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Mutants With Reduced Ca Activation in *Paramecium aurelia*

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Summary. Two heat-sensitive "pawn" mutants of *Paramecium aurelia* are capable of avoiding reactions when grown at 23 °C but not at 35 °C. Electrophysiological analyses show that Ca activation is reduced in the mutants even when they are grown at 23 °C. The maximal rate of rise and the peak of the evoked action potential (Ca-spike) in the mutants are smaller than those of wild type in a K-solution. After suppression of K conductance by either TEA^+ or Ba^{++}, the action potentials of the mutants peak at the same level as that of wild type. However, the maximal rate of rise of the mutants remains only about half that of wild type. Thus, the mutations affect Ca activation but not K activation.

Incubation at a high temperature (35 °C) further reduces Ca activation to almost zero in the mutants but has little or no effect on wild type. This almost complete loss of Ca activation explains the lack of avoiding reactions when the mutants are grown at high temperatures. A double mutant containing two heat-sensitive mutations shows extremely reduced Ca activation even when grown at 23 °C.

Single-gene mutations can have dramatic effects on the excitable membrane. In *Paramecium*, behavioral mutants are now available (Kung, Chang, Satow, Van Houten & Hansma, 1975). Several types of these mutants appear to have modifications in different ion channels (Kung & Eckert, 1972; Satow & Kung, 1976*a*, *b*; Satow, Hansma & Kung, 1976; Schein *et al.*, 1976[1]). The first mutants to be physiologically analyzed is a "pawn" mutant. This mutant, carrying a mutation at the *pwB* gene, is incapable of the "avoiding reaction" (Jennings, 1906) in the face of proper stimuli (Kung, 1971*a*). Avoiding reactions result from ciliary reversal, which is correlated with an increase in the internal Ca^{++} concentration (Naitoh & Kaneko, 1972) normally due to the Ca^{++} influx associated with the Ca-action potential of the paramecium membrane (Naitoh & Eckert, 1974). This mutant has little or no excitability and the defect is most likely in the mechanism for Ca-activation (Kung & Eckert, 1972).

1 Schein, S.J., Katz, G.M., Bennett, M.V.L. 1976. Electrophysiological basis of the pawn trait, behavioral mutants of *Paramecium aurelia. (in preparation)*

Over 170 lines of pawn mutants have been isolated in various mutagenesis experiments and with different screening methods. They are found to carry mutations on three complementation groups (Kung, 1971*b*; Chang, Van Houten, Robles, Lui & Kung, 1974; Schein, 1975, 1976[2]). Allelic variants exist, i.e., mutations on the same gene give different degrees of phenotypic expression. This is most obvious in mutants of the *pwA* genes. Chang *et al.* (1974) are able to classify mutants of this group into four degrees of leakiness in their behavioral expressions. The most leaky are the mutants whose behavioral defects are almost undetectable when grown at room temperature but become pawns after incubation at a higher temperature. These are the temperature-sensitive pawns (*ts*-pawns) (Chang & Kung, 1973*a*, *b*). We have surveyed the defects of two *ts*-pawns grown at different temperatures and have shown that the ability to generate spontaneous Ba^{++} action potential remains when they are grown at room temperature but is lost at high temperatures (Satow, Chang & Kung, 1974).

In this paper, the excitability of the *ts*-pawns in three solutions is quantitatively evaluated. Action potentials are triggered by current stimulations. The peak of the mutants' action potentials and their maximal rate of rise are compared to those of wild type. A part of this work has been briefly introduced elsewhere (Satow, 1976).

Materials and Methods

Stocks and Culture

All stocks belong to species 4 of *Paramecium aurelia* (or *P. tetraurelia*, Sonneborn, 1975). We used the "heat-sensitive pawns", pawn *C* (stock d4-131, genotype *pwC/pwC*) and pawn *A*1 (stock d4-132, genotype *pwA1/pwA1*) and wild type (stock 51 s) from which the mutants were derived. The double mutant *r*7 (genotype *pwA1/pwA1 pwC/pwC*) was an autogamous F2 line derived from a cross between d4-131 and d4-132 and was genotypically verified by a backcross (Chang & Kung, 1973*b*).

Cells were cultured at room temperature (23 ± 1 °C) or in an incubator at 35 ± 1 °C in the Cerophyl medium bacterized with *Aerobacter aerogenes* 20 hr before use (Sonneborn, 1970). All physiological experiments were performed at room temperature. Cells grown at 35 °C for 17 to 24 hr were used within one hr for the experiments. We used only robust cells in log-phase growth.

Solutions

All solutions contained 1 mM $Ca(OH)_2$ and 1 mM citric acid adjusted to pH 7.15 to 7.25 with 1.2–1.3 mM Tris (tris(hydroxymethyl) aminomethane). Addition of 4 mM KCl, 4 mM

2 Schein, S.J. 1976. A non-behavioral selection for pawns, mutants of *Paramecium aurelia* with decreased excitability. Genetic and phenotypic characterization *(in preparation)*

TEA-Cl (tetraethylammonium chloride, Aldrich Chemical Co.) or 4 mM $BaCl_2$ gave the K, TEA or Ba solution, respectively. The K solution was the "adaptation solution" in which the cells were penetrated by microelectrodes. After penetrating the cell, the K solution was sometimes replaced by TEA or Ba solutions. TEA solution was used within 24 hr after dissolution.

Recording

Recordings were done using the electrophysiological techniques previously described (Satow & Kung, 1976*a*). Microelectrodes with 100 to 130 MΩ resistance were filled with 500 mM KCl. These techniques are basically those of Naitoh & Eckert (1972) with a modified ground electrode and a continuous perfusion. Membrane potential is recorded with a *Bioelectric* P1 system having the PF3 amplifier placed close to the specimen. The rate of rise of the action potential was obtained by differentiating the potential change with a circuit having a time constant of 200 μsec. A second inflection of the dV/dt, rising to a peak, marks the surge of net inward current. In our system, this peak occurs not less than 6.5 msec after the beginning of current injection. In some cases no peak is seen and the dV/dt trace shows a steady decline after the small initial surge due to passive response to the injected current (e.g., bottom panel of Fig. 5*A*). We consider the peak dV/dt (maximal rate of rise) as zero in such cases since we are interested only in the regenerative response.

Results

The Evoked Action Potential in K-Solution

Fig. 1 shows the action potentials of wild type, pawn *A1* and pawn *C* evoked by different strengths of outward current. The peaks of the action

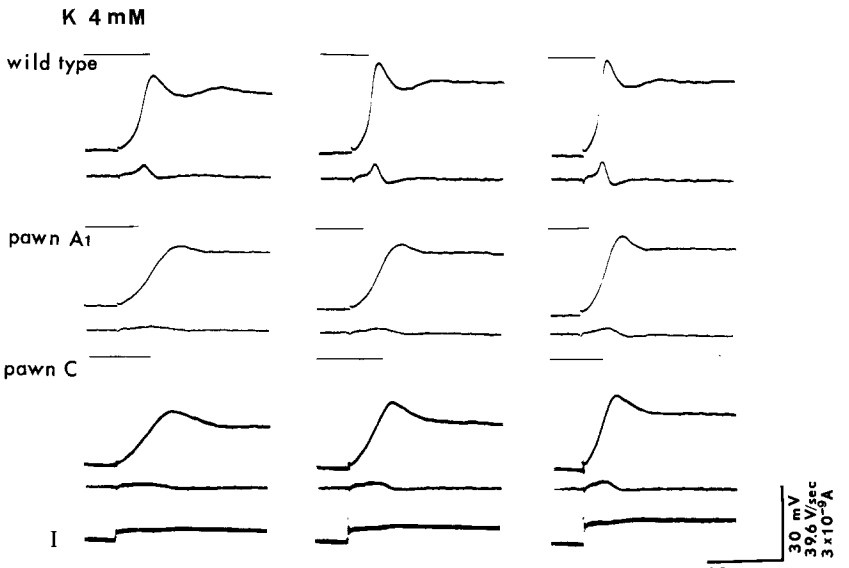

Fig. 1. Evoked action potentials from wild type, pawn *A*1 and pawn *C* bathed in 4 mM K solution. The traces in each frame are 0-level, potential (V) and dV/dt from the top. The bottom traces marked I are the outward current injected (5, 7 and 10×10^{-10} A from left)

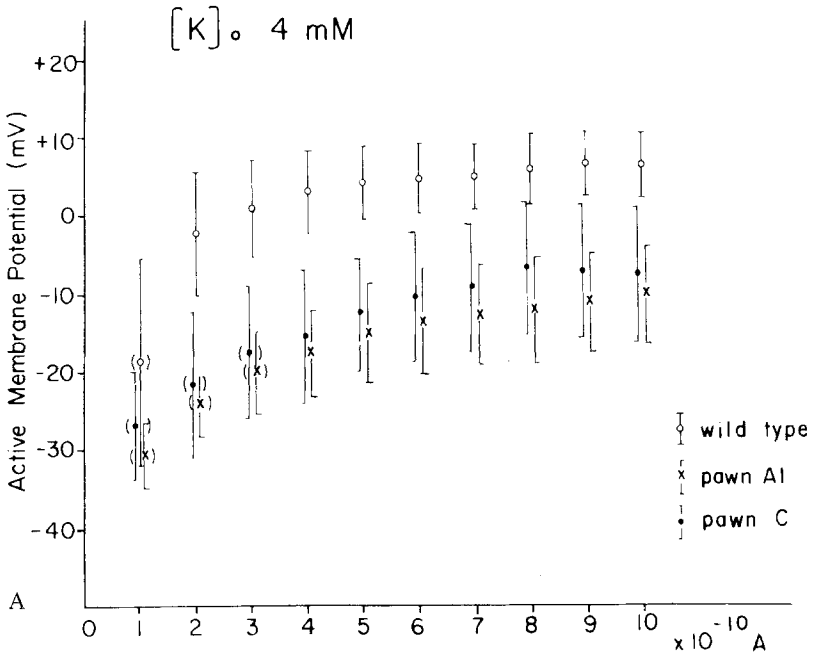

Fig. 2. (*A*) Active membrane potentials (peak level of action potential) with different current strengths from wild type ♀, pawn *A* ⚥ and pawn *C* ♦ in 4 mM K solutions (mean ± SD). Points in brackets include potential responses without peak. Note that the peak levels of the mutants are lower than those of wild type. (*B*) The maximal rate of rise with different current strengths from wild type ♀, pawn *A*1 ⚥, pawn *C* ♦ and △ *pwA1-pwC* in 4 mM K solution (mean ± SD). Note the maximal rate of rise of the mutants (pawn *A*1 and pawn *C*) is about half that of wild type

potentials are lower in the mutants than in wild type. The maximal rate of rise (seen as the peak on the dV/dt trace) of the mutants is also smaller than that of wild type. The relation between the peak potential (active membrane potential) and the injected current strength is shown in Fig. 2*A*. Small outward currents often do not trigger an action potential, especially in the mutants. Maximal potential at these current strengths are enclosed in brackets in Fig. 2*A*. Fig. 2*B* shows the relation between the maximal rate of rise and the current strength used to evoke the action potential. The mutants have a higher threshold for excitation. 4×10^{-10} A is required before an active response is observed in the mutants, whereas 2×10^{-10} A is enough for wild type. With strong currents the maximal rate of rise of the action potential in the mutants is about half that in wild type. With the strongest current in this experiment (10^{-9} A), the maximal rate of rise of wild type is 6.2 ± 0.3 V/sec and that of the mutants is 3.4 ± 0.5 V/sec in pawn *A1* and 3.2 ± 0.6 V/sec in pawn *C*. The rising curves in

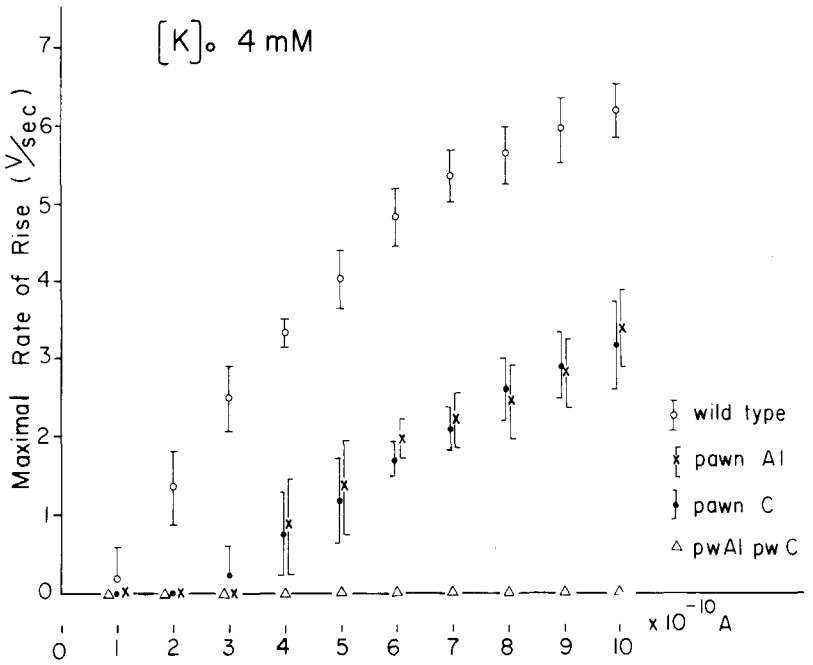

Fig. 2*B*

Fig. 2*B* suggest that even higher rates of rise can be induced by current stronger than 10^{-9} A. 3×10^{-9} A current shows that this is in fact the case in both wild type and the mutants (data not shown). Such currents are nonphysiological and detrimental to the cells.

The Evoked Action Potential in TEA-Solution

TEA^+ is a well-known blocker of K conductance and has been shown to be effective in paramecium membrane (Friedman & Eckert, 1973; Satow & Kung, 1976*a*, *b*). Fig. 3 shows the action potential triggered by smaller current strength from cells bathed in TEA-solution. The peak potential is higher and the maximal rate of rise is higher in TEA than in K solution. (*Compare* Fig. 3 with Fig. 1). This is true for both wild type and the mutants. The peak potentials and the maximal rate of rise against current strength are plotted in Fig. 4 *A* and *B*, respectively. The peak potentials of the mutants are not significantly different from those in wild type except in the region of small currents. The maximal rate of rise in the mutants (4.3 ± 0.4 V/sec in pawn *A1* and 4.6 ± 0.5 V/sec in pawn *C*), however, is only about half that in wild type (8.2 ± 0.4 V/sec) with strong

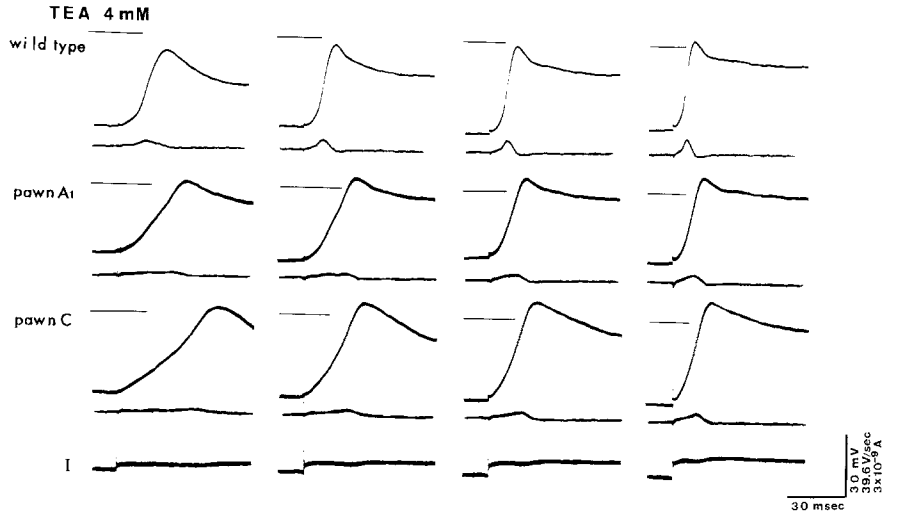

Fig. 3. The evoked action potentials from wild type, pawn *A*1 and pawn *C* in 4 mM TEA solution. The three traces in each frame are 0-level, *V*-trace and *dV/dt* trace from the top. Current strength: 3, 5, 7 and 10^{-10} A from the left. Note that the peak levels of the mutants near those of wild type but the maximal rate of rise of the mutants is clearly smaller than that of wild type

current stimulation (10^{-9} A). The slope of the curves showing the relation of current strength to the maximal rate of rise in TEA solution is similar to that in K solution (Fig. 2*B*).

The Maximal Rate of Rise of Ba Action Potential

Besides carrying the action current, Ba^{++} also suppresses the K^+ conductance in normal (Naitoh & Eckert, 1968) and mutant paramecia (Satow & Kung, 1976*a*, *b*). We have previously recorded the spontaneous action potential of the *ts*-pawns in Ba solution (Satow, Chang & Kung, 1974). Fig. 5*A* shows the action potential evoked by 10^{-9} A current in wild type and the mutants. The maximal rate of rise of the mutants is again only about half of that of wild type (4.3 ± 0.4 V/sec in pawn *A1*, 4.4 ± 0.4 V/sec in pawn *C* and 8.0 ± 0.6 V/sec in wild type). Since there is spontaneous Ba action potential, the threshold of excitation cannot be measured accurately, although it is certainly lower than the threshold determined in TEA or K solution. *Paramecium* membrane rests depolarized when Ba solution is used to replace other bath solutions *(see below)*. The series of spontaneous action potentials make the accurate estimation of resting level in Ba

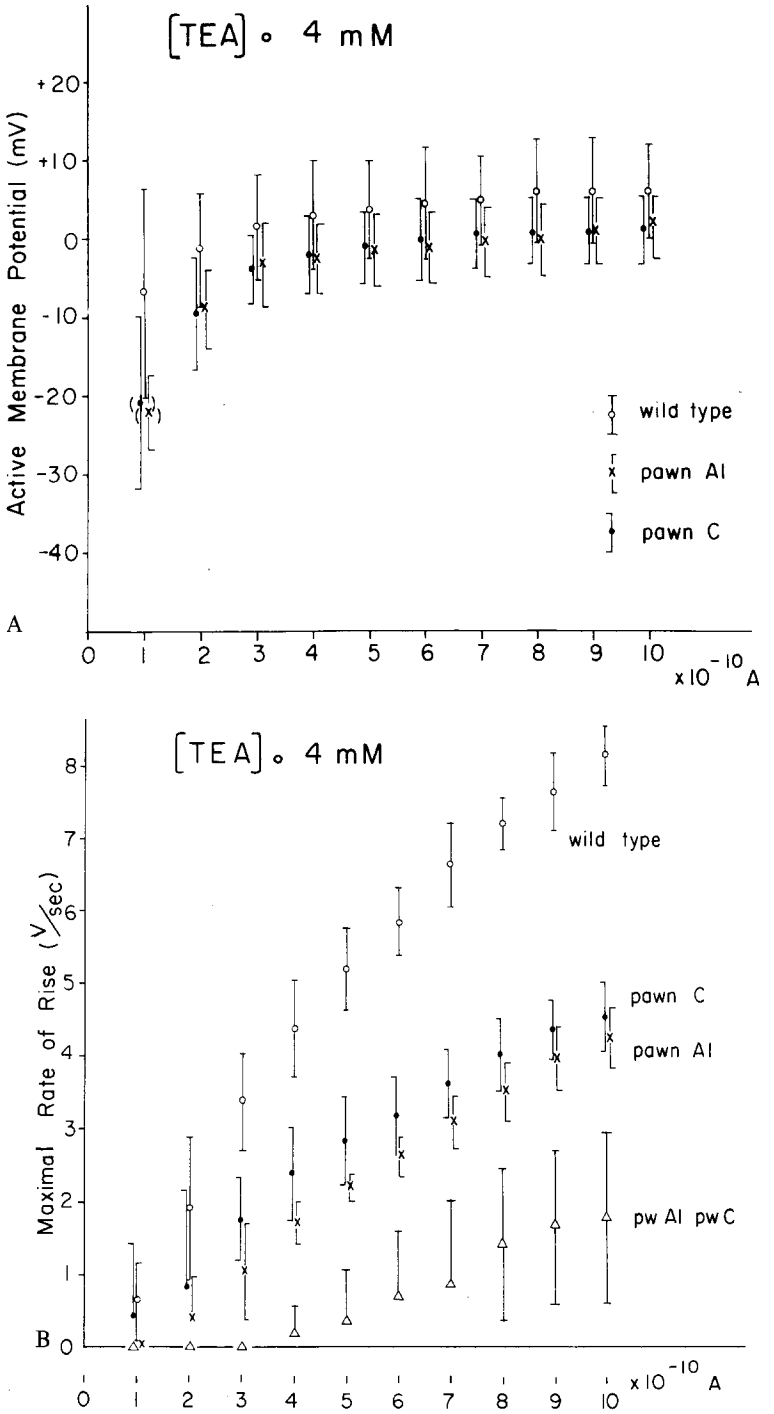

Fig. 4. The active membrane potentials (peak potential) (*A*) and the maximal rate of rise (*B*) with different current strengths from wild type and the mutants in 4 mM TEA-solution. The active membrane potentials of the mutants are not significantly different from those of wild type. The maximal rate of rise of the mutants (pawn *A*1 and pawn *C*) are about half that of wild type, and *pwA1-pwC* double mutant has a very small active component

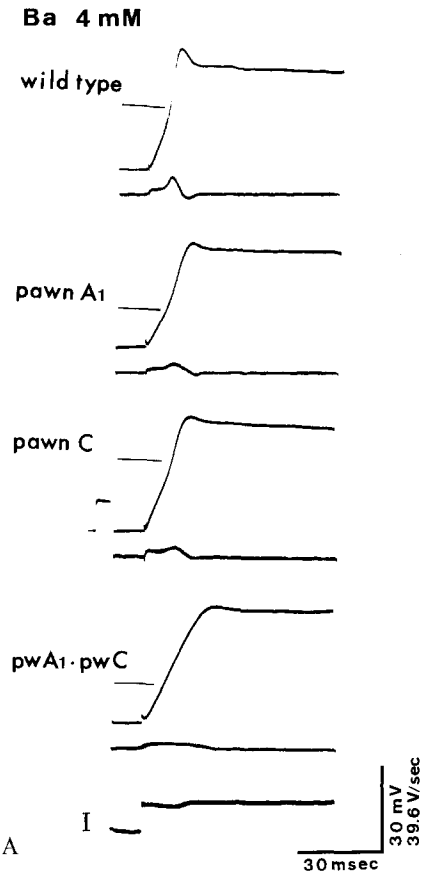

Fig. 5. (*A*) The evoked action potentials with strong current (10^{-9} A) from wild type, pawn *A*1, pawn *C* and *pwA1-pwC* in 4 mM Ba solution. Square pulse on the *V* trace in pawn *C* is 10 mV, 5 msec calibration. Note that the active component is smaller in the mutants. There is no active component in *pwA1-pwC* double mutant. 0-level of each frame is determined with the electrode in 4 mM Ba solution. (*B*) The maximal rate of rise against current strength from wild type ○, pawn *A*1 ×, pawn *C* ● and *pwA1-pwC* △ in 4 mM Ba solution. Note the maximal rate of rise of the single mutants (pawn *A*1 and pawn *C*) is about half that of wild type

solution difficult. This is reflected in the variations of resting level among strains in Fig. 5*A*.

The Effect of Incubation at High Temperature

The potential responses to injected current are recorded from cells grown at 35 °C. The action potential in wild type was seen in both K (Fig. 6) and Ba solutions (Fig. 7). More variability is encountered among

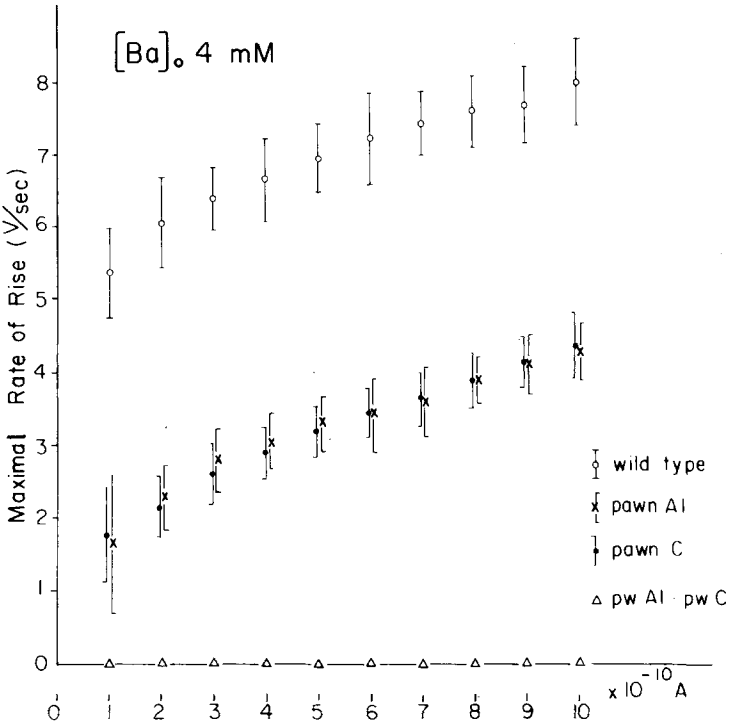

Fig. 5*B*

cells grown at 35 °C than at 23 °C (see standard deviation in figures). The maximal rate of rise in K solution is only slightly affected by the growth temperature in wild type if at all (Figs. 2*B* and 6*B*). The maximal rate of rise measured in TEA solution of wild type also does not seem to be affected by the growth temperature (Figure not shown). However, in Ba solution, the maximal rate of rise is clearly reduced in wild type after growing at 35 °C (Figs. 5*B* and 7*B*).

Although the active component in the mutant grown at high temperature is apparently abolished in K solution (Fig. 6), such mutants sometimes show small but clear active components with strong current in Ba (Fig. 7*B*) or TEA solution.

In both Figs. 6*A* and 7*A*, a small peak of the potential is observed in the mutants before the steady-state response appears. This is most likely due to the K-delayed rectification, which is presumably not affected by the mutations or the growth temperature.

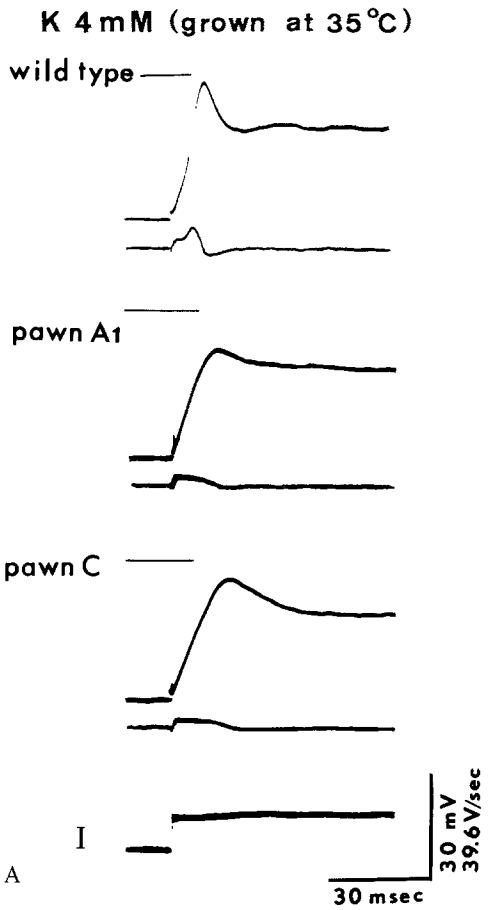

Fig. 6. The current evoked response in 4 mM K solution of wild type, pawn *A*1 and pawn *C* after the cells were grown at 35 °C. (*A*) shows the evoked action potentials with a strong current (10^{-9} A). (*B*) The maximal rate of rise with different current strengths. The active components in pawn *A*1 and pawn *C* were abolished with high temperature incubation

The Double Mutant

A double mutant bred to contain both mutations, *pwA1* and *pwC* and fails to generate avoiding reactions when grown at 23 °C (Chang & Kung, 1973*b*; Satow, Chang & Kung, 1974). The evoked response was recorded from the *pwA/pwC* double mutant. There was no clear active component in K or Ba solutions (Figs. 2*B*, 5*A* or 5*B*). However, small and highly variable active responses are recorded from double mutants bathed in TEA-solution (Fig. 4*B*).

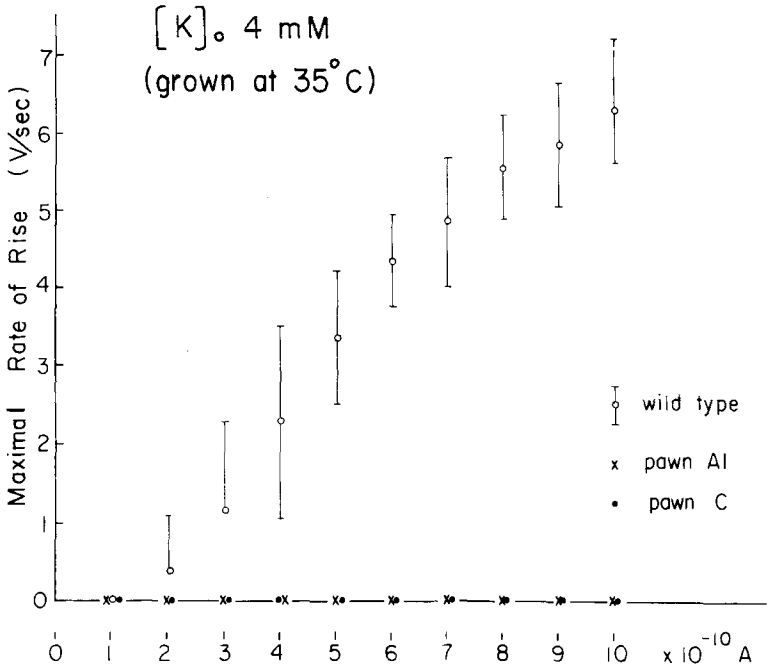

Fig. 6*B*

The Resting Potential and the Membrane Resistance

The resting potentials in wild type, pawn *A1*, pawn *C* and the *pwA1 pwC* double mutant are measured in various solutions (Table 1). There is no statistically significant difference in the resting membrane potential of different strains, grown at different temperatures. Resting levels of all strains become less negative in Ba^{++} (Satow, Chang & Kung, 1974). Repeated firing of Ba-spikes make it impossible to measure the resting level accurately. Resting membrane resistances are measured as the chord resistances with a small hyperpolarizing current (10^{-10}A) (Table 2).

Table 1. Resting potential (mV)[a]

	Wild type	Pawn *A*1	Pawn *C*	*pwA1-pwC*
K 4 mM	−32.5±3.4	−36.9±4.0	−36.6±8.2	−33.4±4.8
K 4 mM (35 °C)[b]	−35.2±7.1	−38.5±4.5	−36.6±9.7	
TEA 4 mM	−38.8±4.9	−36.1±3.5	−36.7±5.8	−32.7±6.2
TEA 4 mM (35 °C)[b]	−33.4±5.0	−36.8±6.0	−33.8±6.8	

[a] Reference level is that measured when the recording electrode is in the K solution (the adaptation solution).

[b] Cells grown at 35 °C for over 17 hr before tested at 23 °C.

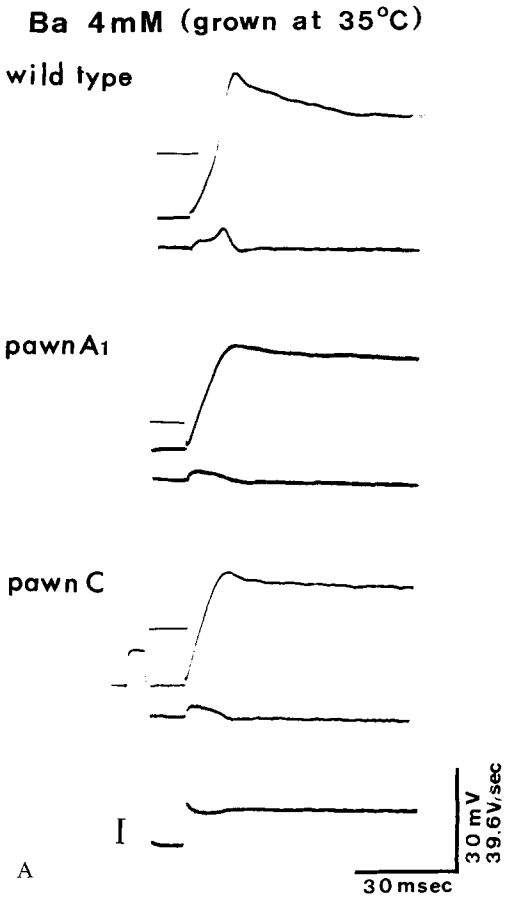

Fig. 7. The evoked responses in 4 mM Ba solution of wild type, pawn *A*1 and pawn *C* after the cells were grown at 35 °C. (*A*) shows the evoked action potentials with strong current (10^{-9} A). (*B*) the maximal rate of rise against different current strengths in 4 mM Ba solution are shown. The evoked responses of pawn *A*1 and pawn *C* in *A* do not have active components (0-level of each frame is determined with the electrode in 4 mM Ba solution)

Table 2. Membrane resistance (MΩ)[a]

	Wild type	Pawn *A*1	Pawn *C*	*pwA1-pwC*
K 4 mM	56.0±19.0	65.1±19.9	54.6±17.3	45.9±16.0
K 4 mM (35 °C)[b]	46.0±10.0	68.5±19.0	48.7± 6.6	
TEA 4 mM	63.2±14.0	76.2± 9.9	69.8±15.5	60.1±12.5
TEA 4 mM (35 °C)[b]	83.0±22.1	75.1±17.1	89.2±20.5	
Ba 4 mM	93.3±21.1	110.3±23.0	105.1±17.3	96.4±32.8
Ba 4 mM (35 °C)[b]	89.1±15.9	99.2±35.2	78.0±16.0	

[a] Chord resistances measured at steady state 0.3 to 1 sec after the initiation of the small hyperpolarizing current (10^{-10} A).
[b] Cells grown at 35 °C before tested at 23 °C.

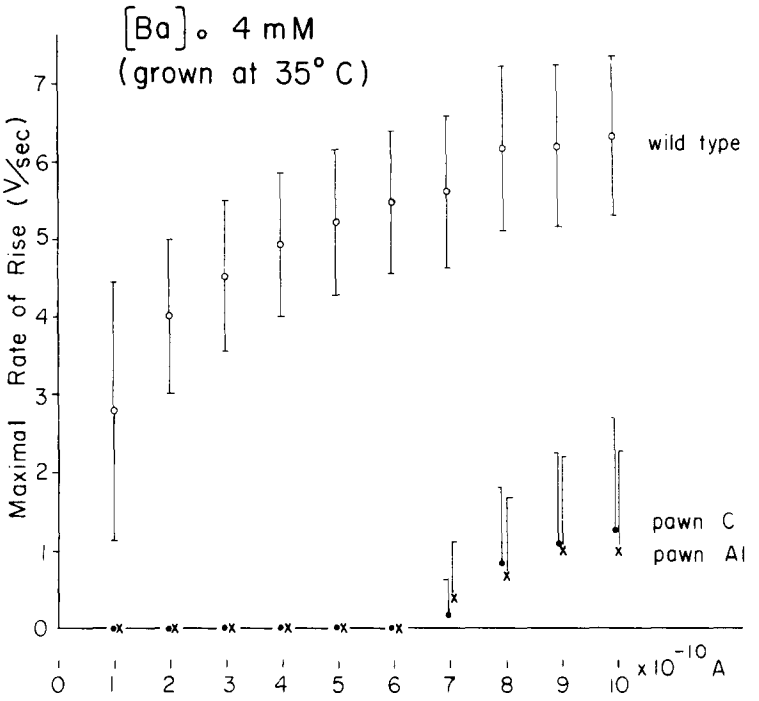

Fig. 7*B*

Membrane resistances of all strains increase when TEA or Ba solution replaces K solution in the bath. As stated above Ba^{++} makes the resting potential less negative and such depolarization is expected to decrease the membrane resistance. The observed increase in resistance is therefore especially significant in the case of Ba solution. Given the large within group variance, no significant differences in membrane resistance of different strains grown at different temperatures are detected. Since the size of the cells is identical in all strains, the resistances of different strains can be compared directly. The $I-V$ relations of injected current and steady-state potential response after the spike show no significant difference between different strains grown at 23 °C or 35 °C. This is true in K, TEA, and Ba solutions. (Figs. not shown).

Discussion

The Peak Potential of the Active Response

The action potential of the *Paramecium* membrane occurs as a result of inward flow of Ca^{++} current (Naitoh & Eckert, 1974) as in barnacle

muscle (Hagiwara & Naka, 1964, X-organ of crayfish (Iwasaki & Satow, 1971) and Retzius cell of leech (Kleinhaus & Prichard, 1975). Since the amplitude of the Ca permeability is not large when compared to the well-known Na activation, the amplitude of the Ca action potential is often graded to the strength of the stimuli (Fig. 2*A*). In barnacle muscle, the peak level of the Ca action potential is determined by the fluxes of both Ca and K ions. The peak can be raised and the action potential approaches all-or-none when the internal Ca ion concentration is reduced by internally injected Ca chelating agent (Hagiwara & Naka, 1964) or when the internal K ion concentration is decreased (Hagiwara, Chichibu & Naka, 1964). Similar concepts have been applied to *Paramecium* (Naitoh & Eckert, 1968). We find that the action potential is less graded and the peak level higher in TEA solution than in K solution when the responses to small injected currents are examined (Fig. 2*A* and 4*A*). This result confirms our previous findings (Satow & Kung, 1976*b*) and leads us to conclude that the peak level of Ca action potential of *P. aurelia* is also partly determined by the K conductance.

The peak potential of the *ts* pawn mutants is lower than that of wild type in the K solution. Either (1) the Ca activation is weakened by the mutations or (2) the K conductance has increased in the mutants. In TEA solution, this difference becomes much smaller (Fig. 3 and 4*A*). This finding, however, does not help us to distinguish between the two possibilities. While it is possible that the mutation-enlarged K conductance is blocked by TEA^+ such that the spikes are now normalized, it is equally likely that blockage of normal K conductance allows the mutation-weakened Ca activation mechanism to become more efficient. Fig. 3 shows that the mutant peaks arrive later than the wild type peaks upon the same stimulations. This finding favors the first hypothesis because if the mutations only affect the K conductance and if TEA is efficient in blocking the K conductance, the mutant spikes should not be delayed.

The Maximal Rate of Rise of the Action Potential

The rate of rise of the action potential is a better estimate of membrane excitability than its peak potential. The maximal rate of rise of the action potential shows the degree of activation, since it is proportional to the net inward membrane current. (Weidmann, 1955, Hagiwara & Takahashi,

1967). Since we have found no clear difference in the resting membrane potential and resistance (Table 1 and 2) of different strains, the maximal rate of rise of different strains can be compared directly. The maximal rate of rise of the mutants is about half that of wild type in the K solution (Fig. 2*B*) even when the mutants are grown at room temperature. TEA^+ is well known for its action in inhibiting K conductance (Fatt & Katz, 1953, in crustacean muscle fiber; Hagiwara & Watanabe, 1955, in sartorius muscle; Armstrong & Hille, 1972, in myelinated nerve fiber; Keynes, Rojas, Taylor & Vergara, 1973, in barnacle muscle; Friedman & Eckert, 1973, in *Paramecium*). Ba^{++} also inhibits K^+ conductance (Hagiwara, Fukuda & Eaton, 1974; Naitoh & Eckert, 1968). In *P. aurelia*, Ba^{++} depolarizes the resting membrane (*below*) and increases membrane resistance (*below* and Table 2). It also appears to block conductances sensitive to external TEA^+ and those sensitive to internal TEA^+ (Satow & Kung, 1976*a*, *b*). In TEA and Ba solution, the rate of rise of both wild type and the mutants becomes higher (Figs. 4*B* and 6*B*). Yet, the maximal rate of rise of the two mutants is still only half that of wild type. This finding quantitatively argues against the possibility that the mutants have an increased K conductance, instead of weakened Ca activation. Should the former be the case, the rate of rise of the mutants would approach that of wild type when the K conductance was inhibited by TEA^+ or Ba^{++}. Even if the inhibition were not complete, the increase in rate of rise due to TEA^+ and Ba^{++} should be disproportionate and in favor of the mutants. The simplest explanation of the fact that the mutant's rate of rise remains only half that of wild type before and after the application of TEA^+ and Ba^{++} is that the mutations have affected the Ca activation mechanism, making it only about half as efficient.

It is known that the degree of the activation of the membrane depends on the conditioning membrane potential level (Weidmann, 1955; Frankenhaeuser & Hodgkin, 1957; Hagiwara & Takahashi, 1967). However, the reduced Ca-activation of the mutant is not due to a simple shift of the inactivation curve toward a more positive or negative membrane potential level. This is because neither hyper- nor de-polarizing conditioning can restore the mutant-reduced Ca-activation to the wild type level.

It is interesting to note that there exists a mutant in *P. aurelia* (the "TEA^+-insensitive mutants") which has the alternate defect, i.e., an increased K conductance. Like *ts* pawns, it has a weaker spike but its maximal rate of rise can be increased to that of wild type when strong currents are injected or when Ba solution is used. For details of this K-channel mutant *see* Satow & Kung, (1976*b*).

Spontaneous Action Potentials in Ba or TEA Solution

Ba^{++} has two major effects on paramecium membrane. First, it carries the major action current in place of Ca^{++}, such that the peak potential level is determined by the concentration of Ba^{++} and not that of Ca^{++} or K^{+}. Second, in inhibits K conductance (Naitoh & Eckert, 1968). In *P. aurelia*, Ba^{++} depolarizes the resting membrane, increases the maximal rate of rise (Fig. 5*B*) and generates all-or-none action potentials (Satow, Chang & Kung, 1974). These spikes, like those triggered with very small currents (Fig. 5*B*) have a maximal rate of rise of about 5 V/sec in 4 mM Ba solution. The two *ts* pawn mutants (grown at room temperature) are capable of generating the spontaneous action potential in Ba solution (Satow, Chang & Kung, 1974). However, the rate of rise of these spikes is no more than about 1.7 V/sec (Fig. 5*B*). This result indicates that full activation is not needed for spikes of full amplitude if the K conductance is sufficiently low. These spikes of the *ts*-pawns not only have a slower rise but also often oscillate at a plateau for a few seconds before repolarization. This may mean that the mutational defect affects Ca inactivation as well as Ca activation. The action potentials correspond behaviorally to the avoiding reactions. Thus, *ts*-pawns grown at room temperature give slightly prolonged backward swimming corresponding to the delay in repolarization. These mutants also behave differently from wild type when they are challenged with a TEA solution. They give fewer and weaker avoiding reactions than wild type (Chang & Kung, 1976). This behavioral deficiency reflects the higher threshold and lower rate of rise of the mutants in TEA solution.

The "Pawn" Mutations

Mutations on three genes (*pwA*, *pwB*, *pwC*) in *P. aurelia* can generate the "pawn" phenotype. These mutants were first recognized for their inability to swim backward (Kung, 1971*a*). Kung and Eckert (1972) studied a pawn mutant (of the *pwB* group) and concluded that this pawn is defective in its Ca-gating mechanism. This conclusion is extended to other pawn mutants in this study and other work (Satow, Chang & Kung, 1974; Schien, *et al.*, 1976). "Leaky" mutants can be recognized for their deficiency in, but not complete loss of, avoiding reactions (Chang *et al.*, 1974). All leaky pawns at the *A* and *C* loci are temperature-sensitive, i.e., they behave like pawns after growing at high temperatures. The two *ts*-pawns (*pwA1* and *pwC*) studied here represent the extremely leaky phenotype. They are capable of avoiding reactions in various solutions (Chang & Kung, 1973*a*, *b*), although some of the reactions are not quite normal

(above). The electrophysiological details of these mutants given here clearly show that even these extremely leaky pawns have only about 50 % the normal excitability. Heat-sensitive mutants presumably have quasi-normal gene products that are made nonfunctional by heat. The kinetics of phenotypic changes, interpretations and discussions on the nature of the *ts*-pawn gene products can be found in a previous paper (Satow, Chang & Kung, 1974).

Schein (1975, 1976) has also isolated pawn mutants with mutations on three genes. Schein *et al.* (1976) have studied a series of pawn mutants and come to a similar conclusion; i.e., pawns are defective in Ca activation and the degree of leakiness corresponds with the amount of Ca activation.

Pawn and leaky pawn mutants thus form one distinct class of membrane mutants in *Paramecium*. The effect of pawn mutations in *Paramecium* is like the effect produced by the application of tetrodotoxin to membranes with Na spikes. A toxin concentration effect can be simulated by the use of mutants of different degrees of leakiness or the use of double mutant (Figs. 2*B*, 4*B*, and 5*B*). The effect can be activated by high temperatures (Figs. 6*A–B* and 7*A–B*). Mutants of other classes with defective K channels or possibly a Na channel (Satow & Kung, 1976*a*, *b*; Satow, Hansma & Kung, 1976) can now be used in combination with pawns to study the interactions of various ion channels.

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