amplitude distribution of all sampled EPSP's (with nondetectable EPSP's included as 0 to 50 μV) (see Fig. 2) shows a clear trend toward detectable EPSP's in animals that have received the single sensitizing (dishabituating) stimulus.

Our results indicate that the profound and long-lasting synaptic inactivation that accompanies long-term habituation can be rapidly overridden by a single sensitizing stimulus. This is surprising, since normal (spontaneous) recovery from long-term synaptic depression requires more than 3 weeks (*1*). Also surprising is the good correspondence between the environmentally induced alterations of the reflex behavior and the concomitant alteration of the efficacy of the monosynaptic connections between the sensory and motor neurons. This correspondence is probably due to two factors: (i) the simplicity of the neural circuit of the behavior and (ii) the fact that the memory for habituation (and its ability to be counteracted by another simple form of learning, sensitization) does not seem to reside in the properties of a complex network, but in the changes in efficacy of a single locus: a set of identified monosynaptic sensory connections. That the modulatory capability of sensitization is exerted at this locus seems particularly adaptive, for it allows the profound depression of sensory inflow induced by habituation to be rapidly overriden by changing environmental demands.

The ability of sensitization to rapidly reactivate inactivated synaptic connections sets limits to the possible mechanisms underlying long-term habituation. In particular, it tends to exclude drastic alteration in synaptic morphology, such as complete anatomical disconnection between sensory and motor neurons. An anatomical reconnection required by the restoration of transmission would likely take more time than 2 hours. The results are more consistent with either a subtle ultrastructural or even submicroscopic rearrangement, whereby some aspect of the transmitter release process is depressed yet is capable of rapid restoration.

Sensitization in *Aplysia* has comparable effects on short- and long-term habituation, which suggests that these two forms of plasticity may share a common underlying mechanism. Klein and Kandel have found that the enhanced transmitter release produced by sensitization at the sensory-to-motor synapses results from a facilitation of voltage-sensitive Ca^{2+} current in the sensory neurons (*10*). Habituation seems to be mediated by an inverse process, a reduction in the Ca^{2+} current (*11*). If the cellular mechanism of long-term memory for habituation simply reflects a long-term inactivation of voltage-sensitive Ca^{2+} current in the sensory neurons, it could, in turn, serve to explain how a single sensitizing stimulus, acting to enhance Ca^{2+} current, could produce a rapid restoration of synaptic transmission.

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5. Within-group comparisons were made by means of Wilcoxon matched-pairs, signed-ranks tests; between-group comparisons by means of Mann-Whitney U tests. All tests were two-tailed.
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Appetitive Learning in 1-Day-Old Rat Pups

Abstract. *One-day-old rat pups learned to probe upward into a terry-cloth–covered paddle when they were rewarded with small infusions of milk into their mouths. In the presence of two paddles, discriminable on the basis of odor and position in the test container, the pups learned to probe into the paddle that provided them with milk. These experiments demonstrate (i) that milk may serve as a reinforcer to deprived rat pups and (ii) that pups as young as 1 day are capable of appetitive learning.*

Recent studies of the development of learning and motivation have revealed unexpected capabilities of infant rats (*1*–*5*). The immature brain appears to be an adequate substrate for many functions previously thought to emerge only later in an animal's development. For example, infant rats can show long-term retention of early aversive odor conditioning (*1*) and inhibition of learned responses (*2, 3*). We now report that 1-day-old rats can learn an appetitive response and, further, can use this response in making (and later reversing) a two-choice discrimination.

The basis of this demonstration was the finding that rat pups could feed independently of the mother (*4*). Newborn rats deprived of food and water for 24 hours ingested large quantities of milk placed in a puddle in front of them or infused into their mouths through an intraoral cannula. This intake of milk was accompanied by a significant behavioral activation, characterized by mouthing, probing, rolling, locomotion, and upward reaching, all suggesting that milk had an arousing and possibly rewarding effect. In the present study, we attempted to determine whether milk would serve as a reinforcer for the deprived newborn rat, and at the same time to evaluate the learning capabilities of the pup. Recently, there have been several convincing demonstrations of early appetitive learning in rats as young as 1 week of age. Such pups will increase their runway running speed or learn a Y-maze discrimination for the opportunity to suckle from a nonlactating mother (*2, 5*). Unfortunately, such paradigms are only suited to pups at least 7 days old because younger animals do not locomote readily. Another approach was required to assess learning in newborn pups.

The task that we developed took advantage of an upward probing response that occasionally occurs when milk is infused into the young pup's mouth (*4*). Pups were required to probe upward into a terry-cloth-covered paddle above them. Pushing up into this paddle closed a microswitch and activated an infusion pump, which delivered a 3- to 4-μl infusion of milk into the pup's mouth through an intraoral cannula (Fig. 1).

In each experiment, newborn rats were allowed 6 to 8 hours of suckling after birth and were then deprived overnight (*6*). Half an hour before being placed in a Styrofoam test container, the intraoral cannula was implanted (*7*). The test container was housed inside an incubator, which was maintained at a warm temperature (33°C) and high humidity.

In the initial learning experiment, each experimental pup ($N = 6$) received a pulse of milk every time it probed into the paddle. Same-sex littermates served as the following controls ($N = 6$ in each group): (i) a yoked control, which was in an identical test container (with paddle) and which received milk only when the experimental pup probed into its paddle; (ii) a deprived control, which was also in an identical container (with paddle) but which received no milk; and (iii) a fed control, which received milk whenever the experimental pup responded but which had no paddle in its container. During an extinction period at the end of testing, each animal in this last group was put into a test container with a paddle, and its responses were compared with those of the experimental animal and the yoked control. No attempt was made to shape responding in any of the pups. Pups were placed in the containers and were tested for 12 hours with milk reward. They were then tested for a further 6 hours in an extinction period with no milk infusions.

Pups learned to probe into the paddle for milk reward. In every case, the experimental pup pushed up into the paddle many more times than the littermate yoked control or the deprived control [mean 12 hour totals: experimental, 341; yoked control, 119; and deprived control, 120; $F(2,10) = 17.6$, $P < .001$]. Typical cumulative records of responding by an experimental pup and its controls are shown in Fig. 2A. The record indicates that for the first few hours, rates of responding for the experimental animal and its controls were similar, but that after 3 hours, the experimental pup began to probe at a much higher rate than either of the controls. The record indicates that the experimental pup frequently showed bursts of responding, followed by occasional long periods without responding. These periods of inactivity may reflect satiation, fatigue, or periods of sleep. Experimental pups usually maintained, but did not gain, weight over the training period, although they might have if a more nutritionally adequate diet had been provided and if more milk had been given for each response.

During extinction, the experimental pups again responded more than any of the controls [mean 6 hour totals; experimental, 96; yoked control, 51; and fed control, 38; $F(2,10) = 4.14$, $P < .05$] (*8*). There was variability among pups in the extent to which their responding declined over the 6-hour period. One pup showed a sharp decrease after the first hour, but another maintained a high rate of responding over the entire period. Typically, however, pups showed decreased responding, with occasional periods of responding at high rates. This variability may reflect fluctuations in state of arousal and activity (perhaps leading to spontaneous recovery of responding), which were no doubt influenced by the increasing state of deprivation imposed by extinction.

These results indicate that deprived 1-day-old rats are capable of learning a relationship between response and reinforcer. Would such pups also learn to choose a paddle that rewarded them with milk from one that did not, a task that requires a more complex form of learning? To evaluate the pup's ability to learn a discrimination, a new group of pups was placed in test containers with two paddles. A different odor was placed on a terry-cloth triangle below each

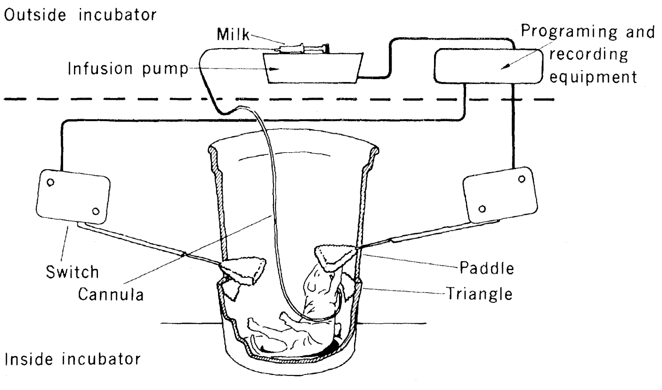

Fig. 1. Diagram of the apparatus used for learning experiments. The test container was a Styrofoam coffee cup (6-oz capacity, 4.5-cm inside diameter at the bottom). Terry-cloth-covered paddles extended into the container and were mounted 3 to 4 cm from the floor. An upward force on the paddles of 3 to 3.5 g (approximately 50 percent of a 1-day-old pup's body weight) was required to close a microswitch. In the first experiment, pups were provided with only one paddle. The two-paddle condition shown here was used in the discrimination experiment. A pump for infusing milk into the pup's mouth and recording equipment were located outside of the incubator.

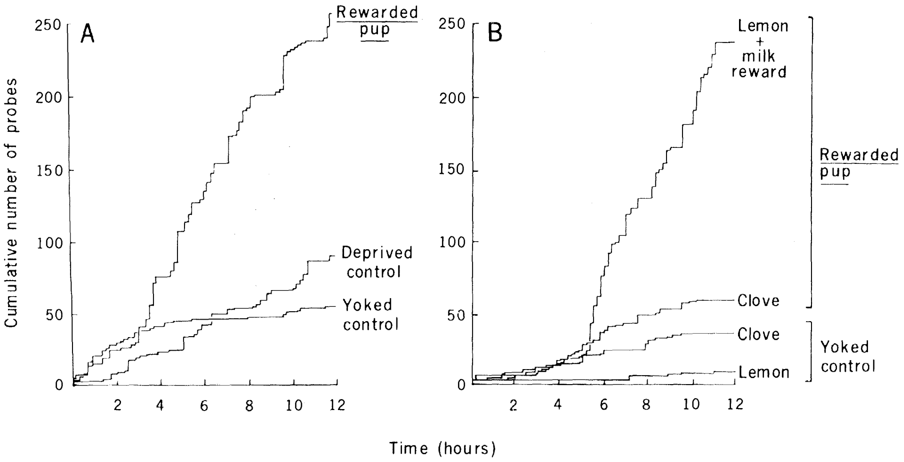

Fig. 2. (A) Cumulative number of probes (in 10-minute intervals) into the paddle made by a representative 1-day-old pup that was rewarded with a small oral milk infusion for each probe. The littermate "yoked control" and "deprived control" pups made considerably fewer probes over the 12 hours of testing. (B) Cumulative number of upward probes (in 10-minute intervals) made by a representative experimental pup in the second experiment. The pup appeared to have learned to discriminate the paddle that rewarded it from the one that did not. This pup's littermate yoked control did little probing and did not show a discrimination.

paddle. In six cases we used lemon and clove and in two others, perfume and cedar. For the experimental pup, probes into the paddle labeled with one odor resulted in a milk infusion, and probes into the paddle labeled with the other odor produced no milk. The odors were counterbalanced across groups of subjects to control for the possibility that odor preferences might affect the pups' responding. A same-sex littermate in an identical container served as a yoked control in the discrimination learning experiments. The yoked control received milk each time the experimental pup received milk; its paddles were labeled with the same odors but were ineffective in producing milk. Pups were given 16 to 24 hours to acquire the discrimination.

In six of the eight cases (*9*), the experimental pup probed much more frequently into the paddle that produced the milk reward than into the ineffective paddle [mean 16 hour totals of 153 versus 68 responses, $t(7) = 2.38$, $P < .025$]. The yoked control, in contrast, showed a lower rate of responding on both paddles [$t(7) = 2.12$, $P < .05$] and also failed to discriminate between the two (mean 16 hour totals of 38 versus 57). Typical cumulative records for an experimental pup and its yoked control in the discrimination situation are shown in Fig. 2B. The initial responding by the experimental pup resembled that by the yoked control. After 5 hours, however, the experimental pup was responding at a much higher rate on the paddle that produced milk infusions, whereas the control showed no preference. Some animals appeared to learn the discrimination in an hour or two, while others took up to 12 hours to learn.

We reversed the paddles for five of the experimental pups that had shown clear discriminative responding. Probes into the previously ineffective paddle were now rewarded, and those into the previously rewarding paddle were not. In all five cases, the experimental pup first probed more frequently into the paddle that had previously resulted in reward [mean 1 hour totals of 25 versus 7, $t(4) = 3.55$, $P < .025$], but eventually shifted its responding to the other paddle. After 10 hours of reversal, four of the five pups were probing into the new rewarding paddle at a higher rate than the previously rewarding paddle [mean hour 10 probes of 21 versus 6, $t(3) = 13.89$, $P < .001$]. The fifth pup increased responding on the new rewarding paddle but responded at a high rate on the other paddle as well. The pups varied in how quickly they reversed the discrimination: one pup reversed its paddle preference within 2 hours, whereas others required up to 8 to 10 hours to show a clear reversal. This persistence in responding to the previously rewarding paddle, despite occasional reinforced responses on the new rewarding paddle, indicates that pups were not simply remaining near the paddle that recently produced milk reward but as a result of their original training were differentiating between the paddles, probably on the basis of odor or position in the test container.

In both experiments, it is possible that the milk reward was simply activating the experimental pup in the presence of the rewarding paddle, thereby increasing the probability of responding on this paddle because it was nearby. Several lines of evidence argue that such "response facilitation" does not account for the learning shown by the experimental animals, although it may make a contribution to the learning process. (i) During extinction of the single-paddle response, the rate of responding by experimental pups continued to be higher than the rate of responding by controls, although no milk was given. (ii) During the reversal of the two-paddle discrimination, the experimental pups showed an initial high rate of responding (for up to 8 to 10 hours) on the paddle that had previously produced a milk reward, even though probing into this paddle was not rewarded and hence not facilitated by milk. (iii) The problem of response facilitation can perhaps be best addressed by examining the extent to which experimental pups initiated responding on a paddle; such a measure is relatively free of the direct effects of milk-elicited behavioral activation. When initiations of responding were examined (initiations being defined here as responses or bursts of responses separated in time by a minimum of 30 seconds), the experimental pups initiated more responding than the yoked controls in the single-paddle situation [$t(5) = 4.3$, $P < .005$]. In the discrimination paradigm the experimental pups initiated more probing on the rewarding paddle than on the nonrewarding paddle [$t(7) = 2.57$, $P < .025$]. Response facilitation may be an important aspect of the first phase of the learning process, but it cannot explain the higher rates of initiation shown as responding develops in the experimental pups.

These data provide strong evidence for appetitive learning in 1-day-old rats. Not only are newborns sensitive to the consequences of their behavior, as indicated in the single-paddle situation, but they can also learn a two-choice discrimination. Thus, milk not only activates or arouses the deprived neonate, but is also responded to in an appetitive or motivated manner, at least as indicated by the willingness of the pups to probe into the paddle for it. We have chosen a response, reinforcer, and stimulus that are probably highly associable by infant rats. This can create interpretational problems because, for example, the response is actually elicited at times by the reinforcer. Questions about the relationship between classical and instrumental conditioning may thus arise. Nevertheless, the way in which these relationships between responses, reinforcers, and stimuli change during development may reveal much about the nature of behavioral plasticity. This paradigm provides an excellent means of examining the development of appetitive and regulatory behaviors in newborn rats, as well as a means of evaluating, from birth, the ontogeny of learning, memory, and discriminative abilities.

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7. The cannula consisted of a piece of fine polyethylene tubing (PE-10, Clay-Adams), with a small (1.6-mm diameter) heat-formed flange at one end. A flat "washer" (0.20-mm diameter), made by heating PE-50 tubing, was fitted over the cannula near the flange to prevent the cannula from pulling through the tongue during long periods of testing. Cannulas were installed 1/2 hour before training started. A curved piece of fine stainless steel wire was passed up through the fleshy portion of the lower jaw and the nonflanged end of the cannula was friction-fitted over the wire coming out of the pup's mouth. The cannula was then pulled flat against the tongue. Installation required only 10 to 15 seconds and was relatively nontraumatic. See W. G. Hall and J. S. Rosenblatt [*J. Comp. Physiol. Psychol.* **91**, 1232 (1977)] for details.
8. Since the deprived control pups were severely deprived by the time the extinction period began, we did not include them in the extinction study.
9. In the two cases in which discrimination failed, the two experimental pups had each responded fewer than ten times on the reward paddle and had apparently not learned to probe for milk reward. Nonetheless, the reported statistical tests were performed on data from all eight pairs of animals.
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