ing the hawk, and other randomly occurring variables may influence a hawk's selection of prey as well. (I was unable to monitor, let alone control, these extraneous variables.) In experiments involving simultaneous oddity, a common mouse is more likely to be exhibiting attractive, randomly occurring behavior than the odd mouse. For example, if mice of two colors are presented in a ratio of 9 : 1, the common mouse is nine times as likely to show a given attractive behavior at any given moment. In my experiments involving sequential oddity, common and odd mice each have equal probability of showing a given behavior. Thus the influence of oddity may have been obscured by random, irrelevant variables in my previous experiments involving aggregations of prey.

It is possible that reinforcement plays a role in determining the results of present and previous experiments, but I doubt it. The possibility exists because birds were not fed until after the last experiment of the day, and the color of the last mouse taken thus might act as positive reinforcement for a preference of that color. However, in all experiments the birds were fed regardless of the color of the last mouse and thus there is no reason why two birds in part A, and three birds in part B, shifted preferences. Further, a detailed analysis of previous experiments (*1*) in which mice of both colors were always available showed no correlation between the color of the mouse last taken on one day and the first mouse taken the next, when the influence of the long-term SSI was taken into account. Last, to test the possible role of reinforcement, Belle was run for ten additional days beyond those listed in part B of Table 1. In these 10 days, the mouse selected in the choice situation (11th trial) was invariably white. The bird was not allowed to feed but was then presented with a single gray mouse which it captured and the bird was then fed. In spite of the reinforcement for gray, the bird continued to choose white mice for the 10 days.

Since the birds were not permitted to feed on the first ten mice on each day, lack of reinforcement acting against the selection of that color may have been influencing the results. This also seems unlikely because the birds continued to prey upon mice in a situation where choice was not possible, in spite of the fact that they were not reinforced. Further evidence against the hypothesis can be seen in the length of runs of captures of a given color of mouse, without reinforcement, in my previous experiments, in which the birds always had a choice between gray and white. In these experiments the birds showed runs of captures of a given color of mouse greater than ten on 34 percent of the possible occasions, in spite of the fact that this often meant that the birds were continually selecting the odd or uncommon color of mice. In the 5440 individual trials, mice were presented in a ratio of 9 : 1 for 55 percent of the time, 8 : 2 for an additional 18 percent, and 7 : 3 for yet another 13 percent. Lengthy runs, particularly of the odd or uncommon color, are unlikely because of the various random influences on selection discussed above. The relatively high incidence of lengthy runs, in spite of the variety of factors which militate against them, suggests that negative reinforcement is not important in determining prey selection in my experiments.

The data thus seem to indicate a preference for odd mice in five out of six birds tested. In the remaining bird (Walda), a long-term SSI appeared to be the most important factor determining prey selection, a factor which also influenced the results from the other five birds.

Many observations and experimental results fit into the generalization that predators select odd prey (*4*), but unequivocal data are lacking. Goshawks (*Accipiter gentilis*) have been shown to select the uncommon color morph out of flocks of domestic pigeons (*Columba livia*), but odd colored pigeons can be considered to be conspicuous relative to the flock in which they fly (*5*). Other evidence for the selection of odd prey is essentially anecdotal. My results suggest that oddity is an important factor in prey selection. Odd animals are almost invariably unfit animals and a prudent predator would select unfit animals out of a prey population (*6*). Selection of unfit animals would benefit both predator and prey and tend to drive the predator-prey system to optimal yield.

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# Learning: Rapid Aversive Conditioning in the Gastropod Mollusk *Pleurobranchaea*

Abstract. *Untrained* Pleurobranchaea *feed voraciously when presented food and withdraw from electrical shocks. We trained experimental animals in ten trials spaced 1 hour apart to withdraw from food alone by electrically shocking them if they fed or were indifferent to food. The greatest increase in the number of learned withdrawal responses occurred within 12 hours after conditioning, and was accompanied by long-lasting increases in the threshold and latency of feeding responses. Control animals, which received food and shock alternately (unpaired) every half hour, showed considerably weaker changes than experimentals. These control responses quickly returned to initial levels after conditioning.*

Invertebrates offer attractive experimental material for investigations aimed at the cellular basis of associative learning inasmuch as they show simple, controllable behaviors and possess correspondingly simple nervous systems (*1*). Among these animals the mollusk *Pleurobranchaea* is uniquely suited for study because it exhibits strong associative learning as shown by classical and avoidance conditioning of its feeding behavior, and because it is the only animal in which evidence of learning can be detected in completely isolated nervous systems (*2*). The behavioral changes produced to date in *Pleurobranchaea*, however, require many days of conditioning to become firmly established. Such long periods of time present serious technical difficulties for experiments aimed at pinpointing the quality, time of consolidation, and locus of the cellular and physiological events that underlie learning. For this reason, we have sought and now report on long-lasting behavioral changes, attributable to associative learning, which can be produced in only 1 day. The effectiveness of the present conditioning paradigm, together with the suitability of the behaviors of *Pleurobranchaea* for physiological analysis (*3, 4*), provides a new and useful experimental system for studying learning.

Specimens of *Pleurobranchaea californica* (MacFarland) of about equal size and having a mean weight of 175 g were obtained off the coast of southern California. The animals were maintained individually in transparent plastic trays (6.5 liters in volume) so that they could be viewed from different angles, and in fresh running seawater (1 to 2 liters per minute at 16°C). All the animals were fed about 1 percent of their body weight of cleaned squid mantle once each week before the experiments, and were not fed during the experiments. *Pleurobranchaea* is a voracious, cannibalistic carnivore that is capable of ingesting amounts of food equivalent to about 10 percent of its body weight every 3 to 5 days, and therefore the feeding regime maintained the animals motivated for feeding. After acclimation to the laboratory, the animals were randomly sorted into experimental ($N$ = 29) and control ($N$ = 30) groups. Each experimental animal was matched with a control animal of approximately equal feeding threshold (described below), and their trays were placed side by side so that they were exposed to the same laboratory conditions. The conditioning and postconditioning observations described below were pooled from five replicate experiments, all of which yielded similar results.

In each of ten conditioning trials, spaced 1 hour apart, experimental animals were given about 5 ml of a standard solution of squid homogenate [designated $10^0$ concentration, see (*4*)], which was applied gently over the oral veil of the animal at a rate of about 0.2 ml/sec via a syringe fitted with a tube extension; we did not contact the animals while they were given food stimuli. The food solutions contained a small amount of Procion yellow dye as a visible marker so that the flow and contact of the food onto the animals could be observed. The feeding behavior consists of orientation movements, extension of the proboscis, and bite responses (*2, 3*), all of which can occur coordinately in a bite-strike response as shown in Fig. 1B. We studied the effect of conditioning on the most obvious of these, the proboscis and bite or strike responses. If the animals showed one of these responses or were indifferent to food within a 180-second period of observation, they were given electrical shocks (60 seconds; 70 volts; 10-msec d-c pulses at 15 per second). If the bite or strike response occurred, they were given the shocks immediately, but if neither of these responses occurred, and if a sustained withdrawal response was not obtained, the shocks were given after 180 seconds. The shocks were applied across the head and oral veil by means of spanning metal electrodes held several millimeters away from the animal, but contact was often unavoidable. Evidence of effective shocks was convulsive contraction of the body, lowering and contraction of the rhinophores and oral veil, and movement of the head away from the source of the shocks (Fig. 1, C and D). Control animals were run concurrently with experimentals and received as much stimulation, but they were given food and shock alternately (unpaired) every half hour.

During the early stages of conditioning, our paradigm resembled classical conditioning, since the to-be-conditioned food stimulus preceded shock by a relatively fixed interval governed by the latency of the bite-strike response. Classical conditioning requires (*5*): that the unconditioned stimulus (UCS) reliably elicits the unconditioned response (UCR); that the conditioned stimulus (CS) does not elicit the UCR; that the animals do not habituate to repeated presentations of the CS at interstimulus intervals used in the training regime; and that the conditioned response (CR) resembles the UCR. Shock was an appropriate UCS since it invariably elicits withdrawal responses (UCR). Food was an appropriate CS since, as shown by over 99 percent of about 50,000 observations made here and in previous investigations (*2, 4*), squid stimuli elicit nonhabituating feeding responses rather than withdrawal responses. And, as shown below, the CR resembles the UCR.

During conditioning, the behavioral observations were conducted blind. One person who did not know the identity of the animals presented the food and noted their responses. After this observation, a second person who knew the identity of the animals applied shock accordingly if the subject were an experimental animal or withheld shock for one-half hour if the subject

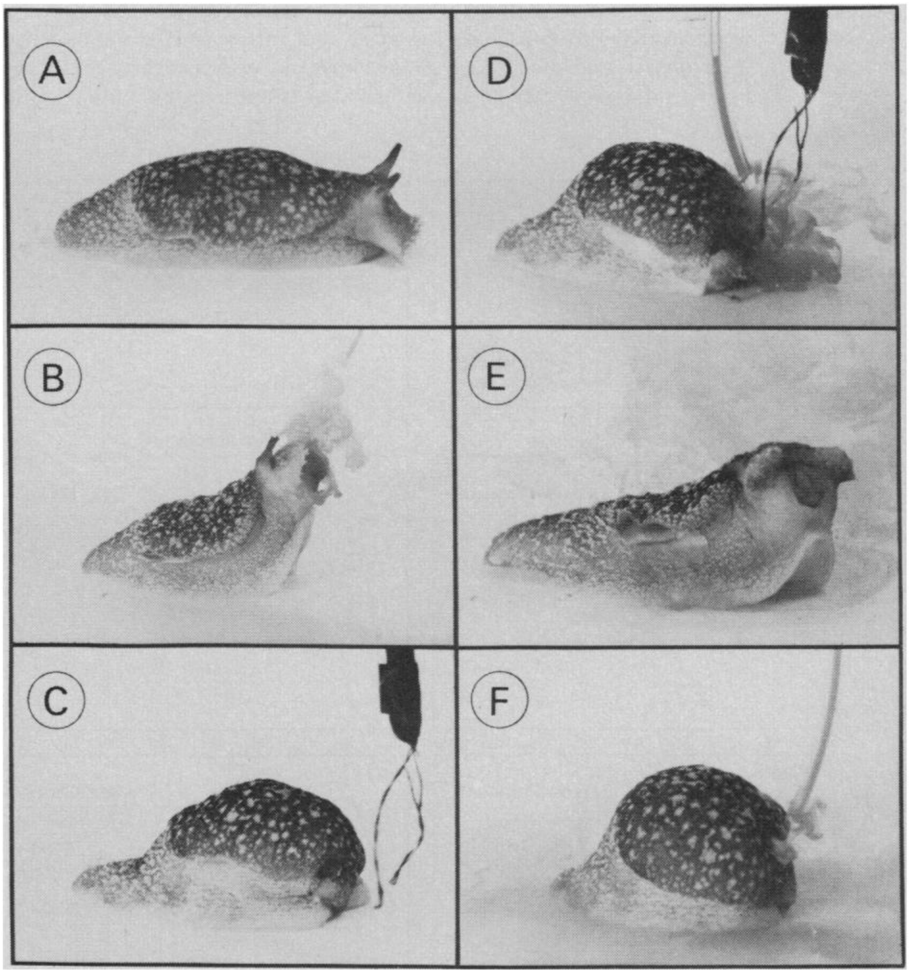

Fig. 1. Behavioral observations. (A) A specimen of *Pleurobranchaea*, about 7 inches (7.8 cm) in length, shown locomoting from left to right in the photograph. Structures pointing upward on either side of the head are the rhinophores; the oral veil, resembling a cowcatcher, is below and in front of the rhinophores; the mouth area lies below the oral veil. (B) Bite-strike response of naive animal to food: the proboscis, extended beneath the outstretched rhinophores and oral veil, and the lifted foot are aimed in almost a leap toward the descending food. (C) Unconditioned withdrawal response to shocks applied via spanning electrodes. (D) Shock-elicited withdrawal response during conditioning when food was present. (E) Approach-avoidance response. The animal is shown postured ambivalently between a withdrawal and a feeding response: the rhinophores and oral veil are withdrawn, the foot has begun to swing away, and the proboscis is only slightly extended (the lips of the mouth eventually moved in a tiny, distasteful bite). (F) A fully conditioned withdrawal response elicited by food (compare with B).

were a control. Knowledge of these operations with shock was withheld from the person who made the initial observations.

Figure 1 illustrates the behavioral changes that occurred during conditioning. Food alone initially elicited only a few conditioned withdrawal responses (CR), but as conditioning progressed, food began to elicit approach-avoidance responses (Fig. 1E) and then many withdrawal responses (Fig. 1F). Figure 2A shows, however, that the control animals as well as the experimentals exhibited such behavioral changes; only on trial 9 was there a statistically significant difference (*6*) between the experimental and control animals ($P < .01$; on all other trials $P > .05$; $\chi^2$ test).

In contrast to these results, we obtained strong differences between experimental and control animals after conditioning. The observations were begun 12 hours after the last conditioning trial and were repeated every 24 hours thereafter. In these observations, we measured the threshold and latency of the proboscis and bite-strike responses, and the withdrawal response levels. To measure thresholds, the animals were placed individually in a seawater-rinsed glass tray containing fresh seawater. Tenfold serial dilutions of the $10^0$ concentration of the squid homogenate were then applied gently over the oral veil by means of Pasteur pipettes fitted with squeeze bulbs. One milliliter of each of six dilutions was applied in each trial, beginning with a $10^{-5}$ dilution and advancing a seriatim toward the maximum concentration ($10^0$) until the proboscis and bite-strike responses were obtained. The presentation of each solution required about 15 seconds. In thousands of threshold measurements conducted in these and in previous (*4*) experiments it has been found that the proboscis extension response has about a tenfold greater sensitivity than the bite-strike response. In the event that neither of these responses was obtained with even the maximum concentration, a threshold value of $10^{+1}$ was assigned to the proboscis response, and a value of $10^{+2}$ was assigned to the bite-strike response; this avoided the mathematically meaningless notion of infinite thresholds in the computation of means. These assigned values are probably conservative since it is assumed that if the proboscis extension did not occur in response to the $10^0$ concentration it would have occurred in response to the next tenfold greater concentration ($10^{+1}$) were it available, and similarly that the bite-strike response would have occurred in response to the following tenfold greater concentration ($10^{+2}$).

Immediately after the threshold determinations, the order of animals was repeated for measuring latencies. For these measurements, the animals were retained in their home trays. A slow, continuous flow (0.2 ml/sec) of the $10^0$ concentration of food was gently presented over the oral veil; as in conditioning, we noted at this time whether the animals withdrew or fed to food. The latency of feeding movements was taken as the time from the contact of the food on the animal to the time of the first noticeable proboscis extension and bite responses.

All the postconditioning observations were conducted double-blind. The location of the animals in the laboratory was changed twice in succession by different people so that each day neither the last person who moved the animals nor the persons making the observations knew whether the animals were experimentals or controls.

Figure 2B shows that by 12 hours after conditioning there were significantly strong differences between the number of withdrawal responses obtained from the experimental and control animals ($P < .01$; $\chi^2$ test), and that the withdrawal level of the experimental animals had greatly increased while the level of the controls decreased to near the preconditioning level. These differences persisted for 132 hours without shock reinforcement before extinguishing. Increases in the response levels of the experimentals after conditioning has been observed in previous learning studies on *Pleurobranchaea* (*2*). This improvement in performance is of interest here, for it indicates that much of the consolidation of the learned response occurs in just a few hours after conditioning. The quick recovery of the controls suggests that the withdrawals obtained from them during conditioning may have been caused by sensitization which is known to produce relatively short-term effects (*7*).

Figure 2, B to E, shows that the changes in the withdrawal behavior of the animals were accompanied by equally strong changes in the feeding behavior. We recorded significant differences between the experimental and control animals in the latency and threshold of the extension of the proboscis and in the latency of the bite-

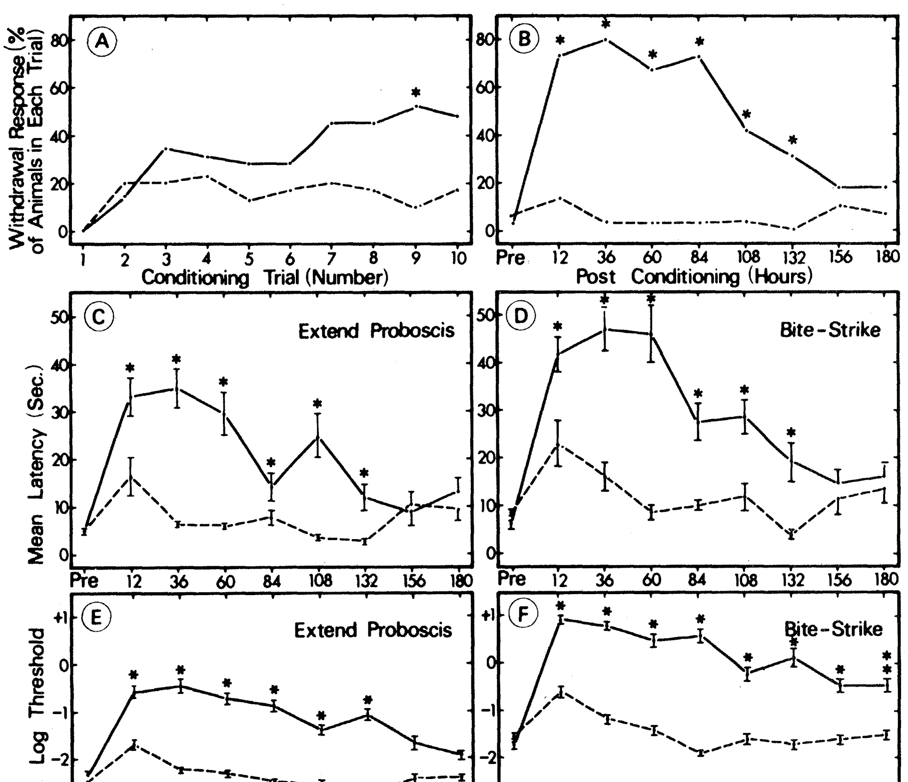

Fig. 2. Pooled data from five replicate experiments. (A and B) Percent of experimental animals (solid curve; $N = 29$) and control animals (dashed curve; $N = 30$) that withdrew from food in each conditioning trial (A) and at different times postconditioning (B). Asterisks indicate statistical differences between experimental and control groups significant at $P < .01$; differences at other points are not significant ($P > .05$); $\chi^2$ test. (C to F) Postconditioning observations on feeding behavior. Measurements of latency and threshold of the proboscis response (C and E) and of the bite-strike response (D and F) were first made at 12 hours postconditioning; preconditioning values are also shown (at *Pre*) on the abscissa. In C to F, single asterisks indicate statistical differences between experimentals and controls significant at $P < .01$, double asterisks indicate $P < .02$; differences at other points are not significant ($P > .05$); Mann-Whitney U test. Vertical bars indicate standard errors.

strike responses for 132 hours after conditioning ($P < .01$; Mann-Whitney U test). The threshold of the bite-strike response of the experimental animals, however, continued to be significantly different from the threshold of the controls for over 180 hours (Fig. 2F; $P < .01$ through 156 hours, $P < .02$ at 180 hours; Mann-Whitney U test). After 180 hours the observations on extinction were terminated so that the animals could be reconditioned in other experiments (*8*).

The results of this investigation demonstrate that pairing or contiguity of food (CS) and shock (UCS) are necessary to produce the long-term changes in the withdrawal and feeding behavior of the experimental animals. As further required in classical conditioning (*5, 7*), experiments designed to control for pseudoconditioning and sensitization, in which food and shock were presented unpaired to the control animals, did not yield the same long-term behavioral changes as were produced by paired stimulation. We interpret these data to show that the rapid and long-lasting behavioral changes of the experimental animals are attributable to associative learning.

The ability of the present paradigm to generate rapid learning of food aversion is striking, particularly when one considers that no alternative, safe food was made available and that the animals were maintained in a relatively starved condition. The drive to feed is usually so strong in *Pleurobranchaea* that feeding thresholds remain stable even after electroconvulsive shocks (*4*). It would be expected, therefore, that food stimuli would be strong reinforcers of behavior and that hungry animals would be difficult to train to avoid food. Previous experiments on *Pleurobranchaea* show, however, that a paradigm which used a food stimulus to reinforce conditioned feeding responses to mild tactile stimulation was much less effective than the food aversion paradigm we used here.

This apparent contradiction may be placed into perspective by viewing the stimulus-response repertoire of *Pleurobranchaea* in the context of behavioral hierarchy which describes how an animal will behave when confronted simultaneously by stimuli that elicit different responses (*4*). This hierarchy may also be viewed as a representation of the drive or motivation-related value of different stimuli with respect to the total behavior of the animal. The hierarchy of three of the behaviors of *Pleurobranchaea* in increasing dominance, and the inferred value of the associated stimuli in increasing strength, is: withdrawal, elicited by mild tactile stimulation; feeding, elicited by food; and escape, elicited by strong noxious stimulation. The results of the learning studies on *Pleurobranchaea* are consistent with the notion that the reinforcing power of a stimulus is governed by its drive or motivational value (*7*); tactile stimulation has been used only as a CS, food as a CS or UCS, and noxious stimulation only as a UCS. As a first approximation, we can say that the lower the position of a stimulus on the hierarchy the greater will be its utility as a CS, while the higher its position the greater will be its effectiveness as a UCS—as a reinforcer. Thus, in the context of the behavioral hierarchy of *Pleurobranchaea*, it is not unexpected that strong noxious stimulation would be a more effective reinforcer than food.

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## Acidity in Rainwater: Has an Explanation Been Presented?

The recent report by Likens and Bormann (*1*) certainly points out the manifold ecological problems, both present and potential, that are associated with the possibility of acid rain. It cannot be disputed that the tall smokestacks introduced in recent years disperse the emitted $SO_2$ over a broader area. That is precisely the philosophy behind tall stacks. Whether there has been a concomitant increase in acidity in the rainwater of rural areas in the northeastern United States has not been documented in the data of Likens and Bormann since, as shown in their figure 1, the *p*H remained relatively constant over an 8-year period.

The authors, however, do show in their figure 2 a striking 70 percent decrease in $SO_4^{2-}$ since 1950 in rainwater at Ithaca, New York. This change is probably correctly attributed to the shift in use from coal (high in sulfur) to natural gas (low in sulfur). The statement is made (correctly) that the drop in the sulfur concentration is difficult to reconcile with the proposed (not substantiated) recent increase in acidity. The contention is then made that high particulate loadings from the combustion of coal in the past could have caused neutralization of the acid sulfate. Likens and Bormann further conclude that the installation of particle-removing devices in tall smokestacks eliminates these alkaline substances, consequently permitting appreciable quantities of $SO_2$ to be converted to acid.

This argument cannot be correct. As an example, coal could contain 3 percent sulfur (6 percent as $SO_2$) and 15 percent ash (composed of 50 percent $SiO_2$, 30 percent $Al_2O_3$, 15 percent $Fe_2O_3$, and 3 percent CaO). The alumina and silica are quite inert, and there is just not enough material left to neutralize the $SO_2$. The only component of any possible significance is $Fe_2O_3$. Even if we consider the highly unlikely overall reaction

$$3\,SO_2 + Fe_2O_3 + \frac{3}{2}\,O_2 \rightarrow Fe_2(SO_4)_3$$

it would require essentially all of the $Fe_2O_3$ to consume an appreciable amount of the $SO_2$. Since there are so many competing processes, it is most unreasonable to assume that a reaction could appreciably proceed when the reactants are present in a near stoichiometric relationship. Furthermore, 25 years ago particulate-removal devices in operation in the northeast, if they were mechanical, removed > 50 percent of the ash or, if electrostatic (in common usage), > 75 percent. Modern electrostatic