor the temperature of the block and the Peltier module beneath it.

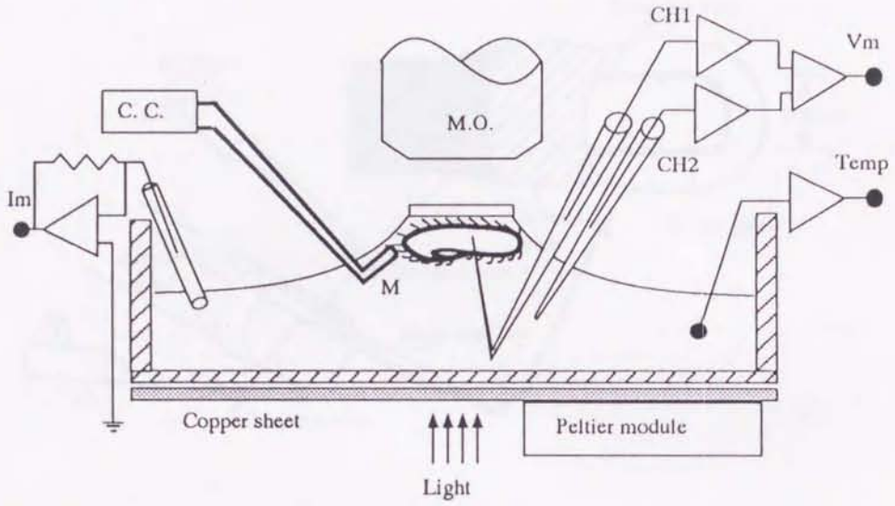


Fig. 4. The experimental setup to measure the membrane potential and the membrane current response to the thermal stimulus in *Paramecium caudatum.* The membrane potential of a specimen impaled by a glass capillary was measured as a difference of the potential at the tip of the glass microelectrode impaling the specimen (Ch1), and the medium potential measured at the tip of the microelectrode put close to the specimen (Ch2). The medium potential is virtually grounded through the current sink connected to the current-voltage converter. Under voltage clamp condition the membrane potential was clamped by a negative feedback loop, which is consisted of the membrane potential monitor and the current injection amp. The feedback current was given to the specimen through third microglass electrode shown in left-hand side. The current through the membrane of the specimen was monitored the current-voltage converter. The thermal stimulus was given by a heat production at the tip of the microheater (M) put close to the specimen by a constant current pulse. The experimental vessel was put on a copper sheet (1 mm thickness) and the temperature of medium in it was controlled by the thermistor and the peltier module. All the micromanupulation was done under the microscope (m.o.).

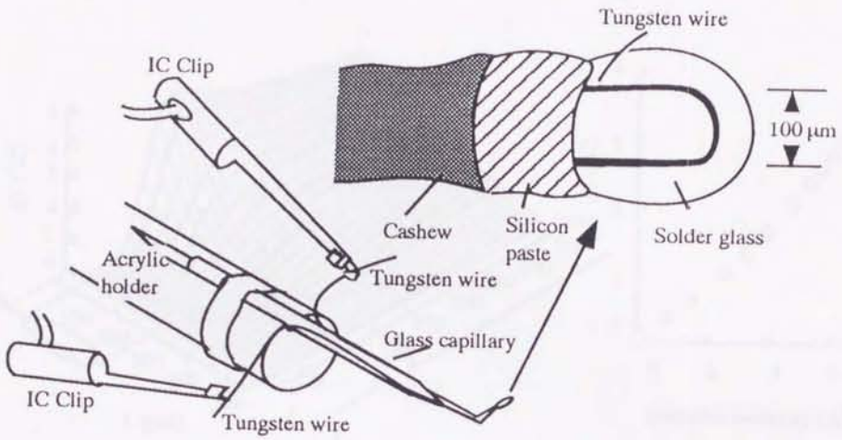


Fig. 5. Diagrammatic illustrations of a microheater for thermal stimulation of a specimen of *Paramecium* impaled by microelectrodes. See the text for details.

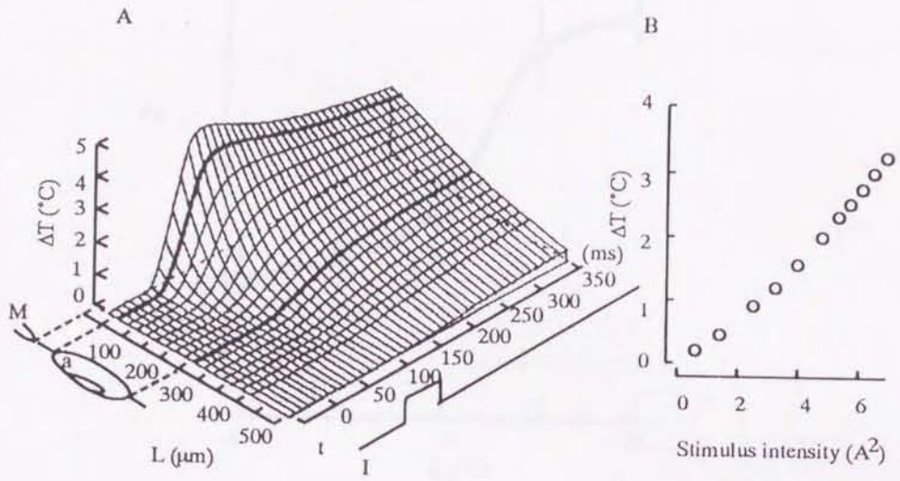


Fig. 6. (A) Time courses of the change in temperature of the solution (ΔT) at different distances from the tip of the heater (L) after application of an electric current pulse (I: 2.6A, 50ms) to the heater. t; time after onset of the electric current pulse (ms). The schematic illustration of *Paramecium* to the left of the L-axis shows the position of an impaled specimen in the experimental chamber relative to the tip of the microheater (M). Its anterior (a) and posterior ends are 50μm, and 250μm from the heater, respectively. The time courses of Δt at both ends of the animal are shown with thicker lines. (B) The relationship between the peak rise in temperature in a region 50μm from the tip of the microheater and the square of the current intensity applied to the heater. Each symbol is the mean of three measurements. Error bars are omitted, since they are smaller than the symbol.

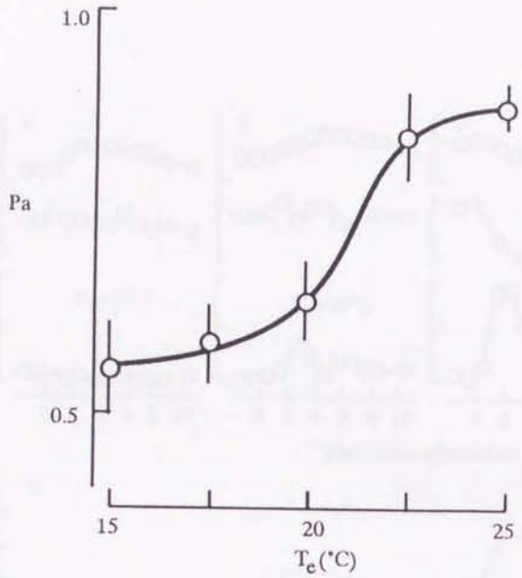


Fig. 7. The strength of the avoidance response (Pa) exhibited by *Paramecium caudatum* encountering a boundary to a higher temperature region is plotted against the temperature of the lower temperature region (T_e). Each symbol is the mean (±S.E.) for five measurements, each of 10-50 specimens. The line of best fit was drawn by hand. Pa is the ratio of the number of specimens that failed to enter the boundary region to the total number of specimens encountering the boundary. See the text for details.

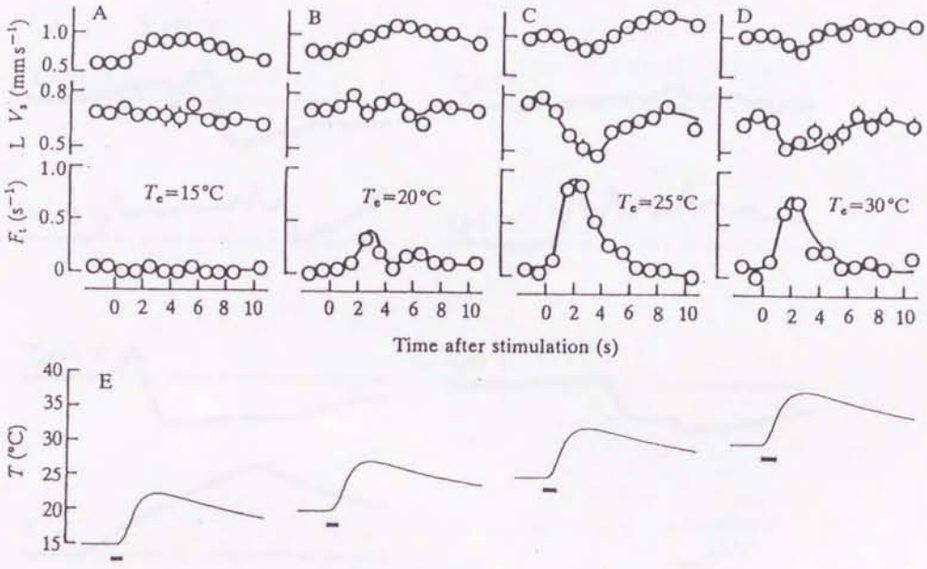


Fig. 8 . Responses of *Paramecium caudatum* to a sudden rise in the temperature of their surrounding medium from various experimental temperatures (T_e): A, 15 °C; B, 20 °C; C, 25 °C; D, 30 °C). V_S, swimming velocity; L, linearity of the swimming path; F_t, turning frequency. (E) The time course of the change in temperature. Each symbol is the mean (±S.E) of 30-50 measurements of 30- 50 different specimens. Error bars are omitted when they are smaller than the symbol. The line of best fit was drawn by hand. See the Materials and methods section for details concerning the evaluation of motile activities.

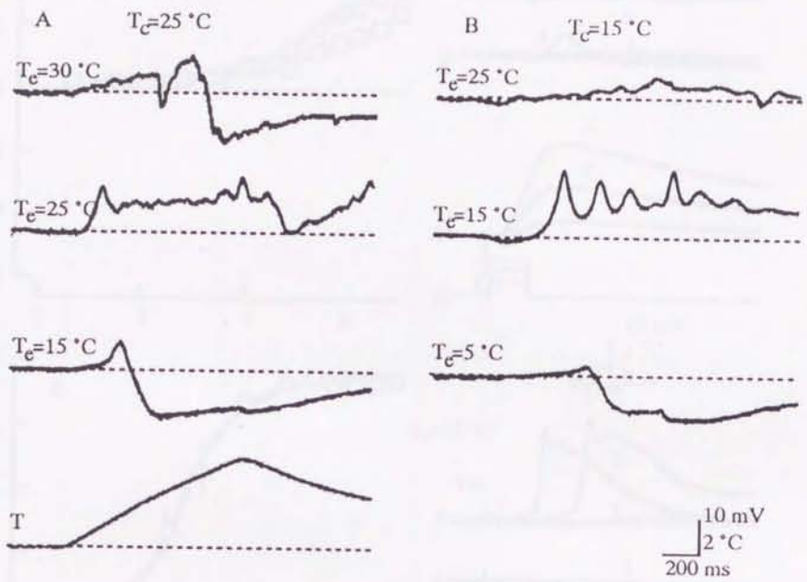


Fig. 9. Membrane potential responses of *Paramecium caudatum* to an overall thermal stimulus. (A) Responses of a specimen cultured at 25 °C. T, time course of the change in temperature measured at the mid-point of the specimen. (B) Responses of a specimen cultured at 15 °C. Numbers to the left of each trace indicate T_e (°C).

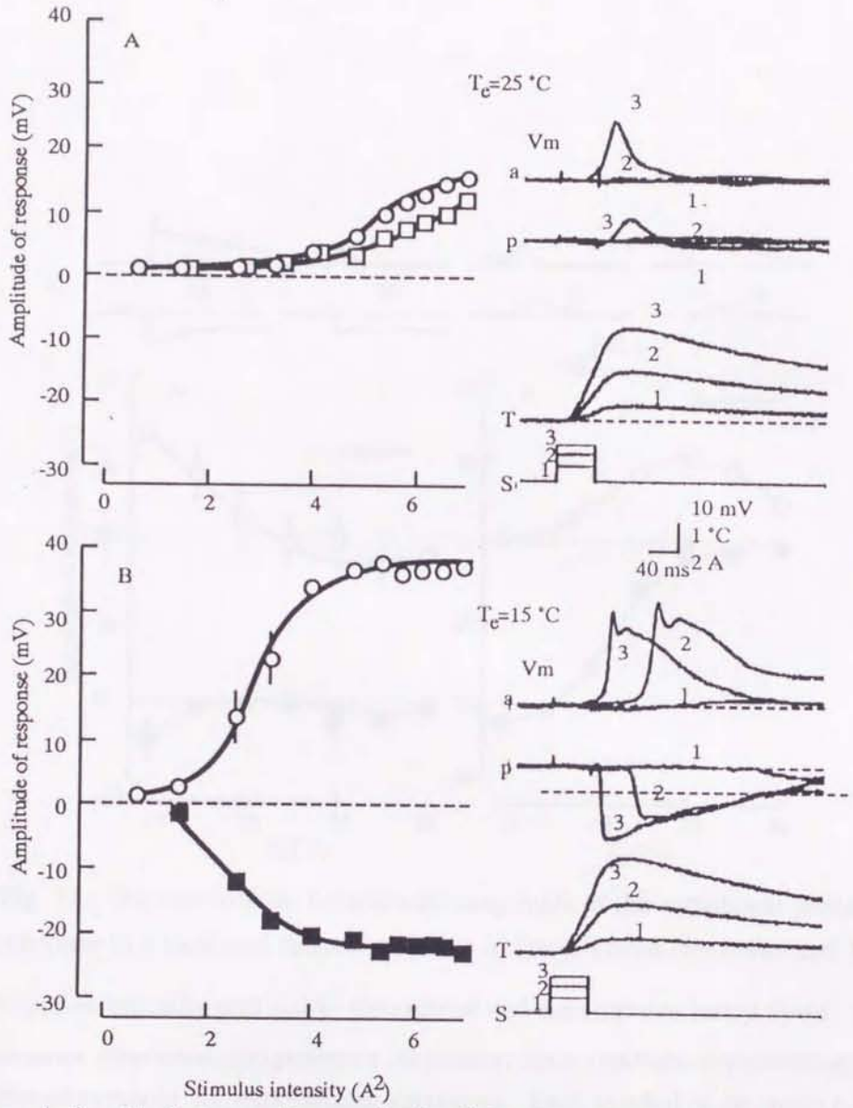


Fig. 10. The relationship between the amplitude of the membrane potential response of *Paramecium caudatum* to a localized thermal stimulus and the stimulus intensity obtained at two different values of T_e (A, 25 °C; B, 15 °C). The stimulus intensity is given by the square of the amplitude of the electric current pulse applied to the microheater (A^2). Pulses was 50 ms long. Circles, anterior responses; Squares, posterior responses; open symbols, depolarizing response; filled symbols, hyperpolarizing response. Each symbol is the mean (±S.E.) of five measurements using different specimens. Error bars are emitted when they are smaller than the symbol. The line of best fit was drawn by hand. Traces to the right are representative examples of the membrane potential response obtained in two different animals, one for T_e=25 °C (A) and the other for T_e=15 °C (B). Vm, membrane potential (a, anterior responses; p, posterior responses); T, change in temperature around one end of the specimen; S, electric current pulse applied to the microheater. The specimens were cultured at 25 °C.

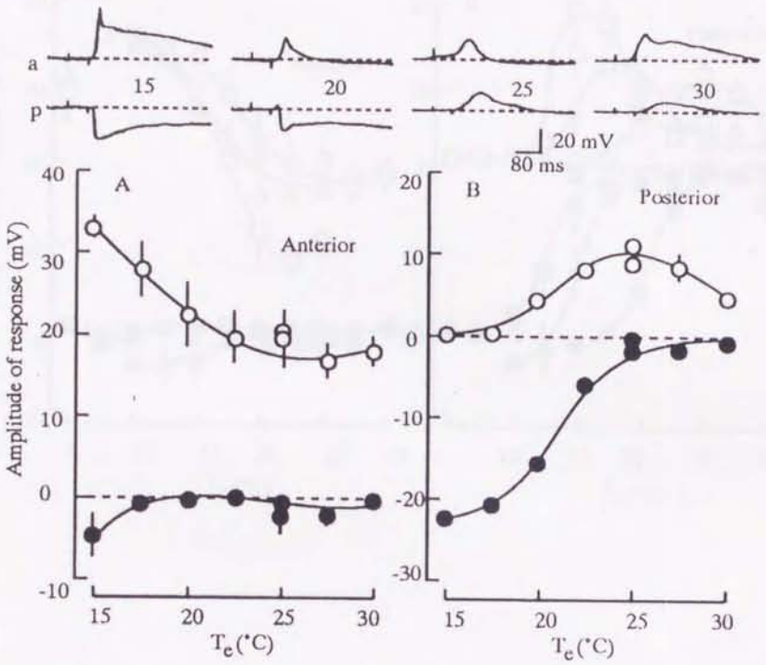


Fig. 11. The relationship between the amplitude of the membrane potential response to a localized thermal stimulus in *Paramecium caudatum* and T_e. The stimulus intensity was $7.0A^2$ throughout and the stimulus lasted 50ms. (A) anterior responses; (B) posterior responses; open symbols, depolarizing responses; closed symbols, hyperpolarizing responses. Each symbol is the mean (±S.E.) of five measurements with different specimens. Error bars are omitted when they are smaller than the symbol. Each series of results consisted of two sub-series, one for values of T_e from 25 °C to 30 °C, and the other for values of T_e from 25 °C to 15 °C. The lines of best fit were drawn by hand. The upper traces show representative membrane potential responses obtained at different Te values (15, 20, 25 and 30 °C)(a, anterior responses; p, posterior responses). Small two successive spike-like artifacts seen on each potential trace correspond to turning on (positive deflection) and turning off (negative deflection) the electric pulse applied to the microheater, respectively.

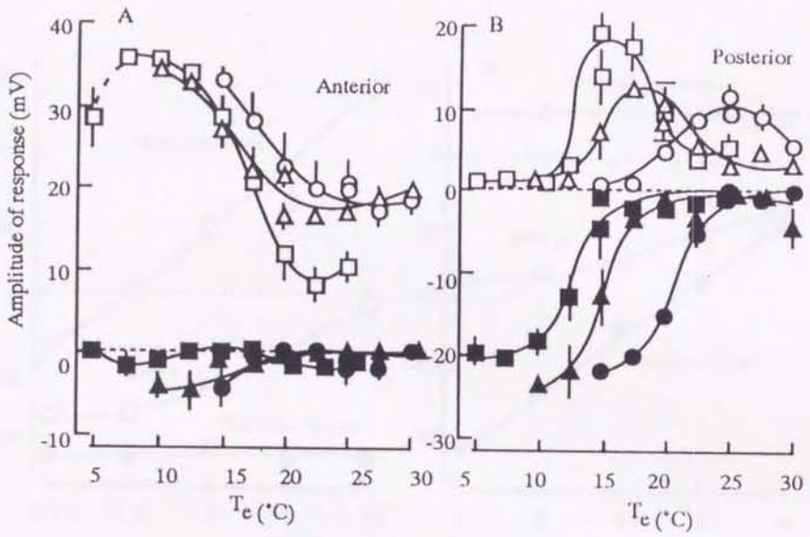


Fig. 12. Influence of the culture temperature (T_c) on the relationship between the amplitude of the membrane potential response to a localized thermal stimulus and T_e in *Paramecium caudatum*. The stimulus intensity was $7.0A^2$ (50ms) throughout. (A) anterior responses; (B) the posterior responses. Open symbols, depolarizing responses; filled symbols, hyperpolarizing responses. Circles, triangles and squares correspond to the responses of the specimens cultured at 25 °C, 20 °C and 15 °C, respectively. Each symbol is the mean (±S.E.) of five measurements using different specimens. Error bars are omitted when they are smaller than the symbol. Horizontal short bars seen at T_e of 20 °C in B indicate the range of S.E. for the open square plot. Each series of results at each T_c consists of two sub-series: one for values of T_e from each T_c to the upper temperature, and the other for temperatures below T_c. The line of best fit were drawn by hand.

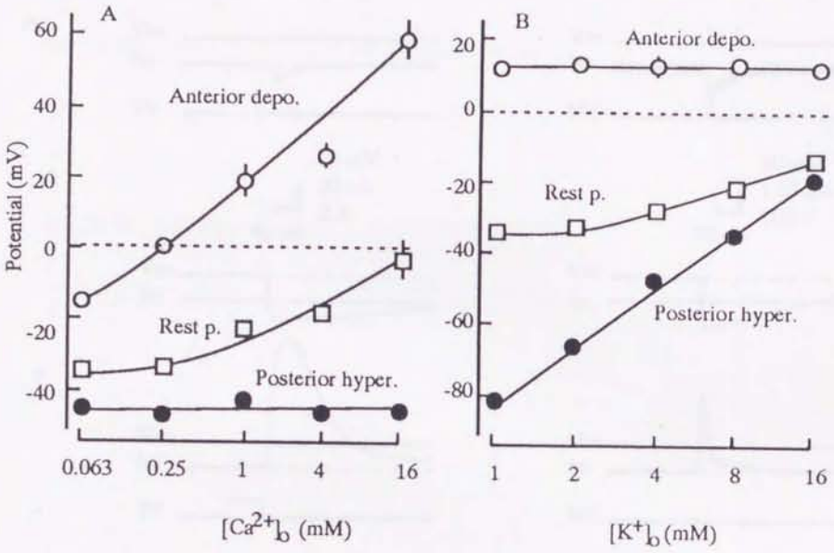


Fig. 13 Relation between strength of the membrane potential response and extracellular concentration of Ca^{2+} (A) and K^+ (B) to a localized thermal stimulus applied to the anterior and the posterior portion of a specimen of *Paramecium caudatum*. Open circle; anterior depolarization. Closed circle; posterior hyperpolarization. Open squarre; resting potential.

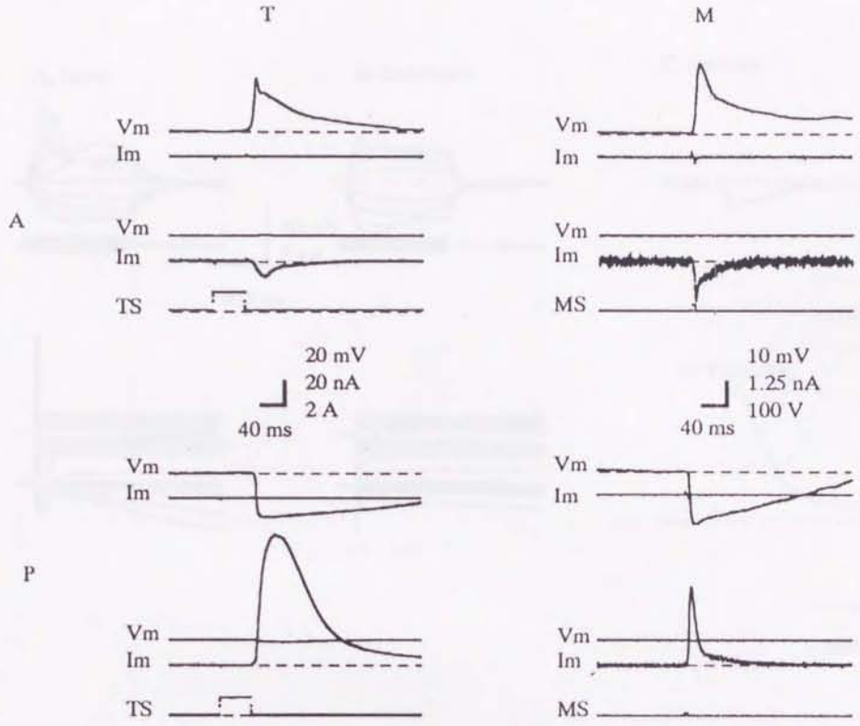


Fig.14. Membrane potential and current responses to thermal or mechanical stimulation of *Paramecium caudatum.* Column T, responses to thermal stimulation; column M, responses to mechanical stimulation. Line A, responses to stimulation of the anterior region of the cell; line P, responses to stimulation of the posterior region of the cell. Vm, membrane potential; Im, membrane current; TS, current pulse applied to microheater for thermal stimulation; MS, voltage pulse applied to a piezoelectric phonocartridge to drive a microneedle for mechanical stimulation. Stimulus strength was 2 A^2 for thermal stimulation, 25 V for anterior mechanical stimulation and 7.5 V for posterior mechanical stimulation. The experimental temperature was 15 °C in thermal stimulation experiments, while it was 25 °C in mechanical stimulation experiments.

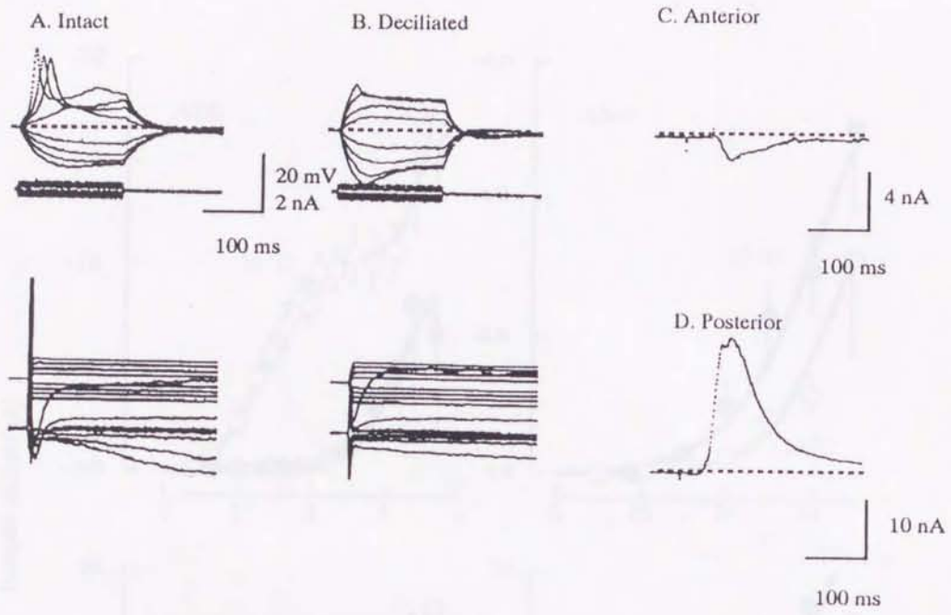


Fig. 15 Representative traces of membrane potential response and membrane current response to injected current and membrane potential change and receptor current to thermal stimulus in intact and deciliated specimen of *Paramecium caudatum*. A. membrane electric response in intact cell. B. membrane electric response in deciliated cell. C and D. thermoreceptor current in deciliated cell.

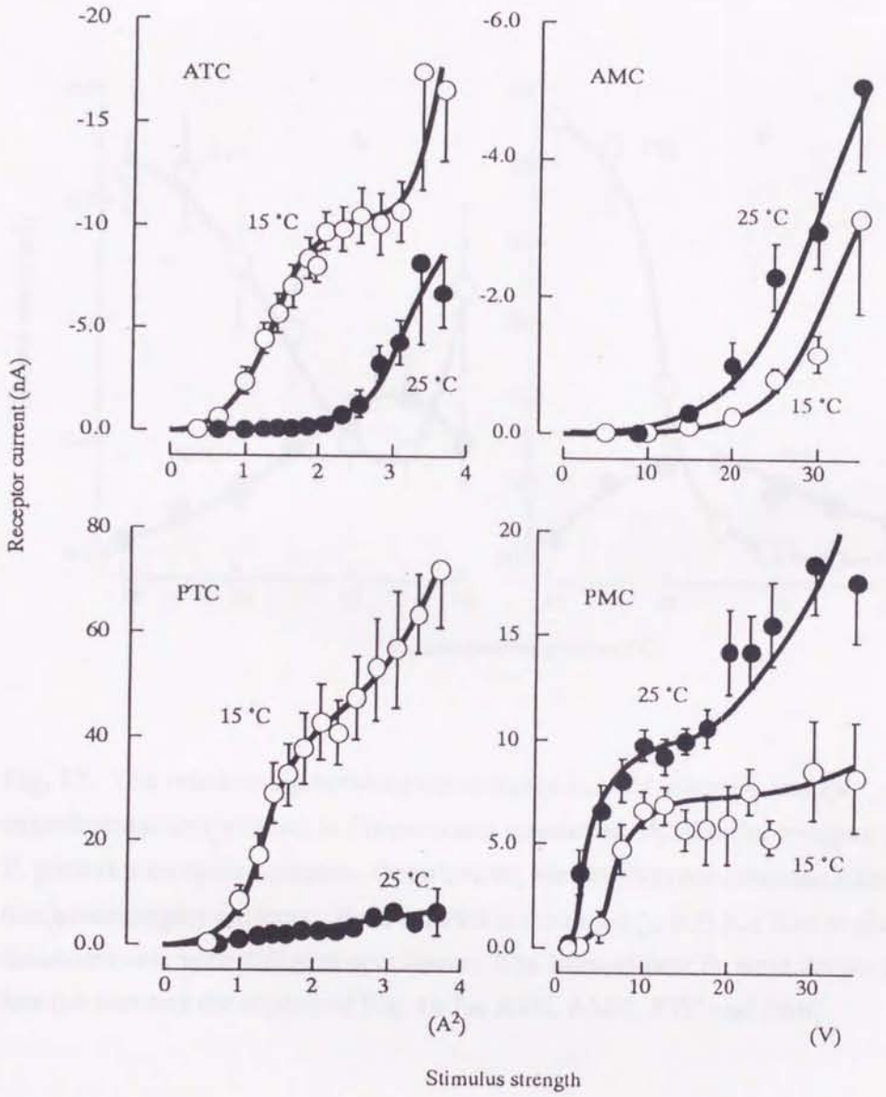


Fig. 16. The relationship between the receptor current intensity and stimulus strength at two different experimental temperatures in *Paramecium caudatum*. The negative current value corresponds to an inward current, while the positive value to an outward current. ATC, anterior thermoreceptor current; AMC, anterior mechanoreceptor current; PTC, posterior thermoreceptor current; PMC, posterior mechanoreceptor current. Open circles, values obtained at the experimental temperature of 15 °C; filled circles, values obtained at the experimental temperature of 25 °C. Each symbol is the mean (± S.E.) of three to six measurements with different specimens. The membrane potential level was held at the resting level. The lines of best fit were drawn by hand.

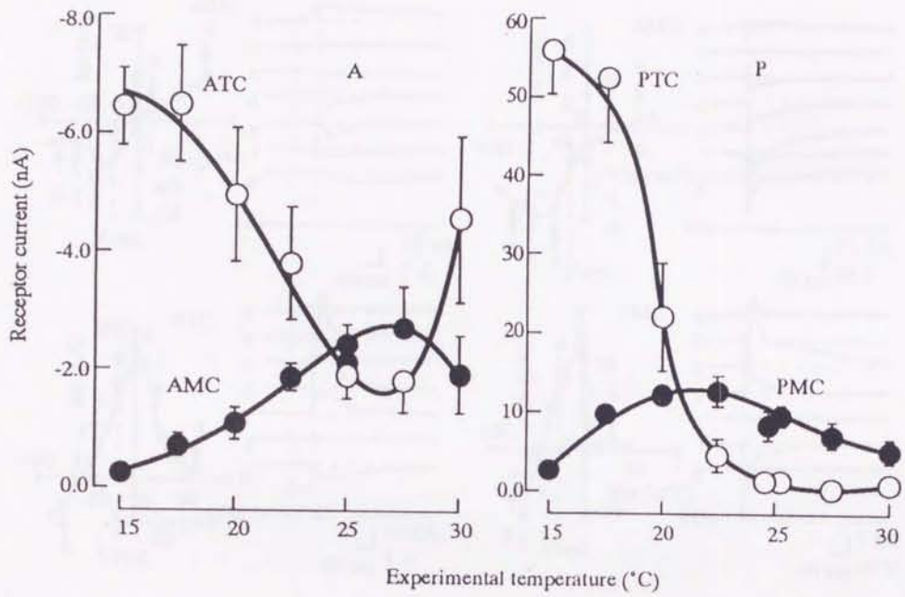


Fig. 17. The relationship between the receptor current intensity and the experimental temperature in *Paramecium caudatum*. A, anterior receptor currents; P, posterior receptor currents. Open circles, thermoreceptor currents; filled circles, mechanoreceptor currents. Each symbol is the mean (± S.E.) of five to sixteen measurements with different specimens. The lines of best fit were drawn by hand. See the text and the legend of Fig. 16 for ATC, AMC, PTC and PMC.

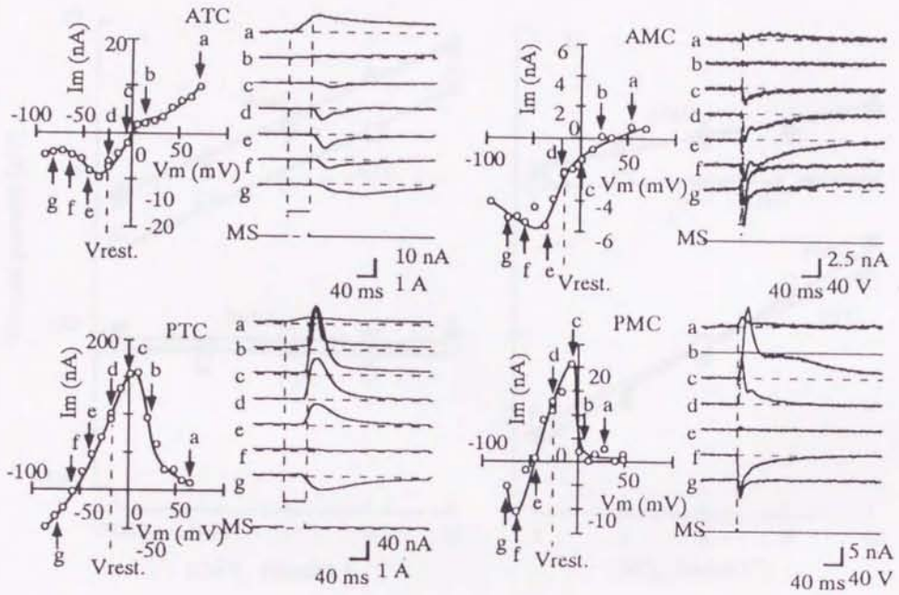


Fig. 18. The relationships between the peak values for the receptor currents and the membrane potential level (the I-V relationship) in *Paramecium caudatum.* Im, receptor current; Vm, membrane potential. Each I-V relationship is accompanied by the corresponding series of receptor current traces. The membrane potential level at which each current trace was obtained is indicated by the arrows. The potential level indicated by a broken line labeled Vrest corresponds to the resting membrane potential level. See the text and legend of Fig. 16 for ATC, PTC, AMC and PMC.

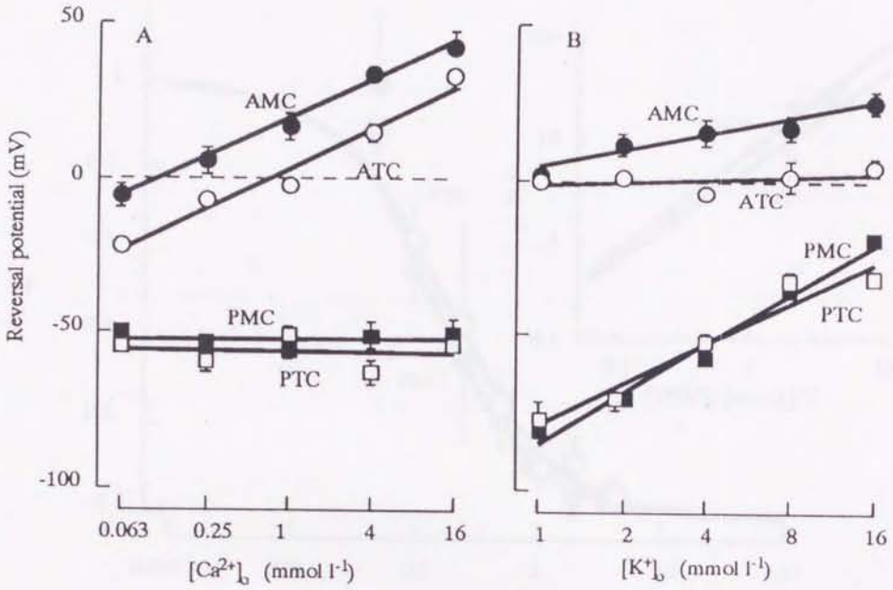


Fig. 19. Effect of external concentration of Ca^{2+} ($[Ca^{2+}]_o$) and that of K^+ ($[K^+]_o$) on the reversal potential levels for the receptor currents in *Paramecium caudatum*. A, Ca^{2+} series experiments, where $[Ca^{2+}]_o$ was changed, while $[K^+]_o$ was kept constant at 4 mmol l^{-1}; B, K^+ series experiments, where $[K^+]_o$ was changed, while $[Ca^{2+}]_o$ was kept constant at 1 mmol l^{-1}. Open circles, ATC; open squares, PTC; filled circles, AMC; filled squares, PMC. Each symbol is the mean (± S.E.) of three to five measurements with different specimens. Regression lines for each series of experiments are drawn. See the legend of Fig. 16 for ATC, PTC, AMC and PMC.

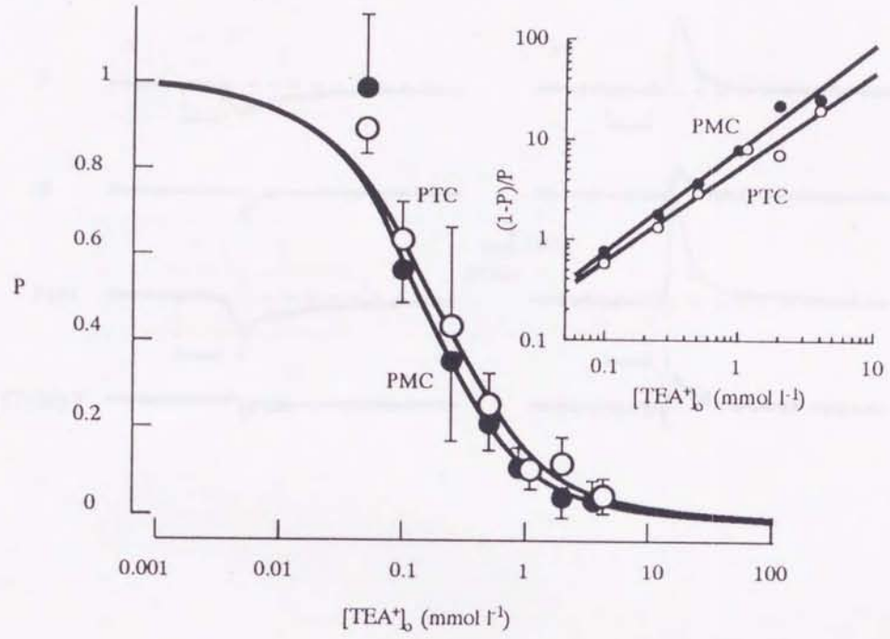


Fig. 20. Effects of external concentration of TEA^+ ($[TEA^+]_o$) on the intensity of the posterior receptor currents in *Paramecium caudatum*. P, the intensity of receptor current in the presence of TEA^+ relative to that in the absence of TEA^+. Open circles, PTC; filled circles, PMC. The inset is the 'Hill-plot' of the $[TEA^+]_o$-P relationship. Each symbol is the mean (±S.E.) of at least five measurements with different specimens. The smooth curved lines were drawn according to corresponding regression lines drawn in the inset. See the text for more details and the legend of Fig. 16 for PTC and PMC.

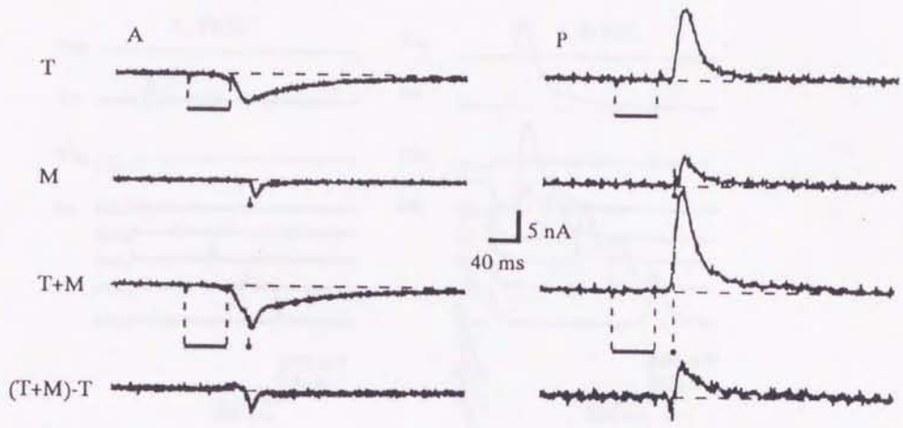


Fig. 21. Traces for the anterior and the posterior receptor currents in one specimen of *Paramecium caudatum*. Column A, receptor currents evoked by stimulation of the anterior membrane; column P, receptor currents evoked by stimulation of the posterior membrane. Line T, receptor currents evoked by thermal stimulation; line M, receptor currents evoked by mechanical stimulation; line T+M, receptor currents evoked by mechanical stimulation applied immediately after thermal stimulation; (T+M)-T, electronically subtracted T from T+M traces. Each horizontal bar indicates the timing and duration of thermal stimulation. Each black dot indicates the time when mechanical stimulation was applied to the cell. See the text for more details.

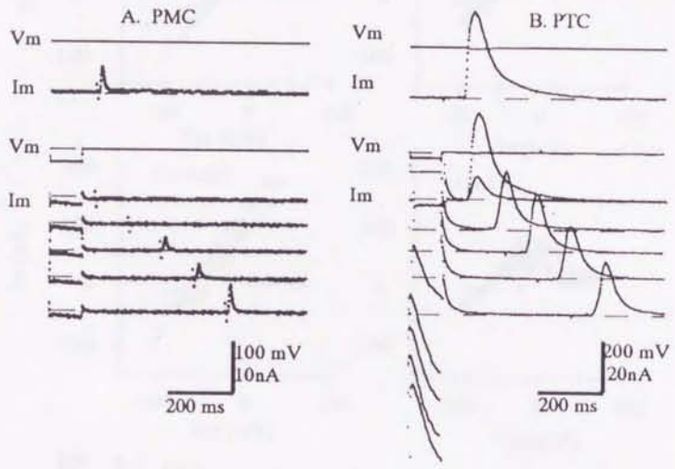


Fig. 22 Representative traces of PMC (A) and PTC (B) for *Paramecium caudatum* after hyperpolarizing voltage pulse. Upper sets of traces, membrane potential (Vm) and membrane current (Im), are responses to mechnical and thermal stimulus without hyperpolarizing pulse under voltage clamp condition. Lower families of traces are receptor currents preceeded by hyperpolarizing pulse (100 ms) with intervals of 50, 100, 200, 300 and 400 ms.

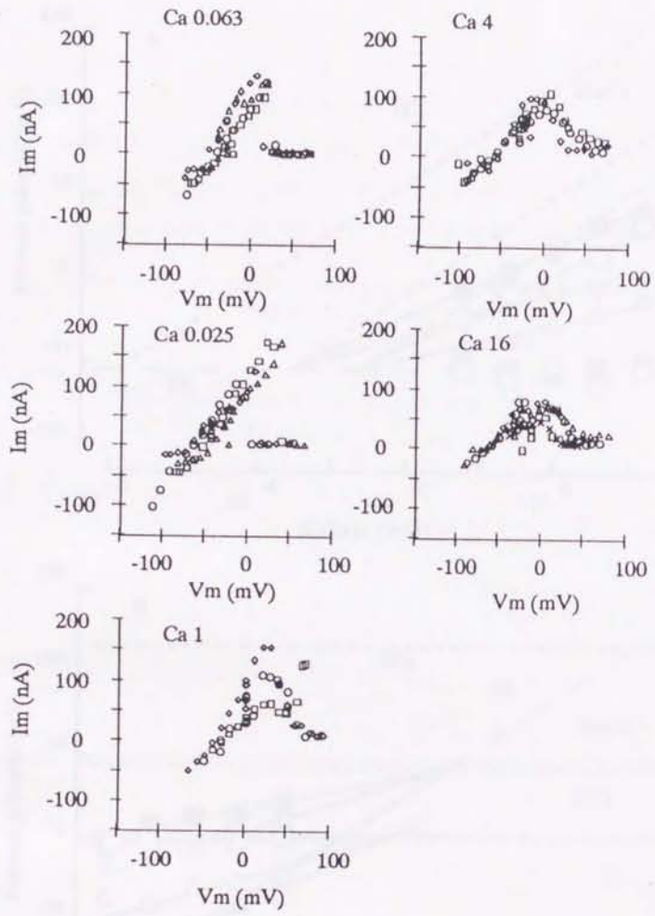


Fig. 23 The current-voltage relationships in various Ca^{2+} concentration ranging from 0.063 to 16 mmol l^{-1} (shown in upper side of each graph) for PTC of *Paramecium caudatum*. K^{+} concentration was kept constant at 4 mol l^{-1}. Each different symbol represents different specimens.

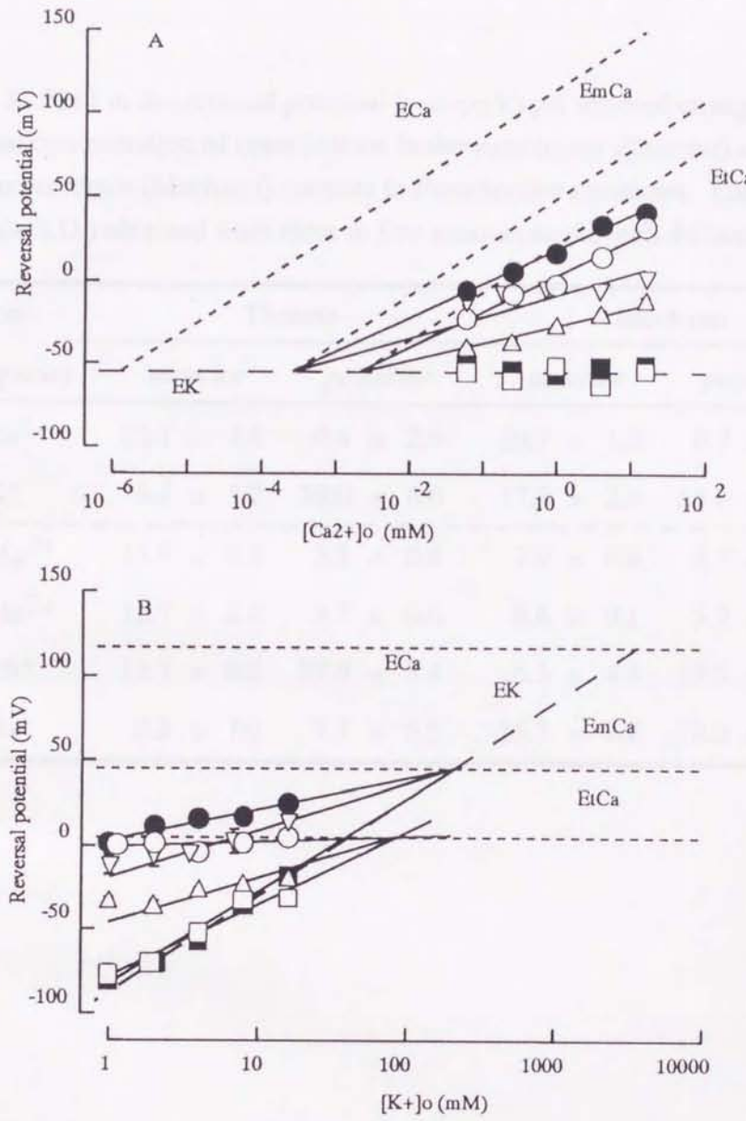


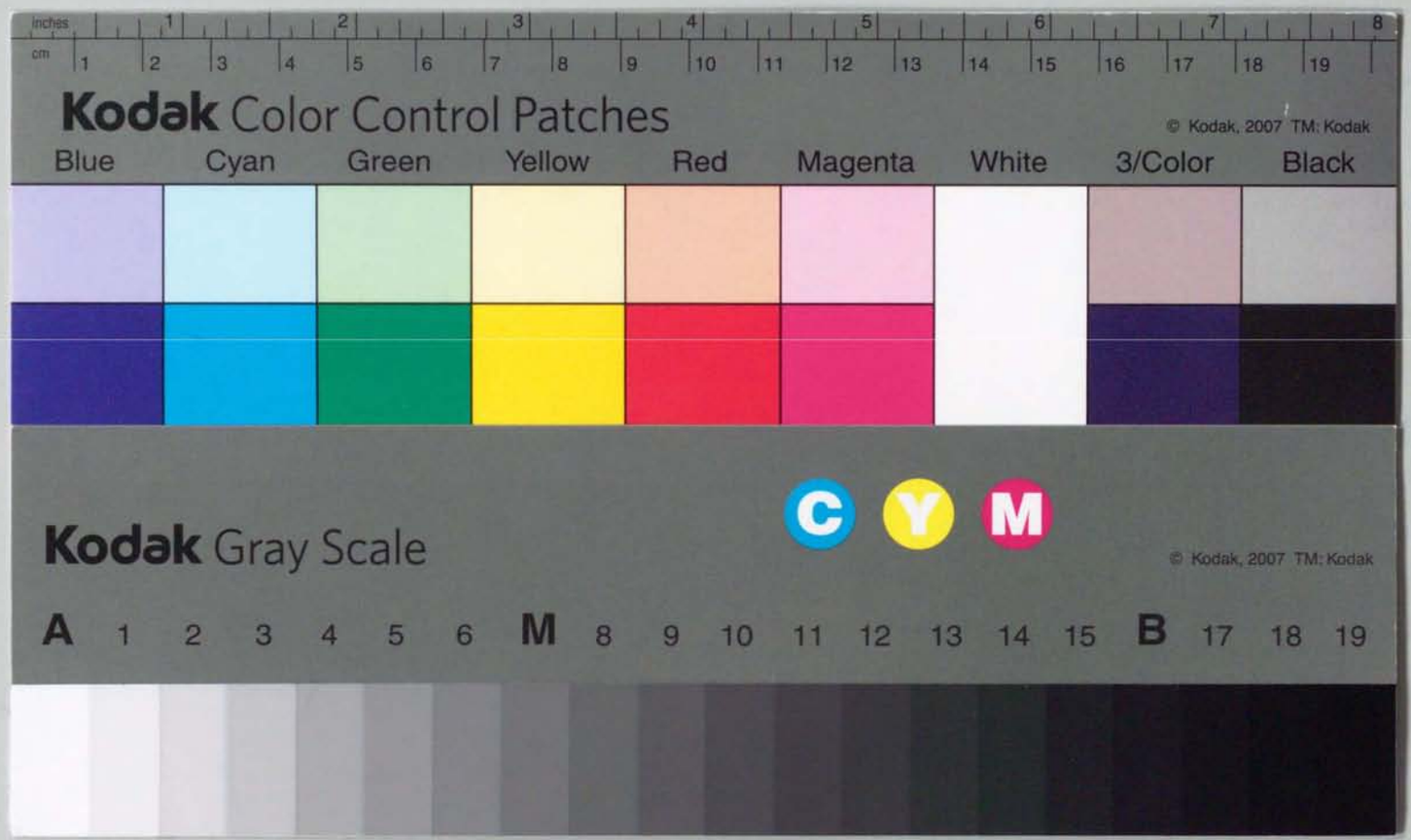
Fig. 24 Relation between reversal potential for thermo- and mechanoreceptor current in *Paramecium caudatum* and extracellular concentration of Ca^{2+} (A) and K^+ (B). Theoretical lines for K^+ equilibrium potential (E_K) and Ca^{2+} equilibrium potential (E_{Ca} assuming $[Ca^{2+}]_i$ below 10^{-6} mol l^{-1} and E^m_{Ca} for mechanoreceptor current and E^t_{Ca} for thermoreceptor current) predicted by Hodgkin and Horowics equation. See Appendix B for more detail.

Table 1. Shift in the reversal potential level (mV) per ten-fold change in the external concentration of some cations in thermoreceptor (Thermo) and mechanoreceptor (Mechano) currents in *Paramecium caudatum*. Each value is the mean (± S.D.) obtained from three to five measurements with different specimens.

ion	Thermo		Mechano	
species	*anterior*	*posterior*	*anterior*	*posterior*
Ca^{2+}	22.1 ± 2.5	-0.4 ± 2.9	20.7 ± 1.2	0.7 ± 1.8
K^{+}	3.4 ± 3.7	39.0 ± 6.0	17.7 ± 2.9	53.0 ± 4.3
Mg^{2+}	13.8 ± 0.8	5.1 ± 0.6	7.9 ± 0.8	8.7 ± 2.2
Mn^{2+}	15.7 ± 2.4	3.7 ± 0.0	8.6 ± 0.1	5.9 ± 1.5
Rb^{+}	13.7 ± 0.5	37.9 ± 3.4	6.3 ± 4.4	35.5 ± 2.5
Na^{+}	0.2 ± 7.9	7.7 ± 5.5	25.7 ± 2.2	-13.0 ± 6.4

Table 2. The fractional conductance (T_X) of the anterior or the posterior membrane of *Paramecium caudatum* to cation X during its subjection to thermal (Thermal) or mechanical (Mechanical) stimulation. Each value is the mean (± S.D.) obtained from three to five measurements with different specimens, and estimated from each corresponding rate of shift shown in Table 1.

ion species		Thermal		Mechanical	
X	(T_X)	*anterior*	*posterior*	*anterior*	*posterior*
Ca^{2+}	(T_{Ca})	0.77 ± 0.09	-0.01 ± 0.10	0.70 ± 0.04	0.02 ± 0.06
K^+	(T_K)	0.06 ± 0.06	0.68 ± 0.10	0.30 ± 0.05	0.90 ± 0.07
Mg^{2+}	(T_{Mg})	0.48 ± 0.03	0.18 ± 0.02	0.27 ± 0.03	0.29 ± 0.07
Mn^{2+}	(T_{Mn})	0.55 ± 0.08	0.13 ± 0.00	0.29 ± 0.00	0.20 ± 0.05
Rb^+	(T_{Rb})	0.24 ± 0.01	0.66 ± 0.06	0.11 ± 0.07	0.60 ± 0.04
Na^+	(T_{Na})	0.00 ± 0.14	0.13 ± 0.10	0.43 ± 0.04	-0.22 ± 0.11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