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3. A. Liberman, F. Cooper, D. Schankweiler, M. Studdert-Kennedy, *Psychol. Rev.* **74**, 431 (1967); I. Mattingly, *Am. Sci.* **60**, 327 (1972). The introduction of concepts from the field of human speech perception should not be interpreted as an attempt to make direct analogies with animal studies. One very important difference is that speech tokens (phonetic segments) are without meaning (semantic reference) in isolation whereas the end points, at least, of continua of animal sounds may convey a message in isolation. The existence of fine gradations in vocal signals is not necessarily matched by the perceptual ability to distinguish among these gradations.
4. The presence of a peak in a discrimination function is, in my opinion, the most reliable operational definition of categorical perception. However, experimental procedures (methods and contexts of the signal presentation and response criteria) can influence human discrimination of intraphonemic tokens within the voicing continuum. In some situations, intraphonemic discrimination is comparable to interphonemic discrimination. Thus, other criteria for categorical perception in humans are currently being used and debated [see, for example, D. Pisoni and J. Lazarus, *J. Acoust. Soc. Am.* **55**, 328 (1974); Sinnott *et al.* (*5*); N. MacMillan, H. Kaplan, C. Creelman, *Psychol. Rev.* **84**, 452 (1977)].
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8. See D. Pisoni [*J. Acoust. Soc. Am.* **61**, 1352 (1977)] and Sinnott *et al.* (*5*) for relevant data from human and monkey studies.
9. Presenting signals in close temporal proximity to trained animals in a quiet environment may enhance their selective responsiveness relative to animals tested under more natural conditions. Failures to discriminate under "ideal" conditions probably reflect limitations (or "special processing") of the auditory system. However, many species fail to respond at all under highly artificial conditions, and we need more comparative data. C. Snowdon (*Brain Behav. Evol.*, in press) discusses the difficulties of comparing the results of animal studies and human studies, which often have different goals as well as methods.
10. Acoustic signals are, however, usually graded in more than one way. They are often produced at the same time as other kinds of signals (for example, visual) especially in close-range communication in primates. Differences in properties which, in isolation, are hard to discriminate may, in consistent combinations with other cues, contribute to the efficiency of discrimination of the whole (*2*, *3*).
11. W. Smith, *The Behavior of Communicating* (Harvard Univ. Press, Cambridge, Mass., 1977).
12. R. Oldham and H. C. Gerhardt, *Copeia* **1975-II**, 223 (1975).
13. If a male produces mating calls near another calling male, one or both animals frequently switch to bouts of pulsed calls until one of the animals either leaves or stops calling.
14. In a preliminary analysis, I found that among 560 pulsed and mating calls recorded in close temporal proximity, about 7 percent could be classified as acoustically intermediate. Calls classified as pulsed had as few as four and as many as seven cycles of modulation. Three-fourths of the intermediate calls had four or more cycles of modulation. They were so classified because the pulsed part of the call was followed by a relatively long unpulsed part. I do not believe that the variations observed in the number of cycles of modulation in pulsed and intermediate calls are correlated with gradations in the level of male aggressive behavior. In other words, a male that produces pulsed calls with seven cycles of modulation is not necessarily more aggressive than a male that produces pulsed calls with four cycles of modulation. A more likely index of male aggression seems to be the frequency with which a male produces bouts of pulsed calls.
15. After the initial pulsatile beginning [the rate of pulsing is much higher than in pulsed calls (Fig. 1A)], the rate of modulation in mating calls is typically around 300 per second. Thus, the amplitude-time envelope appears smoother in comparison with pulsed calls when the same time base is used for oscillographic analysis. The ~50-per-second modulation of pulsed calls appears to be superimposed on the ~300-per-second quasi periodicity of the mating call (*12*).
16. A synthetic call similar to the one illustrated in Fig 1B (UM) attracted female green tree frogs as effectively as a typical, natural mating call [H. C. Gerhardt, *J. Exp. Biol.* **61**, 229 (1974)]. Another study with synthetic calls established that amplitude-modulation of such a synthetic call at rates of about 50 per second and depths of at least 50 percent significantly reduced its relative attractiveness (*17*).
17. H. C. Gerhardt, *J. Exp. Biol.*, in press.
18. One female was first tested with UM versus +5; she later chose +4 over M. Another female chose +2 over +3; she later chose +3 over +4.
19. In a strict methodological sense, these data fail to demonstrate continuous processing because the smallest difference that females discriminate was not determined. Thus, it is possible that if intermediates differed by less than a complete cycle of modulation, then discrimination might differ at different points along the continuum. Nevertheless, in terms of the kinds of gradations that occur in natural signals, it seems reasonable to interpret these results as reflecting more nearly continuous rather than categorical processing. The results of these experiments should be generalized to lower and higher playback levels.
20. A male typically produces pulsed calls (and occasional intermediates) in short bouts of 3 to 25 calls (mean = 10). Other frogs in the immediate vicinity may also be producing mating and pulsed calls at the same time. In the experimental situation, the female was exposed to two kinds of calls without interruption until she responded. The response patterns of the females in the experiment were similar to those of females captured in the field (in amplexus) and released near calling males. Some responded within a few seconds and others took many minutes. The choices made in the discrimination experiments were seemingly independent of the response latency.
21. H. C. Gerhardt, unpublished data.
22. It should be possible to test this hypothesis. S. Perrill, H. Gerhardt, and R. Daniel (in preparation) found that about 16 percent of the calling males in a breeding site had noncalling satellite males associated with them. Preliminary observations indicate that satellite males are attracted (up to a point) by mating calls and repelled by pulsed calls.
23. Suppose, for example, that the UM call were favored only when an intermediate call had at least four cycles of modulation and that the +3 but not the +4 call were more attractive than the M call. If females discriminated between +3 and +4 or even between +2 and +5, these results could be interpreted as demonstrating categorical processing.
24. A greater proportion of nonresponding females were among those tested with the highly pulsed intermediates (+4 and +5) and the M call in this study and other pulsed signals in another study (*17*). The animals usually took longer to respond than females tested with calls from the UM end of the continuum. However, the animals discriminated between +5 and the M call, but failed to discriminate between UM and +1 (*20*).
25. In a two-stimulus test, a nonresponding animal merely fails to provide any information about the relative attractiveness of the two sounds. In a single-stimulus test, a nonresponding animal provides equivocal information about stimulus quality. The failure of an animal to respond in a playback experiment does not necessarily indicate that the stimulus is unattractive.
26. This statement would probably not hold for many other American tree frogs or for other anurans that tend to be opportunistic or explosive breeders. The breeding season of *H. cinerea* lasts from April to mid-August in the southern United States. Males call almost every night during this period, and I have found females night after night, even under drought conditions. The animals usually breed in permanent ponds and lakes. The preference of the female for the mating call over the pulsed call may support the hypothesis that the female assesses call qualities. If she responds to a mating call, there is a much higher probability that she will achieve amplexus with the male producing it than if she responds to a pulsed call which indicates the presence of at least two males. Alternatively, the female may be avoiding aggressive male behavior associated with the production of pulsed calls. Once a female has ovulated, however, she must oviposit on the same night. This probably explains the attractiveness of pulsed calls in the absence of mating calls. Indeed, some female *H. cinerea* respond to the calls of another species, *Hyla gratiosa*, when conspecific calls are unavailable (*12*). The "motivation" of females taken from amplexus may be abnormally high (relative to when they first become responsive to mating calls) and, thus, responses to pulsed calls may be an artifact of the experimental procedure. Nevertheless, the results of discrimination (two-choice) experiments (this study) indicate that these same females make consistent choices between signals with very subtle differences.

27. I thank W. Sherman for designing the special circuitry used to synthesize the stimuli and G. Williamson and G. McClung for the use of Savannah Science Museum facilities. I thank R. Daniel, S. Perrill, S. Hopkins, D. Yaeger, and J. Rheinleander for field assistance and W. Oliver for technical help. I thank R. Dooling, R. Kiester, P. Kuhl, P. Marler, J. Miller, and C. Snowdon for valuable discussions and comments on the manuscript. This work was supported by the NSF grant BNS 73 00795 and NIH career development award NS 00217-02.

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An Ultrastructural Correlate of the Membrane Mutant "Paranoiac" in *Paramecium*

Abstract. *The highly organized array of intramembranous particles, the ciliary plaque, varies from the wild type in size and organization in two stocks of the* Paramecium *behavioral mutant, paranoiac. In one of these stocks, the alteration is dramatic.*

The behavior of paramecia is governed by the membrane potential. When the membrane is at rest, the cilia beat posteriorly and the paramecia swim forward. When the membrane becomes depolarized, the cilia beat in a reversed direction (ciliary reversal) and the cell backs up (*1*). Recent experiments suggest that the ciliary membranes are the specific components which control the direction of ciliary beat because these membranes contain ion channels important in active depolarizations (*2*). A specific structure of the ciliary membrane, the intramembranous plaque consisting of an ordered matrix of membrane particles, is regularly found at the base of the cilia and may contain important sites for ion transport (*3*). One class of behavioral mutants in *Paramecium*, the paranoiacs, is characterized by long periods of continuous ciliary reversal, hence greatly prolonged backward swimming, as an overreaction to sodium. Mutations at any of five loci can result in the paranoiac behavior (*4*). This behavior is cor-

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related with a prolonged membrane depolarization and abnormally large K^+ efflux and Na^+ influx when the mutants encounter a high Na^+ concentration (*5*). We have found alterations of plaque morphology in the two paranoiac stocks examined. In one stock (d4-578) the changes are dramatic.

All stocks used were nearly isogenic and were derived from the wild stock 51, *P. tetraurelia*. The derived stocks were d4-578 (*PaA1/PaA1*), d4-90 (*PaA/PaA*) and d4-93 (*bd/bd*, behaviorally normal, body deformation mutation frequently used to mark genetic crosses). Standard growth conditions were used (*6*). Cells were fixed, washed, frozen, stored, and freeze-fractured, and the replicas were examined by conventional methods (*7*). Forty cilia from 22 to 24 cells each of d4-90, d4-578, and behaviorally normal stocks (51 and d4-93) were examined. To avoid bias, these samples included the first 40 somatic (nonoral) cilia we encountered which had fractured to reveal the P (inner) fracture face 100 to 150 nm distal to the ciliary necklace. The necklace consists of two easily recognized bands of 10-nm particles located in the membrane just at the base of the cilium (*3, 8*). A necklace is clearly visible in Fig. 1c.

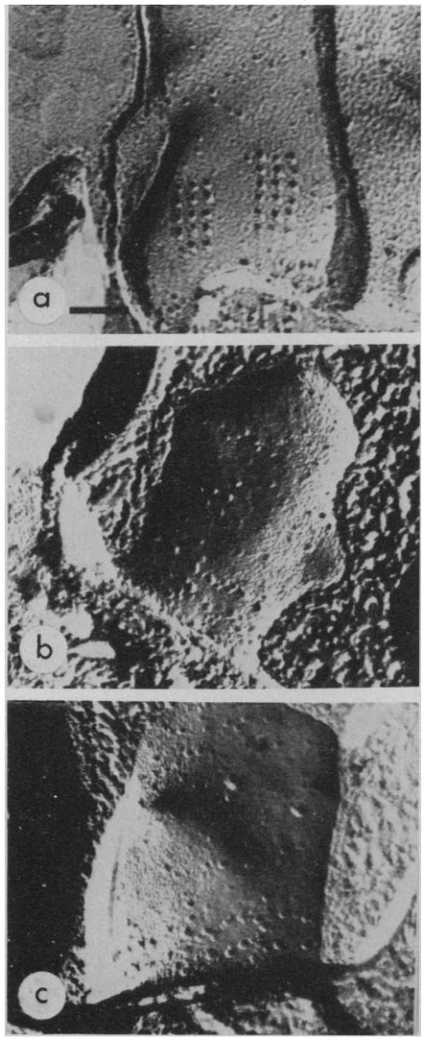

Fig. 1. Ciliary plaques of wild-type and paranoiac d4-578 cells of *Paramecium*. (a) Wild-type cilia regularly have plaques with three vertical columns and usually four to six horizontal rows. (b) Cilia from d4-578 cells characteristically have shorter, sometimes disorganized plaques. (c) In the most extreme case cilia from d4-578 cells have no organized plaques although the necklace is clearly visible. The scale line in (a) measures 100 nm; all figures at same magnification.

Our observations on wild-type cilia confirm earlier reports (*3, 8, 9*). There are invariably three columns of particles per plaque and variable numbers of horizontal rows. For the 56 plaques on the 40 cilia, the range of number of rows is three to seven, with a mean of 4.84 ± 0.73 (± standard deviation) (Fig. 2). The alignment of both rows and columns is regular with virtually no particles out of line (Fig. 1a).

The paranoiac mutant A1, stock d4-578, is strikingly different (Fig. 1b). In the most extreme cases, mutant cilia have normal necklaces but no organized plaques at all (Fig. 1c). Of the 40 cilia examined, 16 (40 percent) have no plaques. Such barren cilia were not observed in the two behaviorally normal stocks. Those cilia which do have plaques have fewer, smaller, and poorly organized ones (Figs. 1b and 2). There are many misaligned particles and sometimes entire columns of particles are missing. Within a plaque, many of the points at which a particle is expected are smooth and cannot be distinguished from the background. The frequency of such points with missing particles is 0.009 in the wild type and 0.164 in this mutant. The deficiency of particles in organized arrays in this region is not compensated by an increase in randomly distributed particles. The observed abnormalities of cilia from log-phase cells do not disappear in cells which have been starved for 18 hours in nonnutritive salt solution.

The paranoiac mutant d4-90, on which many of the electrophysiological and influx experiments have been performed, also has morphological deviations but to a lesser extent than d4-578. The number of rows per plaque field in d4-90 has a mean of 5.03, not significantly different from the wild type, but the range is from 2 to 8 and the standard deviation of 1.07 reflects a significantly different variance from that of the wild type. If one examines only the completely visible plaque fields, one finds nearly a tenfold increase in the frequency of points within a plaque at which particles are missing in d4-90 (8.3 percent) as compared to wild type (0.9 percent). A 2 by 2 contingency χ^2 analysis shows that this increase is highly significant. Examination of the E fracture face reveals no marked increase in the number of particles adhering to that face, indicating the difference is not merely in the pattern of adhesion during fracture. Preliminary results show that paranoiacs with mutations at some of the gene loci other than *PaA* also show abnormalities in plaque morphology. It seems likely that the plaques are related to some bioelectric functions and that their derangement is correlated with the paranoiac syndrome in *Paramecium*.

Plaque morphology is slightly variable even in the wild type and is somewhat sensitive to the conditions under which the cells are cultured. Plattner (*3*) reports seeing wild-type cilia with necklaces and no plaques. Dute and Kung (*10*) confirm this observation. We have not observed this in wild-type cells but have not yet explored the full range of possible growth conditions and physiological states of these cells. We have found that the plaque morphology of stock d4-90 is rather variable from experiment to experiment, sometimes approaching a wild-type pattern and sometimes clearly

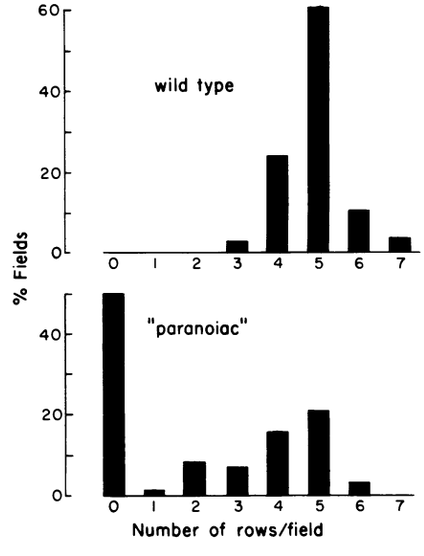

Fig. 2. Frequency of plaques having different numbers of rows of particles. Wild-type cells (51) have normal plaques with about five rows of particles. Paranoiacs (d4-578) have abnormal plaque fields, nearly half of which (27/56) are devoid of particles, that is, zero rows per field (*11*). Only plaques or plaque fields not obscured by shadow, fold, or fracture were tabulated. When there were few or no particles, the fields defined by the proper distance from the "necklace" were examined.

distinct. The behavior of d4-90 cells in response to sodium, as measured by duration of continuous ciliary reversal, also exhibits a significant range as indicated by the figures given in published reports (*5*). Stock d4-578 cells always have yielded plaque patterns readily distinguishable from the wild type. In a "blind" experiment with six coded cultures of wild-type cells and d4-578, we successfully identified each on ultrastructural bases alone.

Our finding that a mutation of a known excitable membrane characteristic is correlated with a clear ultrastructural abnormality of the membrane does not indicate the particular function or functions of the particles or the plaques, nor does it indicate a causal relation between plaque morphology and paranoiac phenotype. It strongly suggests an involvement of these structures in Na^+ influx or K^+ efflux but does not demonstrate whether this correlation is primary, that is, whether the membrane particles represent ion gates or channels, or is secondary. An eclectic approach, combining genetical, biochemical, and electrophysiological analyses may lead to a clarification of the meaning of ciliary plaque variation in the function of the excitable membrane.

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7. Cells were prepared for electron microscopy by harvesting log-phase cells from culture fluid, fixing them in 4 percent glutaraldehyde in 0.05*M* sodium cacodylate buffer, *p*H 7.2, and washing them into fresh buffer and adding glycerin over 12 hours to a final concentration of 24 percent. After fixation, the cells were kept at 4°C. The fixed cells were centrifuged, frozen in liquid Freon, and stored in liquid nitrogen. We used the freeze-fracture techniques of H. Moor and K. Mühlethaler [*J. Cell Biol.* **17**, 609 (1963)].
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9. C. A. Sattler and L. A. Staehelin, *J. Cell Biol.* **62**, 473 (1974).
10. R. Dute and C. Kung, personal communication.
11. This figure is derived by comparing the number of observed plaques on the 40 cilia from d4-578 (27) to the number observed on the same sample size of wild-type cilia (56).
12. Supported by grants from Wells College and the Research Corporation to B.J.B. and B.C.B. and NIH grant R01GM29714-03 to C. Kung, University of Wisconsin. We acknowledge C. Kung's support and comments. We also thank M. V. Parthasarathy (Cornell University) and the Midwest Regional Primate Center (University of Wisconsin) for the use of facilities, A. M. Srb (Cornell University) for advice on the manuscript, R. Dute (University of Wisconsin) for the use of unpublished observations, and A. Shilepsky (Wells College) for assistance with statistical analyses.

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Endorphins: Naloxone Fails to Alter Experimental Pain or Mood in Humans

Abstract. *In 30 human subjects, experimental pain was produced by either ischemia or cold-water immersion. In a double-blind procedure, intravenous doses of up to 10 milligrams of naloxone hydrochloride in saline were indistinguishable from similarly administered saline alone. There were no effects on subjective pain ratings, finger plethysmograph recordings, or responses to mood-state questionnaires. These laboratory procedures do not activate any functionally significant pain-attenuating or mood-altering effect of endorphins.*

The existence of endogenous morphinelike substances (endorphins) in several species, including man (*1*), raises questions about their function. Narcotic antagonists, substances that competitively replace endorphin molecules at receptor sites, reverse and block opiate effects. The failure of the antagonist naloxone even at high doses to have any effect on individuals not addicted to opiates suggests that in the normal state endorphin receptors are unoccupied. However, the ability of naloxone to diminish (i) analgesia produced by electric brain stimulation (*2, 3*) and by acupuncture (*4, 5*) in both animals and man, and (ii) stress-induced analgesia in rats (*6*) suggests that endorphins are released during these procedures. On the other hand, naloxone has no effect on hypnotic analgesia in humans (*7*). Naloxone also does not alter the threshold for escape of trained animals from an electric shock (*8*), but it reduces latency to escape when mice and rats are exposed to a hot plate for the first time (*9, 10*). In humans, naloxone at low dosage does not alter the response to painful electric shocks (*11*). In 12 subjects, we found no effect of naloxone on experimentally induced ischemic pain, but we noted an apparent increase of anxiety after naloxone (*12*). In the two experiments reported here, with 30 additional subjects, we have used both ischemia and cold-water immersion to produce pain. Naloxone had no effect on pain or mood in either procedure.

Subjects for the cold-water (N = 18) and ischemic pain studies (N = 12) were male and female volunteers in equal numbers. Informed consent was obtained. Each subject experienced the painful stimulus at three sessions at 24-hour intervals.

In the cold-water technique (*13*), pain was produced by immersing the dominant hand in a circulating bath of water at 10°C. Subjects rated the pain every 30 seconds on a 10-point scale. A plethysmograph recorded the digital pulse from the index finger of the nondominant hand. As shown by Wolf and Hardy (*13*), pain increases for the first 2 minutes, then decreases. Pulse amplitude decreases (indicating vasoconstriction) within a few seconds after the hand is immersed, then gradually returns to baseline as the pain decreases. If endorphins are involved in these adaptive responses, both the decrease in reported pain and the return of the pulse amplitude to baseline should be blocked by naloxone. Double-blind intravenous injections of saline or naloxone (1 and 10 mg) were administered in a counterbalanced order. The sequence of events at each session was: (i) begin recording the digital pulse, (ii) administer the Profile of Mood States (POMS) questionnaire (*14*), (iii) inject the drug, (iv) wait 5 minutes, (v) immerse the hand for 5 minutes and record subjective pain ratings, (vi) administer the morphine-benzedrine scale (MBG) of the Addiction Research Center Inventory (*15*) (a scale measuring opiate effects), and (vii) repeat POMS administration.

Naloxone had no effect on any of the measures. Both the pain ratings (Fig. 1) and the digital pulse amplitude showed similar adaptive responses after saline and after naloxone. After all of the injections, hand immersion produced an initial acceleration of the pulse, then a slight deceleration. The pulse remained slightly elevated during the entire period the hand was immersed. Naloxone did not affect the scores on either the MBG or the POMS (Table 1). Subjects were not able to differentiate naloxone from saline.

Since we failed to find the same naloxone effect as we had reported (*12*) on the

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