a defensive arousal state, which organizes the animal's behavior for defensive action. This central arousal state [which also appears to be elicited transiently by the US (*13*)] is expressed as a priming of defensive behaviors and a concomitant depression of behaviors incompatible with defense, such as feeding. A model summarizing these results and incorporating previous findings on the effects of the US (*13*) is shown in Fig. 2.

The classically conditioned central state in *Aplysia* resembles states in mammals that have been defined operationally as conditioned fear (*14*). Indeed, the conditioned state in *Aplysia* satisfies functional definitions of fear as a general, preparatory defensive state elicited by stimuli signaling imminent danger (*15*). That such a state can be conditioned in an invertebrate indicates that the capability to anticipate danger and prepare for flexible defensive action is of considerable adaptive value and may be widespread in the animal kingdom. In addition, the discovery of a fearlike state in a simple invertebrate whose nervous system is amenable to neurobiological analysis may permit the use of cellular approaches to explore aspects of aversive learning not accessible to study with behavioral techniques alone. In the accompanying paper, we describe experiments in *Aplysia* in which we begin to examine aversive associative learning on the cellular level (*8*).

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5. The US was a 30-second train of 450-mA (peak-to-peak) a-c (60-Hz) current pulses applied through Ag/AgCl electrodes spanning the head. Each pulse in the train was 1.5 seconds long; interpulse interval was 3 seconds.
6. Tests were given in the following sequence: (i) head withdrawal and feeding, 18 hours after training; (ii) siphon withdrawal and escape locomotion, 22 hours after training; and (iii) inking, 42 hours after training.
7. Animals were tested in the semi-intact preparation used for neuronal studies (*8*). Head withdrawal in paired animals was significantly greater than that in unpaired animals [$t(21) = 3.63$, $P < .005$].
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9. A 15-second train of 45-mA a-c (60-Hz) current pulses applied through bipolar electrodes spanning the tail. Pulse duration was 1.5 seconds and interpulse interval, 3 seconds.
10. A series of 1.5-second shocks (a-c, 60 Hz) were applied at 15-second intervals through Ag/AgCl electrodes spanning the tail in the sequence 50, 100, 150, 200, 250, 300, 350, and 700 mA.
11. Test shocks were applied at 60-second intervals in the sequence 20, 40, 60, 80, and 100 mA through thin capillary electrodes (filled with seawater) held in contact with the side body wall. Again, the median inking sensitivity was greater in paired animals than in unpaired animals [$U(15, 12) = 35$ ($P < .01$].
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Suckling Infant Rats Learn a Preference for a Novel Olfactory Stimulus Paired with Milk Delivery

Abstract. *When presented a novel olfactory stimulus while suckling a passive dam, 11- to 14-day-old rat pups acquire a conditioned preference for that stimulus. The magnitude of the conditioned preference is greater if the pups received milk while suckling than if they did not. The results indicate that infants are capable of learning while suckling and that milk delivery plays a role in this associative process.*

Freud believed infants capable of learning that milk comes from the mother and thought that this was one of the more important reasons the infant sought to maintain proximity with her (*1*). This position has received some support over the years (*2*), but many have criticized it because there has been no clear demonstration that the infant associates the reinforcing properties of the milk it receives while suckling with any stimulus in the environment, including the mother (*3*). I now report what is to my knowledge the first demonstration in the suckling mammalian infant of learning based on milk reward and the transfer of that learning to a situation removed from the suckling environment.

Rat pups are capable of learning a remarkable variety of responses (*4*) but the only attempt to demonstrate that infant rats can learn while suckling the mother was that of Martin and Alberts (*5*). They found that pups suckling a passive dam were unable to learn an illness-mediated aversion to flavored milk unless they were of weanling age, even though much younger pups learn this same aversion if they received the flavored milk when they were not suckling the mother. However, suckling, even in the absence of milk, is rewarding in its own right, so that any experimentally arranged negative contingency might be overshadowed by the inherently positive value of suckling itself. Thus, a question of more immediate importance becomes whether an infant rat might learn a preference for stimuli present in the environment when it suckles and whether mother's milk plays any role in this learning.

The study consisted of two phases. In

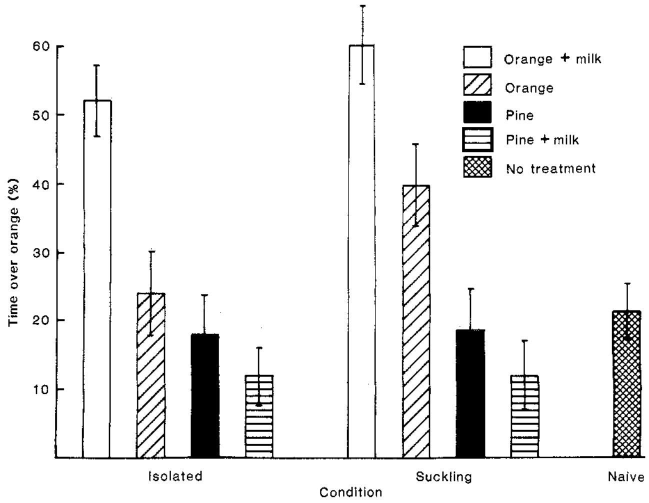

Fig. 1. Mean percentage of time spent in the presence of orange-scented shavings by pups conditioned while isolated or suckling, and by naive pups.

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the first, four groups of 24-hour-deprived Wistar rats, aged 11 to 14 days, were allowed to suckle a passive dam for 1 hour (*6*). Two groups received milk formula during the hour via an intraoral cannula (*7*). These pups received about as much milk during the hour as normally mothered pups do, and delivery of milk was contingent to a large extent on active suckling. One of these two groups was exposed during the hour to an unfamiliar odor, orange extract, in the form of a batch of orange-scented pine shavings suspended above the suckling pup's nose (*8*). The other was exposed to the more familiar odor of unscented pine shavings. (Litters were housed on a bedding of pine shavings.) The third and fourth groups received no milk during the hour they were allowed to suckle, but were exposed to either orange-scented or unscented pine shavings.

Four additional groups were run separately as isolated controls. Pups in each of these groups received the same treatment as their counterparts in the suckling experimental groups except that these pups received their experience while isolated in a plastic tub. Thus, one group received an infusion of milk while exposed to the orange odor, a second group received milk while exposed to the pine odor, and the third and fourth groups were exposed to one of the two odors alone. A ninth group of pups were deprived but received none of the experimental treatments.

In phase 2 of the experiment, conducted 1 hour after the first, pups in all nine groups were given a test in which they were allowed to choose to be in the presence of either orange-scented or unscented pine shavings. The test apparatus was a rectangular arena 21 by 32 by 14 cm with a wire mesh floor. The floor surface was divided into halves by a "neutral zone" (4 cm wide), which ran the length of the arena. A container of unscented pine shavings was placed beneath the floor on one side of the neutral zone and a container of orange-scented shavings was placed beneath the mesh on the opposite side. Pups were given three consecutive 2-minute tests. A pup was considered to be on one side or the other when its snout and front paws left the neutral zone. The amount of time the pup spent on the orange side, the pine side, and in the neutral zone were recorded.

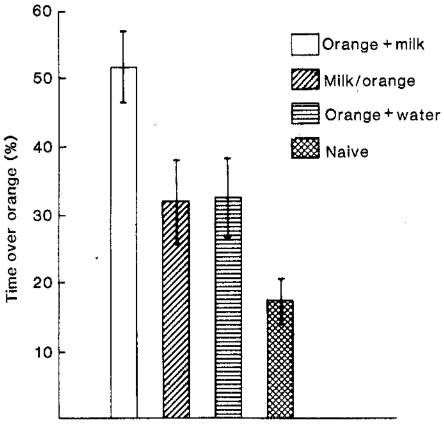

Fig. 2. Mean percentage of time spent in the presence of orange-scented shavings by pups receiving orange and milk simultaneously, milk followed by orange, orange and water simultaneously, or no treatment.

The natural, "uncontaminated" response to the unfamiliar orange scent was revealed by the percentage of time experimentally naïve pups spent on the orange side. These pups found the orange odor mildly aversive, spending only 23 percent of the total test time on that side (Fig. 1). Compared with the amount of time spent on the pine side (48 percent), this was a statistically significant behavioral aversion [dependent $t(19) = 2.39, P < .05$]. Pups that had received an infusion of milk in the presence of unscented pine shavings and pups that had been exposed to the unscented shavings alone also displayed an aversion to the orange odor (orange, 15 percent; pine, 61 percent) regardless of whether they were exposed while suckling or while isolated [dependent $t(79) = 6.78, P < .01$].

Of the two isolated groups exposed to the orange odor, only the group exposed to orange while receiving milk displayed an enhanced preference for the orange odor relative to the two groups exposed to pine [independent $t(29) = 7.34, P < .01$]. Exposure to orange alone (or to pine in the presence or absence of milk) did not result in a comparable increase in the percent of time spent over the orange side. Thus, isolated 11- to 14-day-old rat pups are capable of acquiring a conditioned olfactory preference on the basis of milk reinforcement (*9*).

Of most interest is the performance of the suckling pups exposed to orange alone or to orange and milk together. The behavior of pups in both of these groups indicates that, relative to controls, they acquired a conditioned preference for the orange odor [percentage of time on orange side for pups exposed to orange and to pine independent $t(39) = 5.75, P < .01$] (Fig. 1). This preference, however, is significantly more marked in pups that received milk while exposed to the orange scent than in pups that did not [independent $t(19) = 2.69, P < .05$] (*10*). Thus, (i) suckling, even in the absence of milk, is rewarding for 11- to 14-day-old rat pups; (ii) suckling in the presence of milk is more rewarding; and (iii) the suckling 11- to 14-day-old rat, whether in the presence or absence of milk, is capable of associating the rewarding aspects of suckling with a novel (and in this case aversive) stimulus, learn a preference for that stimulus, and display that preference in an environment outside the suckling situation.

Three additional groups were run as an auxiliary experiment. Pups in the first of these were allowed to suckle for 60 minutes; during the first 30 minutes they received milk in the absence of orange odor, and during the second 30 minutes they were exposed to the orange without receiving milk. This "backward" contingency is a standard conditioning control designed, in this instance, to help determine whether milk delivery enhances the pup's attention to the orange odor without necessarily associating the odor with delivery of milk. (Enhanced attention to the odor might result in rapid habituation to its aversive characteristics, which might in turn influence a pup's behavior in a choice test.)

The second group was allowed to suckle for 30 minutes in the presence of the orange odor while receiving tap water instead of milk. This contingency was designed to help determine whether the delivery of any fluid during exposure to the orange odor might result in differential attention to the odor.

The third group was treated exactly as the orange plus milk suckling group in experiment 1 except that they were allowed to suckle for 30 rather than 60 minutes (*11*). All three groups were thus exposed to the orange odor for 30 minutes and received either milk or water for 30 minutes. Testing was conducted as before. Neither the backward group nor the water group preferred the orange side as much as the orange plus milk group did [independent $t(28) = 2.90, P < .01$] (Fig. 2). In fact, the orange preference of the backward and water groups was nearly identical to that of the orange-alone suckling group of experiment 1. These data suggest that associative learning, not differential attention and habituation, resulted in the enhanced preference seen in the orange plus milk groups (*12*).

These findings demonstrate that infants can learn about milk delivery at the mother's breast. Previous work with human infants (*13*) and puppies (*14*) has suggested that such learning is possible by showing that infants suckling artificial nipples readily accommodate their sucking to various contingencies of milk delivery. This response ability could be taken to reflect associative learning, although in most cases nonassociative factors such as simple activation or sensitization cannot be ruled out; no independent test of learning was performed (*15*). Similarly, findings that rat pups prefer to consume fluids and solid foods that have been adulterated with the flavor of the mother's diet (*16*) might reflect simple habituation to the previously novel taste rather than associative learning.

More recent studies showing that 11- to 17-day-old rat pups perform more efficiently in runways and mazes when reward is the opportunity to suckle dry nipples generally support the current finding that suckling for milk is more rewarding than dry suckling (*17*). They do not, however, necessarily demonstrate the pup's ability to form an association *while* suckling. If one views instrumental and classical conditioning as different processes, performing a response in order to suckle (like performing a response in order to eat) is conceptually quite different from acquiring a response during suckling (which might be more analogous to acquiring an association while actually eating). Thus, the two most noteworthy findings of my experiments are that infant rats acquire new associations while actually suckling and receiving milk and that they display these newly acquired conditioned preferences in a situation removed from the suckling environment. These results bring us closer to an understanding of what an infant might learn as a natural consequence of suckling.

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6. Each of these four groups, and each of the groups mentioned later, consisted of ten pups, five of each sex. Pups within each group were drawn from eight or nine different litters. During deprivation, pups were placed in a warm (36°C), moist (50 percent humidity) incubator. The dam was rendered passive by an intraperitoneal injection of urethan (2 g in 10 ml, 1 ml per 100 g of body weight), which also blocks the naturally occurring milk-ejection cycle [D. W. Lincoln, A. Hill, J. B. Wakerley, *J. Endocrinol* **57**, 459 (1973)]. The dam was placed on her side in a warm (32°C) plastic tub. Pups suckled one at a time.
7. The intraoral cannula was installed at the start of the deprivation period. It consisted of a length of fine polyethylene tubing (PE-10, Clay Adams) with one flanged end, which rested on the pups tongue. The cannula was placed toward the rear of the pup's mouth and did not interfere with nipple attachment or sucking [W. G. Hall and J. S. Rosenblatt, *J. Comp. Physiol. Psychol.* **91**, 1232 (1977)]. Milk was delivered through them to the rear of the tongue, where the nipple usually rests during suckling, and was responded to as if it came from the nipple. The milk formula siphoned to the pup was commercially available half-and-half. Siphoning was accomplished by suspending a 1-ml syringe 30 cm above the suckling pup, attaching the external lead of the cannula to the syringe, removing the plunger from the syringe, and filling it with milk. Pups received an average of 0.75 ml of milk during the hour, but, because the frequency and intensity of the pups' sucking affected the rate of flow, some received as little as 0.45 ml and some as much as 1.25 ml. Isolated pups (pups not suckling the mother) received an average of 0.54 ml, with considerably less variance. Pups did not "stretch" while receiving milk.
8. Scented shavings were prepared by sprinkling 2.5 ml of orange extract (A & P) onto 500 ml of pine shavings and shaking them thoroughly. During conditioning, a small batch of shavings (1 to 2 g) was held by a clip 2 cm from the pup's nose. If the shavings were placed closer to the nose or scented more strongly, the pups would back-treadle and sometimes come off the nipple; although they would almost always reattach, conditioning was almost always unsuccessful.
9. Similar effects in even younger pups were first reported by I. B. Johanson, W. G. Hall, and M. H. Teicher (paper presented at the annual meeting of the International Society for Developmental Psychobiology, Atlanta, 2 to 4 November 1979).
10. The percentage of time spent on the pine side by pups in these two groups, as well as pups in the isolated orange + milk group, was conversely less. For the suckling orange + milk group this figure was 19 percent; for the suckling orange group, 37 percent; and for the isolated orange + milk group, 24 percent.
11. Pups received an average of 0.40 ml of milk during the 30 minutes and an average of 0.65 ml of water. In the backward group, about 5 minutes passed between the time milk delivery was terminated and the time the orange odor was introduced.
12. It is still theoretically possible that simultaneous exposure to milk and odor results in differential attention to the odor (and thus, possibly, differential habituation) in a way the backward or water groups do not address. Discrete presentations of milk and odor, both simultaneously and in control contingencies, might help clarify this issue, but such procedures, like most classical conditioning paradigms, are still subject to the objection that differential attention and habituation might result only in those conditions in which associative learning is most likely [P. J. Durlach and R. A. Rescorla, *J. Exp. Psychol. Anim. Behav. Process.* **6**, 175 (1980)]. A study based on sensory preconditioning procedures, in which the value of the milk reward is changed from positive to negative after conditioning, seems a promising way to address this problem.
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Difference in Brain Densities Between Chronic Alcoholic and Normal Control Patients

Abstract. *The densities of the brains of 11 chronic alcoholics were compared with those of 11 age-matched normal control subjects. Densities were determined from the density numbers generated by computerized tomography at three levels of the brain—the highest level of the lateral ventricles and the next two higher levels—with adjustments made to control for possible artifacts in the data. The advantage of the dominant hemisphere over the nondominant hemisphere was lessened in alcoholics.*

The relationship between alcoholism and structural changes in the brain has been of great interest. Most recently, computerized tomography (CT) scans of chronic alcoholics have found larger lateral ventricles and sulcal enlargement, two indices of structural changes in the brain (*1-3*). However, these techniques were not able to take full advantage of the power of the CT scan to reveal localized damage in the brain and thus left open the question of lateralized impairment in the alcoholic, as has been hypothesized in many psychological studies (*4*). Our study was designed to use more sophisticated measures of CT scan density changes to investigate the hypothesis of specific localized changes in the brain of the alcoholic.

In past studies, the ventricular and sulcal measurements have always been indirect measures of actual changes in brain density, on the assumption that decreased brain density will lead to less brain mass and subsequent ventricular and sulcal enlargement. However, one can directly measure the density of brain areas as a whole through the use of a CT scan, since its pictures are simply analog representations of the density numbers generated by the CT procedure. The numbers themselves offer much greater potential than films in terms of localization of dysfunction and the identification of more subtle disorders. The use of the CT scan in this manner is useful in such disorders as brain atrophy (*5*). Our study was designed to determine wheth-

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