MUTANTS WITH ALTERED Ca^{2+}-CHANNEL PROPERTIES IN *PARAMECIUM TETRAURELIA*: ISOLATION, CHARACTERIZATION AND GENETIC ANALYSIS

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ABSTRACT

Dancers are a group of mutants in *Paramecium tetraurelia* whose Ca^{2+} current inactivates poorly and are likely to be defective in the structure of their Ca^{2+} channels. These mutants show prolonged backward swimming in response to K^+ and Ba^{2+} in the medium and were selected by this property in a galvanotactic trough. The dancer mutants are semidominant, and all isolated mutants belong to one complementation group; they are not allelic to any of the previously isolated behavioral mutants of *P. tetraurelia*. The phenotypic change from the homozygous parent to heterozygous F_1 generation takes three to five fissions. There is no evidence of a cytoplasmic factor capable of converting the dancer to the wild-type phenotype, as has been demonstrated in the mutants pawn and *cnr*. We suggest that the dancer locus is a structural gene for the Ca^{2+} channel.

THE excitable membrane contains many proteins that control ion currents across the membrane. Genetic dissection has been used as a means to alter different ionic currents in Drosophila and Paramecium (*e.g.*, HALL 1982; SALKOFF 1983; KUNG 1979; SAIMI *et al.* 1983). The use of mutants can lead to the eventual identification and analysis of macromolecular components of the excitable membrane that control specific ion permeabilities. However, the existing mutations that are thought to affect the structure of specific ion channels are rare. The shaker locus in Drosophila may code for a K^+ channel since one allele alters the kinetics of inactivation of the I_A current, whereas other alleles completely inhibit it (SALKOFF 1983). Neurotoxin-resistant mutants of mouse neuroblastoma cells have also been isolated (WEST and CATTERALL 1979) which may be defective in ion channel structure.

The pawn mutants of *P. tetraurelia* have little or no Ca^{2+} current upon depolarization and, therefore, cannot generate action potentials (KUNG 1979). These mutations (at three loci) may affect structural components of the Ca^{2+} channel, cofactors needed for channel activation, factors required to remove inactivation or elements involved in the proper assembly, insertion or maintenance of the channels in the ciliary membrane.

We recently reported on the electrophysiological characters of a group of mutants, designated dancers, whose Ca^{2+} current inactivates slower and to a

lesser extent and also deactivates slower (HINRICHSEN and SAIMI 1984). In this paper, we describe the method of selection, behavioral phenotypes and genetic analyses of these dancer mutants. Differences between dancer and pawn are consistent with the view that dancer is defective in the structure of its Ca^{2+} channel.

MATERIALS AND METHODS

Stocks and culture conditions: We used *P. tetraurelia*, stock 51s (kappa-free), d4-90 paranoiac A (*PaA/PaA*), d4-91 fast-2 (*fna/fna*), d4-94 pawn A (*pwA/pwA*), d4-95 pawn B (*pwB/pwB*), d4-97 fast 1A (*fA/fA*), d4-131 pawn C (*pwC/pwC*), d4-150 paranoiac C (*PaC/PaC*), d4-152 TEA^+-insensitive (*tea*A/*tea*A) (KUNG 1979), d4-622 pantophobiac (*pantA/pantA*) (SAIMI *et al.* 1983), the trichocyst nondischarge mutant *nd6* (SONNEBORN 1975) and the recently isolated dancer mutants, d4-623 (Dn^1/Dn^1), d4-624 (Dn^2/Dn^2), d4-625 (Dn^3/Dn^3), d4-626 (Dn^4/Dn^4) and d4-628 (Dn^5/Dn^5). Paramecia were cultured at 28° in Cerophyl medium enriched with stigmasterol (5 mg/liter) buffered with sodium phosphates and bacterized with *Enterobacter aerogenes* (SONNEBORN 1970).

Genetic analyses: Standard Paramecium genetic techniques were employed as described by SONNEBORN (1970) for obtaining F_1 through conjugation and F_2 by autogamy. The dancer mutants were initially crossed to the *nd6* marker, a recessive mutant that cannot fire its trichocysts upon treatment with picric acid (SONNEBORN 1975). The *nd6* cells behave like wild-type cells (Y. SAIMI and R. HINRICHSEN, unpublished results).

The complementation tests among the dancer mutants were performed by crossing a dancer carrying the trichocyst nondischarge trait (*nd6*) to another dancer without *nd6*. The F_1 phenotype was scored only in those true crosses in which both exconjugants could fire trichocysts. The cells were taken into the F_2, through autogamy, and both the dancer and nondischarge traits were scored to ensure that cross-fertilization had taken place.

Mutagenesis: The cells were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (4 mg/50 ml) as described by KUNG (1971). Autogamy, to induce homozygosis and growth for six to eight fissions (phenomic lag), preceded the enrichment procedures.

Mutant selection: Differential galvanotactic behavior of mutant cells permitted enrichment of cells that overreacted to weak stimulation. Paramecium orients in a DC field and swims forward to the cathode or backward toward the anode (JENNINGS 1906; MACHEMER and DE PEYER 1977). They can be made to swim forward or backward depending on the ionic composition of the bath. A Lucite trough was constructed (height × width × length: 1.8 × 1.3 × 10.6 cm) with agar-bridged Ag-AgCl electrodes at each end (Figure 1). The side walls of the trough were slit at 2.2 and 2.8 cm from one end, so that two cover slips could be inserted into them, separating the trough into three compartments (A, B and C in Figure 1). The slits, as well as the cover glasses, were greased with petroleum jelly to prevent water leakage. Compartments A and C were filled with the screening solution; approximately $2–4 \times 10^5$ mutagenized cells, which had been washed in a control solution of 1 mM KCl, 1 mM $CaCl_2$, 1 mM HEPES, 10^{-2} mM EDTA, pH 7.2, were placed in compartment B. Forty volts, 50 msec, 5 Hz square pulses (from a Grass S9 stimulator) were turned on before the cover slips were slowly raised by a small forklift which was fabricated with wires and attached to a M3 Narishige micromanipulator. The pulses were strong and frequent enought to orient the cells but weak enough so as not to harm them. A stereomicroscope was placed over the trough to enable easy observation of individual cells in the trough. In an appropriate screening solution the desired mutants should swim away from the wild-type cells: they could swim in the opposite direction, swim for longer distances or stay on one side for longer periods of time.

A 10–15 mM KCl stimulating solution elicits a weak backing response ($\simeq$1 sec) in the wild type, followed by forward swimming. To isolate dancer mutants, the anode was placed at the end of compartment C (Figure 1), and cells that overreacted to the solution and swam backward to the far end of compartment C were selected.

Barium lethality assay: The barium lethality assay was similar to that described by SCHEIN (1976). Cells were placed in the control solution for 5 min and then individually transferred with a

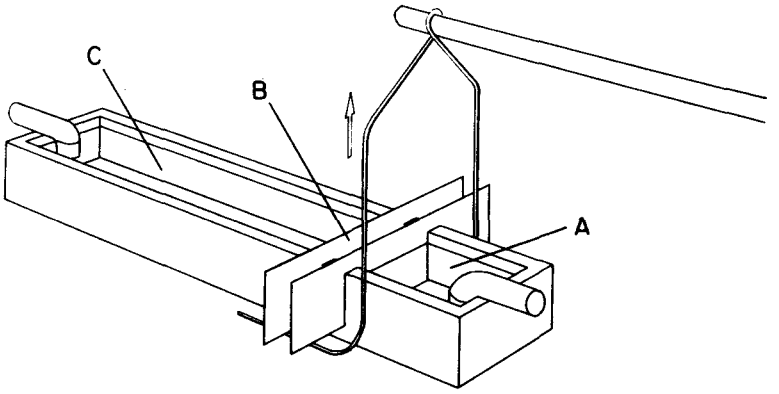

FIGURE 1.—The galvanotactic trough used to isolate behavioral mutants. A suspension of cells is placed in compartment B, and compartments A and C are filled with a screening solution. The electrodes at each end are used to impose a voltage on the trough. Once the current is turned on, the forklift is raised by micromanipulation and the cells are exposed to the screening solution. A stereomicroscope placed above the apparatus (not shown) is used to view the cells swimming into compartment C. See text for details and dimensions.

micropipette to 4–8 mM $BaCl_2$ added to control solution. The cells were monitored intermittently for viability.

Behavioral assays and dark-field photography: Behavioral assays were done according to SAIMI *et al.* (1983). The test solutions contained 10–20 mM K^+, 2–20 mM Na^+, 2–8 mM Ba^{2+} or 5–10 mM TEA^+, each added to the control solution; all were chloride salts and of reagent grade.

Dark-field photographs of cell behavior were made as described previously (HAGA *et al.* 1983). Photographs of 1-sec exposure were made after 2–4 μl of cells were added to 50 μl of solution on a glass plate.

Electrophysiology: The methods used for penetration and intracellular recording were those described previously (HINRICHSEN and SAIMI 1984).

Microinjection: KOIZUMI'S method (1974) of microinjection was modified as described by HAGA *et al.* (1983). Approximately 10% of the donor cell's cytoplasm was injected into the recipient cell.

RESULTS

Phenotypes: We screened for mutants that overreacted to 10–15 mM K^+ in five separate mutageneses. Eight such mutants were independently isolated. All eight isolates, called dancers, gave an exaggerated backing response in a 10–15 mM KCl solution. Five of the lines, designated *Dn*[1], *Dn*[2], *Dn*[3], *Dn*[4] and *Dn*[5], are described here.

In culture media the five dancer mutants were characterized by repeated bouts of sharp, uniform, jerking movements of several body lengths, whereas wild-type cells gave occasional, brief and nonuniform jerks. These reactions were more clearly delineated when the cells were in the stationary phase of growth. The mutants resembled wild type in shape and growth rate.

Dancer mutants only overreacted to solutions with K^+ or Ba^{2+} (Table 1). In a 10 mM K^+ solution, wild-type cells gave only occasional avoiding reactions (Figure 2A), whereas dancer underwent a repeated jerking motion (Figure 2B). In a 20 mM K^+ solution, wild-type cells swam backward for approximately 10 sec, stopped, whirled about their posterior end for several seconds and

TABLE 1

Behavior of dancer mutants in various stimulating conditions

Line	Solution				
	K^+	Ba^{2+}	Na^+	TEA^+	Heat (39°)
+	CBS (17.1 ± 3.2)	Jerks	CBS (14.1 ± 5.1)	Jerks	Jerks
pwB	FS	FS	FS	FS	FS
Dn^1	CBS (62.5 ± 6.4)	Spin (139.3 ± 20.0)	CBS (9.0 ± 3.1)	Jerks	Jerks
Dn^2	CBS (78.5 ± 4.2)	Spin (81.5 ± 9.4)	CBS (12.5 ± 4.0)	Jerks	Jerks
Dn^3	CBS (75.5 ± 3.9)	Spin (62.5 ± 9.1)	CBS (14.3 ± 4.6)	Jerks	Jerks
Dn^4	CBS (57.6 ± 1.9)	Spin (76.8 ± 6.2)	CBS (11.8 ± 2.8)	Jerks	Jerks
Dn^5	CBS (61.5 ± 7.1)	Spin (48.5 ± 2.5)	CBS (13.6 ± 3.7)	Jerks	Jerks

All cells were placed in a control solution for 5 min before transferring individual cells to the test solutions. The test solutions had (in mM) 20 K^+, 20 Na^+, 8 Ba^{2+} or 10 TEA^+ added to the control solution. Cells tested at 39° were placed in a Cambion temperature regulator. The behavior was monitored for up to 3 min. Parentheses indicate the duration of the behavior (sec) ± the standard deviation.

FS, forward swimming; jerks, short, repeated bouts of backward swimming lasting <1 sec in duration; CBS, continuous backing swimming, a continuous ciliary reversal that results in prolonged backward swimming for tens to hundreds of body lengths; spin, the cell spins about the longitudinal axis slowly, either remaining in place or moving very slowly forward.

began to swim forward slowly. The dancer mutants, on the other hand, underwent a long bout of backward swimming (>50 sec) that terminated in a series of short, uniform jerks (the "dance") for approximately 10 sec before they began to swim forward slowly. In 8 mM Ba^{2+} the mutants immediately began to spin slowly in place and continued for >50 sec before they slowly swam forward; wild-type cells displayed only a repeated jerking motion in 8 mM Ba^{2+}. Under all other conditions tested the dancer cells behaved similarly to the wild type (Table 1).

When the electrical properties of the membrane were studied by intracellular recording, a striking difference was observed between the wild type and the dancer. Stimuli, such as outward currents injected through the wild-type membrane, can trigger action potentials whose amplitude and rate of rise are proportional to the strength of the stimuli (the "graded response," NAITOH and ECKERT 1969). A very weak current injected into a wild-type cell elicited a marginal action potential followed by a passive ohmic response (Figure 3A). Dancer, on the other hand, showed a series of all-or-none action potentials after the injection of the same weak current (Figure 3B), *i.e.*, it gave a full response to this barely suprathreshold stimulation. All other membrane electric properties of dancer, such as the resting potential, membrane resistance, time constant and the maximal peak of the action potential, appeared normal. Examined under voltage clamp, the Ca^{2+} current in dancer inactivated slower than in the wild type. The dancer inactivation was also incomplete. The stronger Ca^{2+} current explains the more vigorous action potentials and their resultant avoiding reactions in the mutants. Details of the electrophysiological abnormalities of dancer have been reported elsewhere (HINRICHSEN and SAIMI 1984).

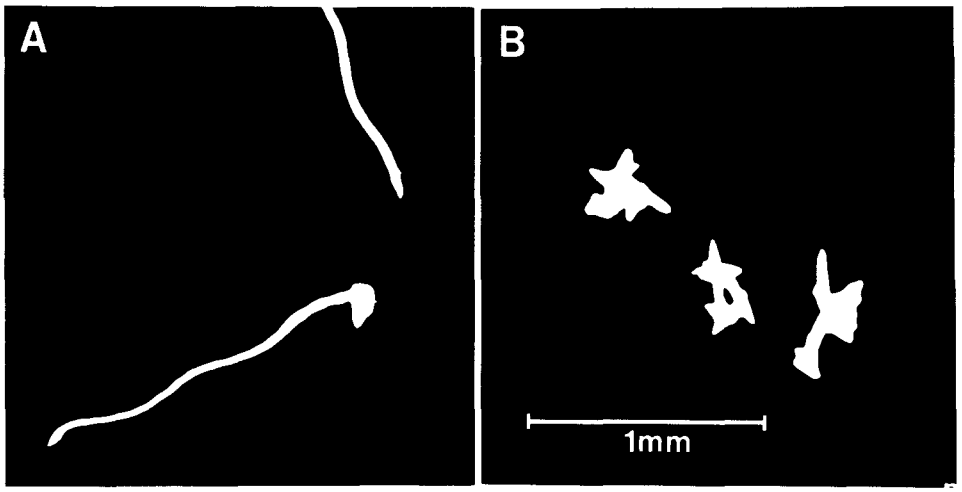

FIGURE 2.—Dark-field photographs tracing the trajectories of the wild-type (A) and dancer (B) behavior. The cells were placed in a solution composed of 10 mM KCl, 1 mM $CaCl_2$, 1 mM HEPES and 10^{-2} mM EDTA, pH 7.2. The two wild-type cells swim forward (loose helices) with only an occasional jerk (the kink at the end of one helix). The three dancer cells, on the other hand, jerk repeatedly (clustered barbs).

Since wild-type cells die in Ba^{2+} (SCHEIN 1976) and Ba^{2+} is known to pass through the Ca^{2+} channel (LING and KUNG 1980), the previous results predicted dancer would die sooner than wild type in solutions containing Ba^{2+}. To test this prediction, individual cells were placed into a solution of 4 or 8 mM $BaCl_2$. The wild-type cells were alive in 4 mM Ba^{2+} for longer than 24 hr. In 8 mM Ba^{2+} they swam for 3–5 hr before they stopped and died (Table 2). The dancer mutants died within 3 hr in the 4 mM Ba^{2+} solution and within 90 min in 8 mM Ba^{2+}. These data are consistant with the idea that the Ca^{2+} channel of the mutant allows more Ca^{2+} (and Ba^{2+}) into the cell.

Genetic analysis: Each dancer line was crossed to a marker line, *nd6*. All five heterozygous F_1 progeny displayed a phenotype that was intermediate between the wild type and dancer (Table 3). Whereas the dancer parent swam backward for >50 sec, ending with the characteristic "dance" in 20 mM K^+, and the wild type showed a 15-sec response, the F_1 cells had a 25- to 40-sec response, also terminating with the "dance." The F_1 response time varied among the five mutant lines; the Dn^1 and Dn^3 mutants gave the greatest response, whereas the Dn^2 and Dn^5 had weaker ones. All five F_1 heterozygotes also gave an intermediate response to 8 mM Ba^{2+}, showing the slow spinning behavior like the dancer parents but not for as long a period of time. The F_1 cells were induced to undergo autogamy and the F_2 cells were scored for their phenotypes, and all five lines segregated in an approximate 1:1:1:1 ratio for the behavioral and trichocyst discharge traits (Table 3). These data indicate each dancer mutant carries a single, partially dominant genetic lesion that is unlinked to the marker gene, *nd6*.

To determine the number of separate loci represented by the five dancer mutants, crosses in all combinations were performed among all of the dancers.

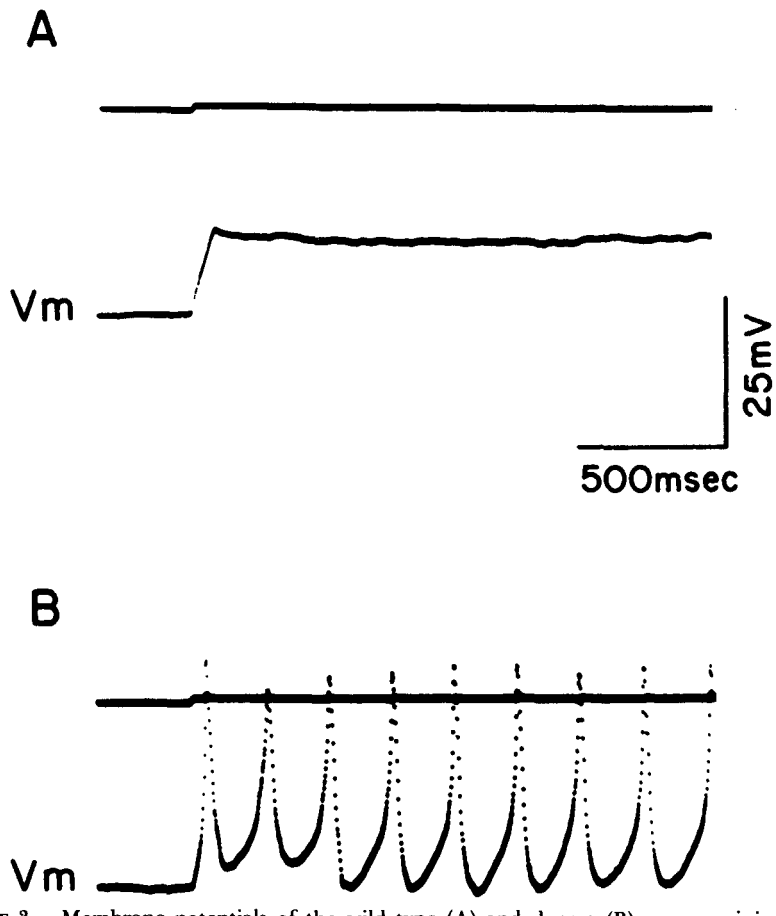

FIGURE 3.—Membrane potentials of the wild type (A) and dancer (B) upon an injection of a sustained, outward, 0.2 nA current. Wild-type cells show a graded action potential and a sustained membrane potential throughout the current injection. In contrast, dancer generates a series of all-or-none action potentials throughout the current injection. The top lines indicate the reference for the membrane potential and the level of current injection.

The results of these ten crosses indicated that only one locus is represented here because all crosses gave F_1 phenotypes like the parents, whereas the marker segregated normally in the F_2 (data not shown). Therefore, the five dancer lines form one complementation group.

In an effort to determine whether the dancer lines were allelic to other behavioral mutants of *P. tetraurelia*, the dancer mutant was crossed to ten different behavioral mutants: pawn A, pawn B, pawn C, Paranoiac A, Paranoiac C, *fnaP*, fast-2, *teaA*, fast 1A and pantophobiac. Dancer was found not to be allelic to any of the behavioral mutants tested (data not shown) because the F_1 phenotypes were typical of the heterozygous dancer phenotype and in most cases the F_2 data clearly showed the segregation of two unlinked genes. The only exceptions were the *PaA* and *PaC* crosses in which the F_2 ratios were distorted in favor of the *Pa* classes. This has been shown previously for Para-

TABLE 2

Barium lethality assay

Ba^{2+}		Viability[a] after				
		30 min	60 min	90 min	180 min	300 min
4 mM	Wild type	+++	+++	+++	++	++
	dancer (*Dn*[1])	++	++	+	−	−
8 mM	Wild type	++	++	++	++	−
	dancer (*Dn*[1])	+	+	−	−	−

The cells were washed into 1 mM K^+, 1 mM $CaCl_2$, 1 mM HEPES, 10^{-2} mM EDTA, pH 7.2, and then transferred to 4 or 8 mM $BaCl_2$ added to the control solution. The test was repeated three times using ten cells per test. The score assigned indicates that >80% of the cells showed that behavior.

[a] Viability was scored as follows: +++, swimming the same as control cells in wash solution; ++, still swimming but slowly; +, alive but on the bottom of the well; −, dead.

TABLE 3

F_1 phenotypes and autogamous F_2 segregations of crosses between the dancer mutants and the trichocyst nondischarge mutant

Cross	F_1 phenotype[a]	Autogamous F_2[c]				P[b]
		+	*Dn*	*nd6*	*Dn and nd6*	
1) *Dn*[1]/*Dn*[1] × *nd6*/*nd6*	Partial dancer	39	56	45	32	0.07
2) *Dn*[2]/*Dn*[2] × *nd6*/*nd6*	Partial dancer	36	31	32	39	0.75
3) *Dn*[3]/*Dn*[3] × *nd6*/*nd6*	Partial dancer	47	41	59	42	0.25
4) *Dn*[4]/*Dn*[4] × *nd6*/*nd6*	Partial dancer	22	19	25	20	0.80
5) *Dn*[5]/*Dn*[5] × *nd6*/*nd6*	Partial dancer	36	30	29	26	0.65

[a] The partial dancer phenotype consists of a prolonged backing response in a 20 mM K^+ solution but only one-half the duration of the dancer parent.

[b] χ^2 values were calculated using the expected ratio of 1:1:1:1.

[c] The viability of the autogamous F_2 isolates was >90% in all crosses.

noiac (VAN HOUTEN, CHANG and KUNG 1977; R. HINRICHSEN and C. KUNG, unpublished results). The reasons for segregation distortion involving Paranoiac are unknown, but explanations involving linkage are not sufficient. Macronuclear regeneration has not been ruled out as a cause.

The phenotypes of the double mutants constructed from these crosses were analyzed (all double mutants were genetically confirmed by backcrossing the putative doubles to the parents). In the case of the double mutant dancer, pawn A and dancer, pawn B, the double mutants showed the behavior of the pawn, *i.e.*, no response to any stimuli. It was expected that pawn would be epistatic over dancer because the pawn mutations are known to have no Ca^{2+}-channel function (KUNG 1979). Pawn C is a temperature-sensitive mutant that exhibits the full pawn phenotype when grown at 35°; but it behaves as a very leaky pawn at 23°, showing approximately one-quarter of the backing response in 20 mM K^+ as the wild type. When the double mutant of pawn C and dancer was grown at 35°, the pawn phenotype was epistatic over the dancer. However,

when the double mutant was grown at 23°, it did not act like pawn C or dancer but showed an approximate wild-type behavior in a 20 mM K^+ solution. This apparent mutual suppression of pawn C and dancer may indicate the averaging of the stronger and weaker Ca^{2+} current of the two mutations. When the dancer mutant was crossed to the other behavioral mutants, the double mutants that segregated in the F_2 displayed the characteristics of both mutants.

Phenotypic change in dancer during conjugation: BERGER (1976) has shown that when pawn A conjugates with the wild type, the phenotypic change of the pawn partner takes place within hours (much before the new genome is expressed). The phenotypic change has been shown to be caused by a cytoplasmic factor (HIWATASHI, HAGA and TAKAHASHI 1980; HAGA and HIWATASHI 1982; HAGA *et al.* 1983). Since both pawn and dancer affect the Ca^{2+} current, it was of interest to determine whether there was also a cytoplasmic factor that influences the behavior of dancer.

We first repeated Berger's experiment but substituting pawn B; the duration of the backing response was measured according to the procedure of HAGA *et al.* (1983). As in the case of pawn A, when pawn B was crossed to the wild type, the pawn partner very rapidly (less than one fission) gained the ability to swim backward (Figure 4A). The wild-type partner, on the other hand, took two to three fissions to gain the heterozygous (*pwB*/+) phenotype which was barely different statistically from the wild type (+/+) phenotype.

Since pawn B could be cured by wild-type cytoplasm, but the wild type was not much affected by pawn B cytoplasm, the phenotypic conversion of the pawn partner was complicated by the cytoplasmic effect and the expression of the new genome. To separate these two effects, we monitored cytogamous pairs, in which case the two cells fused properly (*i.e.*, exchanged cytoplasm) but did not cross-fertilize. The pawn partner showed the ability to swim backward almost immediately but lost it after two to three fissions (data not shown).

The conversion of the heterozygote (*pwB*/+) to the homozygote (*pwB*/*pwB* or +/+) was accomplished by crossing the heterozygous F_1 cells to one another. The phenotypic change from *pwB*/+ to +/+ took only three to five fissions, whereas the change from *pwB*/+ to *pwB*/*pwB* took greater than nine fissions (Figure 4B). Therefore the rates of phenotypic change involving *pwB* are highly asymmetric as in *pwA* (BERGER 1976); it was very rapid going from pawn to wild type and very slow going from wild type to pawn.

We next did the identical experiments using dancer. When Dn^1/Dn^1 was crossed to +/+, there was no rapid effect on the behavior of either partner. This was also confirmed with cytogamous pairs. Since the pawn partner in a cross between Dn^1/Dn^1 and *pwB*/*pwB* could be cured within hours (data not shown), the dancer mutant was not inhibited in the ability to transfer cytoplasm. The phenotypic conversion from Dn^1/Dn^1 to $Dn^1/+$ and from +/+ to $Dn^1/+$ took three to five fissions to complete (Figure 4C). The conversion from $Dn^1/+$ to +/+ or to Dn^1/Dn^1 also took three to five fissions (Figure 4D). Therefore, the rate of phenotypic conversion from parent to F_1 and vice versa was symmetric. This is in direct contrast to the rates of conversion seen in pawn A and pawn B.

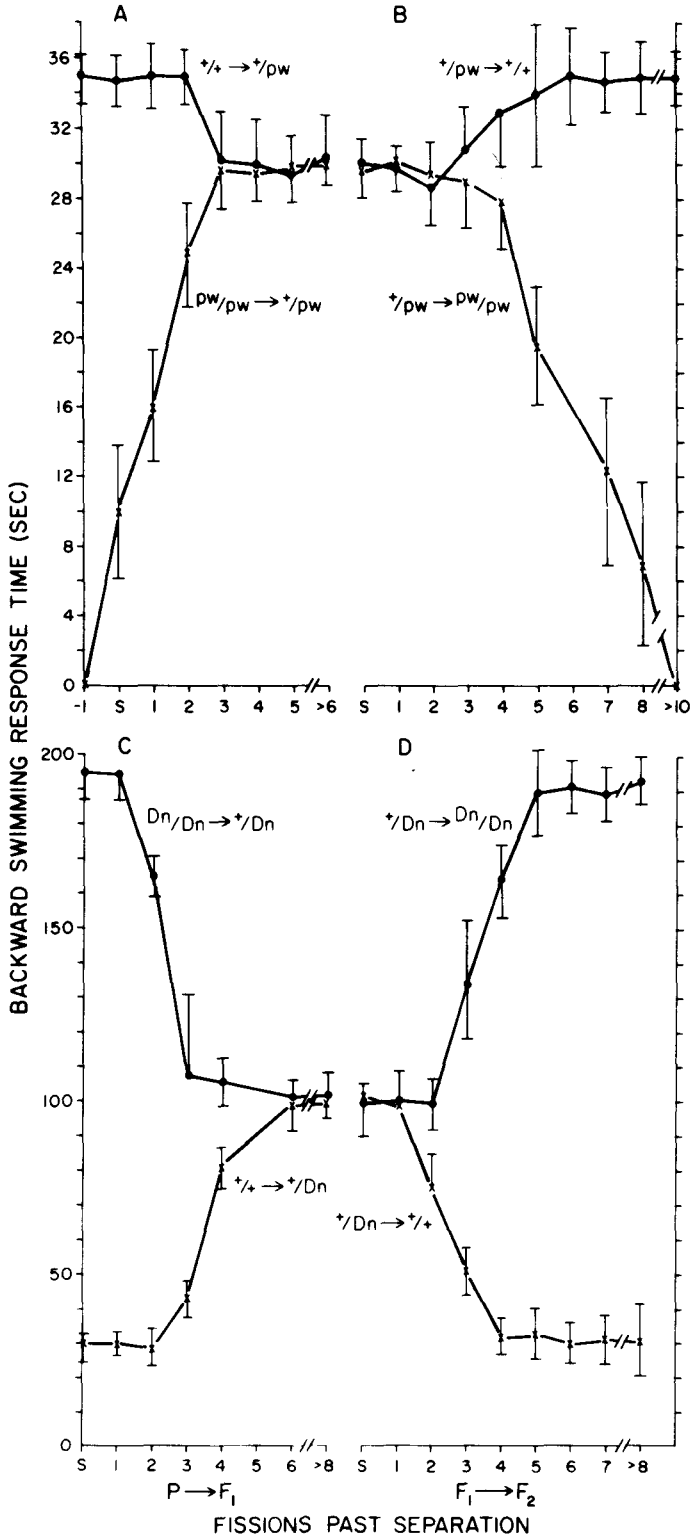

FIGURE 4.—The time course of phenotypic change from parent (P) to F_1 and F_1 to F_2 generation for pawn B (A,B) and dancer (C,D). Pawn B and dancer were crossed to the wild type, and the phenotype (duration of backward swimming) of each exconjugate was monitored. For the conversion from F_1 to F_2, heterozygous cells were crossed (+/*pwB* × +/*pwB* or +/*Dn* × +/*Dn*). The backward swimming response was tested in 20 mM K^+ in Dryl's solution. Both pawn B and dancer experiments were repeated two times, using six pairs of cells both times. The bars represent the standard deviation.

The lack of a diffusible substance from wild type which "cures" the dancer: The experiments indicated that dancer is not rapidly restored to the wild-type phenotype by cytoplasmic exchange during conjugation. To directly establish that dancer was not "cured" by wild-type cytoplasm as in pawn, microinjection of wild-type cytoplasm into pawn B and dancer was performed. The restoration of pawn B was detectable by 2 hr after injection and becomes maximal by 8 hr. The restored activity lasted for 2–3 days and the recipient eventually returned to the inexcitable mutant state (Figure 5A). Identical microinjection experiments transferring wild-type cytoplasm into the dancer mutants had no effect on the dancer phenotype (Figure 5B) indicating the lack of a diffusible material that was capable of restoring the altered Ca^{2+}-channel function in this mutant.

DISCUSSION

The dancer phenotype: Paramecium has Ca^{2+} channels that are voltage activated but are inactivated by intracellular Ca^{2+} (ECKERT and BREHM 1979). The Ca^{2+} channels in the dancer mutants activate properly and have a normal peak inward current, but the channels inactivate more slowly and there is a large sustained inward current (HINRICHSEN and SAIMI 1984). Greater amounts of Ca^{2+} enter the cell as compared with the wild type. This conclusion is consistent with their exaggerated behavior in a K^+ solution (Table 1) and by the fact that dancer cells die sooner in a Ba^{2+} solution than the wild type (Table 2). Furthermore, it has been reported (HINRICHSEN and SAIMI 1984) that the Ca^{2+}-dependent K^+ and Na^+ currents of dancer also are increased, which is expected if there is a greater Ca^{2+} influx into the cell. Consequently, the dancer mutant can be used as a means to "biologically inject" greater amounts of Ca^{2+} into the cell than would normally be allowed.

The prolonged reversal of the ciliary beat of dancer in a 20 mM K^+ solution is explained by the enhanced sustained Ca^{2+} current. Repolarization by means of the Ca^{2+}-dependent outward K^+ current (SAIMI *et al.* 1983) does not occur here because of the high external K^+ concentration where the driving force for K^+ is very small. In 4 mM Ba^{2+} the dancer cells apparently have an increased amount of Ba^{2+} that enters the cell, which results in the slow spinning behavior. Although Ba^{2+} and Ca^{2+} pass through the Ca^{2+} channel, Ba^{2+} may not be as effective as Ca^{2+} in activating the Ca^{2+}-dependent K^+ channel as well as inactivating the Ca^{2+} channel. Thus, the recorded Ba-Ca action potential is prolonged (data not shown). Dancer behaves similarly to the wild type in other stimulating conditions presumably because the enhanced Ca^{2+}-dependent K^+ current counteracts the decreased inactivation of the Ca^{2+} current in the mutant and rapidly repolarizes the cell to prevent long bouts of backward swimming.

The rate of conversion of the dancer phenotype: The rate at which the parental phenotype is converted to the F_1 phenotype following conjugation in Paramecium is a complex process involving both nuclear and cytoplasmic factors. The parental macronucleus (the highly polyploid, vegetative nucleus) is broken down into 30–40 actively transcribing fragments that eventually are destroyed and/or diluted by the dividing exconjugants (SONNEBORN 1975). This allows

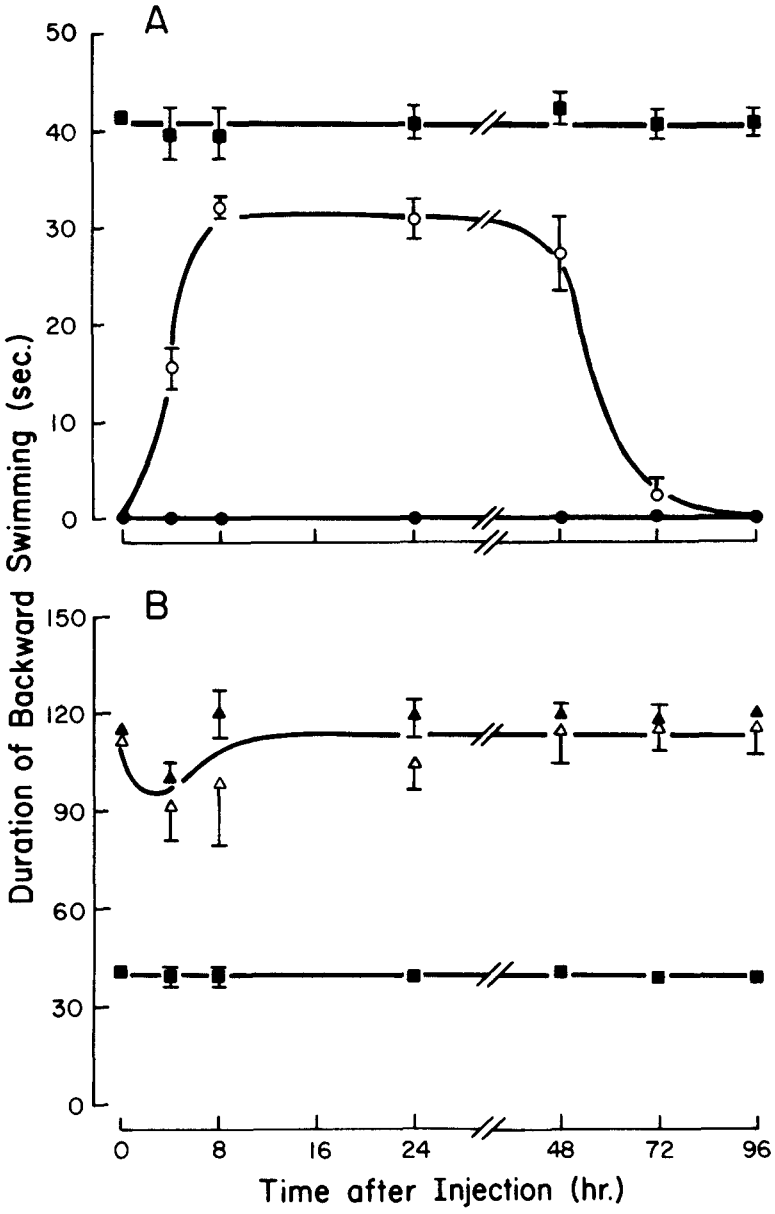

FIGURE 5.—The time courses of phenotypic change of pawn B (A) and dancer (B) following microinjection of donor cytoplasm. The recipients were injected at time 0 with cytoplasm of approximately 10% cell volume taken directly from the donor cells. The recipients were then incubated in a nutrient-depleted medium to prevent growth and division. They were periodically tested for the duration of backward swimming in response to 20 mM K^+ in Dryl's solution. (A) Pawn B cells injected with wild-type cytoplasm (○) began to show backward swimming within 4 hours; pawn B recipients of pawn B cytoplasm (●) never showed backward swimming. Uninjected wild-type cells incubated in the same depleted medium (■) were also periodically tested as controls. (B) Dancers injected with wild-type (△) or dancer (▲) cytoplasm showed no change in phenotype. Although the means of the duration of backward swimming at 4 hr after injection look slightly smaller, they are not significantly different from the duration of the uninjected dancer controls. Uninjected wild-type cells (■) are presented again for comparison. All points show means ± SD (n = 4).

both parental and F_1 genomes to be expressed simultaneously for several divisions after conjugation. There are also parental cytoplasmic components that are diluted at each division. In addition, there is cytoplasmic exchange during conjugation by which products of one mate can be transferred to the other mate. The nature of the gene product, whether it is a rapidly turned over product or a more slowly turned over structural component, should also influence the rate of conversion from parental to F_1 phenotype for a particular trait.

One explanation for the asymmetric rate of conversion seen in pawn A and pawn B is that the pawn cells are lacking a functional factor (*e.g.*, an enzyme) required for normal Ca^{2+}-channel activity. If very little of this factor is required for channel activity, the entry of a small amount of cytoplasm during conjugation from the wild-type mate would be sufficient to convert the pawn to the wild-type phenotype. Even when this cytoplasmic effect is discounted, a small amount of transcription and expression of the newly arrived wild-type allele in the heterozygote would also be sufficient to convert it to the wild-type phenotype. The conversion from the wild-type to pawn phenotype takes longer because there would be wild-type factors remaining for many divisions due to the wild-type macronuclear fragments. Therefore, until all of the wild-type factor is degraded or diluted, the cell would exhibit some wild-type phenotype. In the case of the dancer mutant, on the other hand, there is no evidence for such a cytoplasmic factor that can influence dancer in a matter of hours, both from conjugation and microinjection experiments. Also, going from the F_1 phenotype to the wild type or dancer takes approximately the same amount of time as from either the wild-type or dancer to the F_1 phenotype. Thus, dancer differs from pawn both in the absence of immediate conversion by cytoplasmic exchange during conjugation and having the same rate of conversion going from dancer to F_1 and in the opposite direction.

Is dancer a structural gene for the Ca^{2+} channel? Several pieces of evidence lead us to suspect that the dancer locus is a structural gene for the Ca^{2+} channel in *P. tetraurelia*. (1) The Ca^{2+} channel in dancer shows abnormalities in both inactivation (slower and to a lesser extent) and deactivation, which are separate properties (for details, see HINRICHSEN and SAIMI 1984). An alteration of the structure of the channel itself is most likely required to alter both inactivation and deactivation properties. (2) The semi-dominance of the dancer mutation, as opposed to a completely dominant or recessive mutation, is consistent with a mutation within the Ca^{2+}-channel locus. If there is one copy of Dn^+ product per channel, the F_1 cells would have 50% of the Ca^{2+} channels produced by the dancer allele and 50% by the wild-type allele, thus giving an intermediate phenotype. Mutations in Drosophila, suspected to affect channel structures, were also found to be semidominant (HALL 1982). (3) Since there is little effect of cytoplasmic transfer in dancer and there is a symmetry of the rate of phenotypic conversion from dancer to the wild type and vice versa, *Dn* might well code for a structural component that is diluted and/or turned over slowly. Indeed, SCHEIN (1976) has provided indirect evidence that the Ca channels in Paramecium turn over very slowly. (4) In *P. caudatum* there is a mutant, *cnrB*, that has no Ca^{2+}-channel activity. There is an allele of the *cnrB* locus, $cnrB^{Ks}$

that causes a behavioral phenotype similar to the dancer mutant, *i.e.*, an exaggerated response to K^+ (TAKAHASHI 1979). Since $cnrB^{Ks}$ is allelic to a mutation with no Ca^{2+}-channel activity and $cnrB^{Ks}$ and dancer have similar phenotypes, this may be a further indication that the dancer gene codes for the Ca^{2+} channel, assuming *Dn* of *P. tetraurelia* is the equivalent locus as *cnrB* in *P. caudatum*. (5) We have been able to identify only a single locus for the dancer mutant (this includes five separate mutageneses). The mutable target in the Ca^{2+}-channel structure may be limited, *e.g.*, to one peptide. An extensive search for γ-ray-induced deletion mutants with the dancer phenotype has thus far been unsuccessful, whereas many other γ-ray-induced behavioral mutants have been easily obtained. A deletion in a gene that codes for the Ca^{2+} channel would result in total inactivity of the channel. In this case the extreme dancer phenotype (*i.e.*, even poorer inactivation of Ca^{2+} current) could not be obtained using deletion mutagens. A similar argument has been put forth for the inability to find oubain-resistant γ-ray-induced mutants in vertebrate cells (see discussion in CHAKRABARTI *et al.* 1983). Deletion mutants of the Na^+-K^+ pump would result in inactivation of the pump, eliminating the possibility of a resistance mutant.

Galvanotaxis as a selection technique: Galvanotaxis has been employed previously as a technique to select behavioral mutants in Paramecium (SCHEIN 1976; VAN HOUTEN, CHANG and KUNG 1977; TAKAHASHI 1979). The present work is a systematic application of the technique. Many of the isolations of behavioral mutants in Paramecium previously have been through the use of vertical columns, employing the principle of negative geotaxis (KUNG 1971). The galvanotactic approach has several advantages. (1) It is rapid and allows large numbers of cells to be screened. Approximately 10^5 cells can be tested in a 5- to 10-min period. The vertical column technique, although more cells ($>10^6$) can be screened at once, is more cumbersome and time consuming. (2) By the proper manipulation of the poles, it is easy to select mutants that overreact to stimuli. The dancers are cases in point. This is much more difficult with the vertical column technique because mutants that overreact tend to remain at the bottom of the column along with some wild-type or physically damaged cells. (3) It is easy to screen for temperature-sensitive behavioral mutants because the trough can be placed on a temperature-regulated stage and the temperature of the solution in the trough can be quickly and accurately regulated. (4) With the stereomicroscope situated above the trough the cells can be monitored directly during the selection and one can select any cell that appears to be of interest. The vertical columns are not suited for direct observation.

The galvanotactic trough proved efficient in enriching for the dancer phenotype. In a simulation, when ten dancer cells (Dn^1) were added to 10^5 wild-type cells and the population treated as in the mutant-screening experiment, five dancer cells were collected in compartment C together with only two wild-type cells.

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