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6. Besides single-sinusoid gratings, square waves (bar gratings) were also used in these experiments. The influence of spectrally proximate noise was similar.
7. It must be noted that this expectation will be tempered by two provisions. First, previous results on visual critical-band masking are based on one-dimensional presentations; the experiments reported here deal with two-dimensional displays. Second, in the prior work only single sinusoids or square waves were used; the present material is pictorial. So, provided these differences are unimportant, we may be able to establish a case for critical-band masking in the present experiments.
8. Although the noise amplitudes and bandwidths were the same for the two cases, the noise energies were not thereby made equal. This can be seen by considering that in the two-dimensional Fourier space, the ratio of the areas of the annuli described by the noise bands $4w$ to $7w$ and w to $4w$ are unequal in the ratio 2.2 : 1. Furthermore, the human contrast-sensitivity curve (modulation transfer function) peaks around 7 cycles per degree, and thus the eye's noise-sensitivity is different for the two cases. To obtain equal masking energies in Fig. 3a and Fig. 3b, it is necessary for the average observer to view Fig. 3 from a distance of about 18 times the picture height. For closer viewing distances, the perceived noise in Fig. 3b becomes relatively greater, and the test is even more stringent.
9. We acknowledge the assistance of A. B. Lesk for the computer programming and picture processing. We thank L. R. Rabiner and R. W. Schafer for their consultation on digital filtering techniques, and we are grateful to them and to other colleagues for critical discussions and manuscript review.
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Temperature-Sensitive Pawns: Conditional Behavioral Mutants of Paramecium aurelia

Abstract. *"Pawns" are mutants of* Paramecium aurelia *in which the process of calcium activation during membrane excitation is genetically impaired, with a corresponding loss of avoiding reactions. Mutants are selected that behave normally when grown at 23°C but as pawns at 35°C. The normal excitation can now be disrupted and restored in the same strain at will.*

We have succeeded in selecting temperature-dependent mutants by modifying a previous method based on the principle of chemotactic interference with geotaxis (*1*). These mutants behave normally at room temperature and are virtually indistinguishable from the wild type. When grown at restrictive temperatures, they exhibit behavioral aberrations which can be grouped with the phenotypes of the previously analyzed, temperature-independent behavioral mutants. Of major interest are the temperature-sensitive "pawns" that lose their avoiding reactions completely when cultured at 35°C.

Ciliated protozoa perform the avoiding reaction in response to various stimuli (*2*). This reaction involves a period of backward swimming which results from reversing the beating direction of cilia. Such reversal is correlated with membrane depolarization (*3*). Eckert proposed that the influx of Ca^{2+}, as the result of such depolarization in normal membranes capable of Ca activation, causes the ciliary reversal (*4*). Kung reported the discovery of the behavioral mutant pawn, which was completely lacking the avoiding reaction (*1*). The genetic defect was traced to the specific loss of proper Ca activation during excitation of the membrane (*5*). We then induced and searched for heat-sensitive mutants in order to understand the nature of various mutational effects which may lead to the pawn phenotype and as an expansion of our program to dissect genetically the excitable membrane of *Paramecium*.

We used the standard culture technique (*6*) and mutagen treatment (*1*). The mutagenized exautogamous cells were cultured for a phenomic lag period of four to eight fissions, and the descendant populations were used to select for behavioral mutants. In previous studies, pawns had been selected by first injecting a mutagenized population into the bottom of a screening column filled with solution of high Na^+ concentration and later taking a selected fraction from the top of this column. In this solution, the normal animals in the population exhibited repeated avoiding reactions, moving randomly about the position in which the population was placed. Mutants unable to avoid Na^+ retained the natural tendency to swim upward, completing the negative geotactic migration in a few minutes. Thus the selected fraction collected from the top of the column after migration time was greatly enriched with mutants unable to avoid Na^+, such as the pawns (*1*). Two modifications were made in the present study.

1) Mutagenized exautogamous populations of about 10^4 cells were condensed by centrifugation (250*g*) into 10 ml of culture medium. Each population was gradually adapted into a sucrose solution of a final concentration of 65.2 m*M* through three steps spaced 20 minutes apart. Each step involved slowly dripping 0.5 ml of 500 m*M* sucrose into the culture medium. The populations that had been adapted to sucrose were then gently injected through a lower spout into a column filled with a solution rich in Na (*7*). After the migration time, a top fraction of 5 ml was removed through an upper spout of the column. All cells in this fraction were subsequently cloned separately. Sucrose was used to ensure that the injected fraction was evenly layered at the bottom of the column. This method successfully prevented an undesirable flaring, often encountered in previous studies in which the injected material and the liquid in the column had a similar density (*1*, *8*). In our method, the injected population formed a dense and even layer just below the boundary of the two liquids with the individuals in the population performing repeated avoiding reactions.

2) To screen for heat-sensitive mutants, the above procedure was followed at a restrictive temperature. From the point of exautogamous expansion during the phenomic lag until cloning of the cells from the selected fractions, all steps were performed in a 37°C walk-in incubator. Some clones obtained from such screening were found to be pawns at all temperatures and were phenotypically identical to the previ-

Table 1. Reactions of various strains of *P. aurelia* to different cationic stimuli at two temperatures; Na^+ means that the test medium contains 20 m*M* NaCl and 0.3 m*M* $CaCl_2$; K^+ means 8 m*M* KCl and 0.1 m*M* $CaCl_2$; and Ba^{2+} means 8 m*M* $BaCl_2$ and 1 m*M* $CaCl_2$. All solutions contain 1 m*M* tris buffered at *p*H 7.2. Figure 1 records the reaction of the Ba^{2+} solution. +, indicates the presence of obvious avoiding reactions; −, indicates the complete lack of avoiding reaction.

Strains	Reactions of cells grown at:					
	23°C			35°C		
	Na^+	K^+	Ba^{2+}	Na^+	K^+	Ba^{2+}
51s (wild type)	+	+	+	+	+	+
d4-133 (*ts* pawn)	+	+	+	−	−	−
d4-95 (pawn)	−	−	−	−	−	−

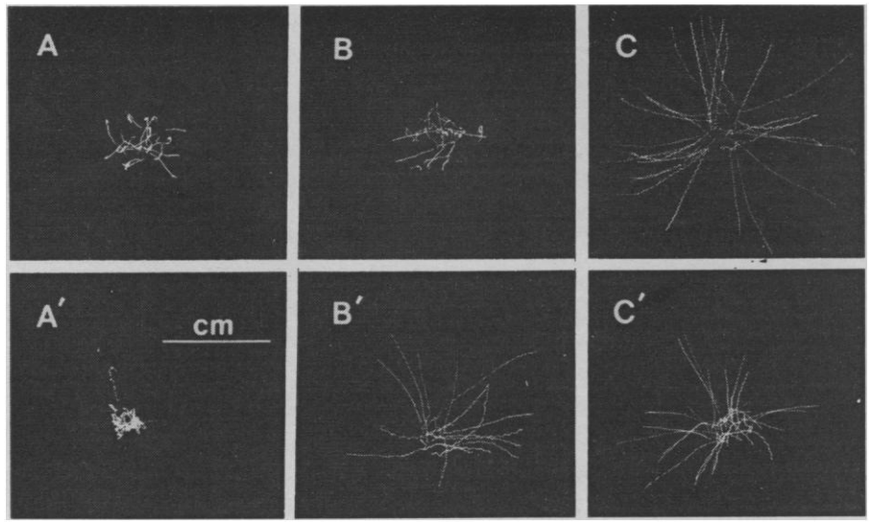

Fig. 1. Behavioral responses to Ba^{2+} solution of three strains of *P. aurelia*. These are dark-field photomacrographs taken at 23° ± 1°C in which the continuous lines record the movement of cells during the 13.3 ± 0.1 seconds immediately after they were put into the Ba^{2+} solution. (A, A′) Wild type (strain 51s). (B, B′) A *ts* pawn mutant (strain d4-133). (C, C′) A temperature-independent pawn mutant (strain d4-95). (A, B, C) Cells grown at 23°C. (A′, B′, C′) Cells were grown at 35°C and were photographed within 30 minutes after they had been taken from the incubation at 35°C. A and A′ show wild type repeatedly avoiding the Ba^{2+} solution. This reaction results in confining the cells to the vicinity of the area in which they were put. C and C′ show the unconditional pawn mutants swimming into the surrounding Ba^{2+} solution along their usual helical courses with no avoiding reaction. This results in the sun-ray patterns. B and B′ show the *ts* pawn mutants behaving like the wild type when grown at 23°C and like pawns when cultured at 35°C.

ously known pawns. However, five clones were heat-sensitive pawns, the types we had set out to find.

One of these heat-sensitive pawns (*ts* pawns), d4-133, was studied in more detail. Its behavior was compared to that of wild type and the unconditional pawn, d4-95. Animals of this strain were virtually indistinguishable from the wild type in their behavior when grown at room temperature (23° ± 1°C). Spontaneous avoiding reactions in culture medium were observed. To test their chemotactic reactions to various cations, several animals were gathered with a micropipette, then transferred into test solutions of defined ionic concentrations. Both d4-133 and the wild types (stock 51s) reacted to Na^+, K^+, and Ba^{2+} with avoiding reactions (Table 1). Depending on the concentrations and ratio of cations in the solutions, repeated quick avoidances or violent avoiding reaction involving a few seconds of backward movement could be observed. The temperature-independent pawns such as d4-95 (*1*, *5*) showed not even weak avoidance in all these solutions and thus swam directly out of the drop of culture medium into the test solutions. When the cells were grown at 35° ± 1°C and examined at 35°C anytime afterward or at 23°C within 2 hours, d4-133 behaved in a manner totally different from the wild type and became indistinguishable from pawn d4-95. Wild type cultured at 35°C avoided the above test solutions, although the reactions were slightly weaker. Pawn d4-133 lost its avoiding reaction after being grown at 35°C. The loss was complete since the pawns then failed to react to various ionic stimuli (Table 1). This complete loss of avoiding reaction suggests a heat disruption of a mechanism general to the excitation process triggered by cationic stimuli. The parts of the process specific to Na^+, which can also be mutated (*1*), are not altered here.

Behavioral reactions can be recorded by a dark-field photomacrographic technique. We used a Polaroid system which is much more convenient than the previous method (*1*, *9*). Briefly, a Polaroid MP-3 Land camera was mounted above and focused on the surface of a glass plate illuminated obliquely with a Tiyoda illuminator in a dark room. Since no light entered the camera directly from the lamp, only objects on the glass plate which reflected the light could provide an image. From 10 to 30 cells in a drop of culture fluid were put in the middle of a thin film of solution containing 8 m*M* $BaCl_2$, 1 m*M* $CaCl_2$, and 1 m*M* tris (*p*H 7.2), placed on the glass plate ahead of time. The shutter of the camera was opened immediately after the drop was added. A Prontor CR-4 ultraslow cable release, overriding the exposure control of the camera, was used in order to attain a shutter opening of 13.3 (± 0.1) seconds. The trajectory of each paramecium during the time of film exposure was recorded as a continuous line (Fig. 1). Figure 1A shows the violent avoiding reactions of wild type (51s) to this Ba^{2+} solution. The animals jerked repeatedly and sometimes swam backward for a few seconds. These are the periodic ciliary reversal and the continuous ciliary reversal described by Dryl and Grebecki (*10*) and correspond to the generation of all-or-none action potentials recorded electrophysiologically in the presence of Ba^{2+} (*11*). Unconditional pawn d4-95, unable to generate such potentials (*5*), also failed to avoid Ba^{2+} (Fig. 1C). The mutants dashed away from the drop of culture fluid in the center into the Ba^{2+} solution in the periphery. Aside from the small irregularities near the center, which represent the passive disturbance right after the drop had been added, all lines were helical paths leading away from the central origin. The *ts* pawn, d4-133, when grown at room temperature, behaved normally as the wild type (Fig. 1B). The small difference between A and B of Fig. 1 is no more than the variation encountered when recording from the same strain. Cells grown at 35° ± 1°C were slightly less active. The wild-type reaction to the Ba^{2+} solution was predominantly the periodic ciliary reversal (Fig. 1A′). Pawn d4-95, when grown at 35°C, remained indifferent to the Ba^{2+} stimulation, although the outward movement was slightly slower (Fig. 1C′). Heat-sensitive pawns, d4-133, when grown at this restrictive temperature, lost their avoiding reactions completely. Their pattern of reaction to Ba^{2+} was distinctly the pattern of pawn, but not that of the wild type (Fig. 1B′).

Heat-sensitive phenotypes are generally regarded as the result of temperature-dependent macromolecular alteration. Yet we should not make direct inference from the *ts* pawn phenotype to the molecular nature of the membrane Ca gate which appeared to be the ultimate target of various pawn mutations. The genetic relations among various pawns and especially the kinetics of phenotypic alterations upon temperature step changes can aid in the probe for the nature of the relevant membrane structure. Electrophysiologi-

cal comparison of pawns of different genetic loci is necessary for further analysis.

The heat-sensitive pawns are interesting material for the studies of membrane excitation because the normal process can now be switched on and off and presumably only one molecular species was altered in the disruption and restoration of excitation.

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Retrograde Amnesia and the "Reminder Effect": An Alternative Interpretation

Abstract. *Recent findings suggest that amnesic agents block the retrieval of stored information. "Reminder" treatments, such as noncontingent punishments given after the production of amnesia for avoidance learning, improve the later retention performance of an animal. The data reported suggest that noncontingent treatments provide an additional learning experience which adds to the retention performance of partially amnesic or poorly trained animals.*

Electroconvulsive shock (ECS) and a variety of other treatments produce retrograde amnesia. Animals treated shortly after training perform poorly on subsequent retention tests. These facts are not in serious dispute. Controversy centers on interpretations of the basis of the retention deficit (*1*). One interpretation is that the treatments produce retrograde amnesia by interfering with time-dependent processes underlying memory storage (*2*). An alternative interpretation of experimentally produced retrograde amnesia is that amnesic treatments produce retrograde amnesia by interfering with the retrieval of stored information. The findings of several studies appear to support the "impaired retrieval" interpretation of retrograde amnesia (*3–6*). In many of these studies, animals are (i) trained on an inhibitory (passive) avoidance task, (ii) given a treatment (such as ECS), (iii) given an initial retention test, (iv) given a noncontingent aversive treatment [usually a noncontingent footshock (NCFS)] in a different apparatus, and (v) are then given a second retention test. The noncontingent aversive stimulation appears to attenuate the retention impairment produced by the amnesic treatment. According to the impaired retrieval interpretation, these findings indicate that the noncontingent aversive treatment serves as a "reminder" and thus enables the animal to retrieve the stored but previously unavailable information.

However, another possible explanation of the "reminder effect" is that the NCFS is itself a weak training experience which an animal with some retention of inhibitory avoidance training will generalize to the task on which it is tested. This generalization hypothesis predicts that the retention performance of animals which would otherwise show poor retention will be increased by the NCFS treatment. Studies on the "reminder effect" have two results that support the hypothesis presented here: (i) the retention latencies of the retrograde amnesia control groups are generally higher than the latencies of the naive level, indicating incomplete retrograde amnesia (*3, 4*), and (ii) retention latencies of the control group that received footshocks (FS) are also increased by the NCFS (*3, 5*). These findings suggest that the NCFS provides an additional training experience which summates with retention of an avoidance task.

We tested the generalization hypothesis directly by (i) comparing the effectiveness of "reminder" treatments on animals with different degrees of retrograde amnesia and (ii) examining the effectiveness of "reminder" treatments on animals that are poorly trained initially but never given an amnesic treatment.

Sixty-day-old male Sprague-Dawley rats (250 to 300 g) (Simonsen) were used. Under Nembutal anesthesia, we implanted stainless steel skull screw electrodes bilaterally over frontal and posterior cortex (2 mm anterior to bregma, 2 mm lateral; 7 mm posterior to bregma, 2 mm lateral) in 21 of the rats. These rats were then allowed a 1-week recovery period. Other animals were unoperated.

All animals were water deprived to 80 percent of their initial weight over a period of 6 to 8 days. The rats were then given 4 days of preliminary training during which they were allowed to lick for a 30-second period from a water tube at one end of a straight alley. The alley was divided into two compartments: a white safe compartment (24 by 14 by 12 cm) separated by a sliding door from a black shock compartment (37 by 14 by 12 cm). Each day the animals were placed in the white compartment and the door was opened, allowing the animals to run to the water tube that protruded from the far end of the black compartment.

On day 5, animals were divided into seven groups (Table 1). (i) The strong-FS control group ($N = 8$) received a 2-ma, 1-second FS administered from a constant current source during the 10th second of licking on day 5. These animals, as well as animals in all other groups were given retention tests 24 hours (T_1) and 48 hours (T_2) later. (ii) The retrograde amnesia control group ($N = 6$) received the strong FS followed 0.1 second later by 6-ma (0.5-second, 60-hertz, sine wave, constant current) stimulation of posterior cortex. This amnesia treatment was chosen on the basis of prior studies that used the same task (*7*). One hour after T_1 the animals were placed in the NCFS apparatus but received no FS. (iii) The retrograde amnesia + NCFS group ($N = 15$) received a 2-ma, 1-second (strong) FS followed 0.1 second later by 6-ma stimulation of posterior cortex. One hour after T_1 each animal received