seen with β-endorphin, were relevant to the euphoric component of drug self-administration, then the indirect excitatory effects on hippocampus merit further examination.

W. ZIEGLGÄNSBERGER*
E. D. FRENCH, G. R. SIGGINS
F. E. BLOOM

*Arthur V. Davis Center for Behavioral Neurobiology, Salk Institute, La Jolla, California 92037*

### References and Notes

1. S. H. Snyder and S. R. Childers, *Annu. Rev. Neurosci.* **2**, 35 (1979); E. J. Simon and J. M. Hiller, *Annu. Rev. Pharmacol. Toxicol.* **18**, 371 (1978); L. Terenius, *ibid.*, p. 189.
2. M. Sar, W. E. Stumpf, R. J. Miller, K.-J. Chang, P. Cuatrescasas, *J. Comp. Neurol.* **182**, 1 (1978); F. E. Bloom, J. Rossier, E. Battenberg, unpublished data; H. Karten, personal communication.
3. R. C. A. Frederickson, *Life Sci.* **21**, 23 (1977); W. Zieglgänsberger and J. P. Fry, in *Developments in Opiate Research*, A. Herz, Ed. (Dekker, New York, 1978), p. 193.
4. R. A. Nicoll, G. R. Siggins, N. Ling, F. E. Bloom, R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1591 (1977).
5. R. G. Hill, J. F. Mitchell, C. M. Pepper, *J. Physiol. (London)* **272**, 50 (1976).
6. M. Segal, *Neuropharmacology* **16**, 587 (1977); J. R. Fry, W. Zieglgänsberger, A. Herz, *Brain Res.* **163**, 295 (1979).
7. S. J. Henriksen, F. E. Bloom, F. McCoy, N. Ling, R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5221 (1978).
8. E. D. French, G. R. Siggins, S. J. Henriksen, N. Ling, *Neurosci. Abstr.* **3**, 291 (1977).
9. D. Taylor, B. Hoffer, W. Zieglgänsberger, G. Siggins, N. Ling, A. Seiger, L. Olson, *Brain Res.*, in press.
10. These data were reported in preliminary form: G. R. Siggins, W. Zieglgänsberger, E. French, N. Ling, F. Bloom, *Neurosci. Abstr.* **4**, 414 (1978); W. Zieglgänsberger, G. Siggins, E. French, F. Bloom, *Dev. Neurosci.* **4**, 75 (1978).
11. Body temperature was kept between 37.5° and 38.5°C. Stereotaxic coordinates for the dorsal HPC recordings were 1.5 to 3.0 lateral to midline, 3.0 to 3.7 mm posterior to bregma, and 2.0 to 3.5 mm in depth. Electrodes for microiontophoretic applications (four- and five-barreled pipettes) were constructed either as complete five-barrel assemblies or from fused omega-dot capillaries (American Glass Co.). The latter were glued alongside a tungsten or a 3*M* NaCl-filled glass recording electrode (*12*). The vertical separation between recording and iontophoresis electrodes was 30 to 180 μm (Figs. 1a and 2c). The drug barrels were filled immediately before the electrode was used with the following solutions: Met$^5$-enkephalin or D-Ala$^2$-enkephalin, 0.03*M*, *p*H 5 in 0.9 percent NaCl solution; β-endorphin, 0.03*M*, *p*H 5 in 0.9 percent NaCl; naloxone hydrochloride, 0.1*M*, *p*H 5; l-monosodium glutamate, 0.5*M*, *p*H 8; acetylcholine chloride, 0.5*M*, *p*H 5; bicuculline, 0.05*M*, with or without 0.9 percent NaCl, *p*H 5; and MgCl, 1.0*M*, *p*H 6. The compounds were applied by cationic currents except for L-glutamate which required anionic current. With the micropressure method (*13*), *p*H of the endorphin solutions was 6.5 to 7.0, and the same barrel as for iontophoresis was used. This system allows a better estimation of the concentration reached at the tip orifice than does iontophoresis, and is advantageous with poorly ionized or relatively dilute solutions. Cells were considered responsive when (i) their firing rates were changed by more than 30 percent, (ii) the effect could be reproduced at least three times, and (iii) the response was not mimicked by polarizing current passed through a barrel containing 2*M* NaCl. Current neutralization (*14*) was used continuously by means of the NaCl barrel. Responses were considered antagonized by a substance if they were reduced by more than 50 percent. Poststimulus time histograms were constructed on a PDP-11/40 computer (*15*).
12. M. Satoh, W. Zieglgänsberger, A. Herz, *Brain Res.* **115**, 99 (1976); W. Zieglgänsberger and J. Bayerl, *ibid.*, p. 111.
13. M. Sakai, H. Sakai, C. D. Woody, *Exp. Neurol.* **58**, 138 (1978).
14. G. C. Salmoiraghi and F. F. Weight, *Anesthesiology* **28**, 54 (1967).
15. Software (FISH; DECUS 11-3200) developed by K. Liebold.
16. Pyramidal cells (sometimes identified by transcallosal stimulation) were characterized by their burstlike firing pattern (*17*). The administration of the opiate agonist at 10 to 150 nA or 1 to 3 atm for 60 to 120 seconds generally led to an excitation after 2 to 10 seconds; responses were dose-related, and outlasted the application by 20 to 50 seconds. Naloxone, administered either by iontophoresis (20 to 100 nA for 60 to 120 seconds before or simultaneously with the opioid peptides) or subcutaneously (1 mg per kilogram of body weight) antagonized the excitatory action of the peptides in 30 of 40 HPC's. About half the HPC's tested by iontophoresis were excited slightly by naloxone.
17. J. B. Ranck, in *The Hippocampus*, R. L. Isaacson and K. H. Pribram, Eds. (Plenum, New York, 1975), vol. 2, p. 207.
18. E. C. Ribak, J. E. Vaughn, K. Saito, *Brain Res.* **140**, 315 (1978).
19. P. Andersen, in *The Hippocampus*, R. L. Isaacson and K. H. Pribram, Eds. (Plenum, New York, 1975), vol. 2, p. 155.
20. D. R. Curtis, A. W. Duggan, D. Felix, G. A. R. Johnston, *Nature (London)* **229**, 1222 (1970).
21. R. A. Nicoll, *Brain Res.* **35**, 137 (1971).
22. A bipolar concentric electrode, tip (200-μm outer diameter) 3.0 mm below the cortical surface, was used, with the same stereotaxic coordinates as the recording electrode, except contralateral. Stimulus intensities (1 to 4 V) used were sufficient to elicit a primary excitatory response followed by an inhibitory silent period of 30 to 120 msec (Fig. 2b).
23. J. V. Nadler, K. W. Vaca, W. F. White, G. S. Lynch, C. W. Cotman, *Nature (London)* **260**, 538 (1976); M. Segal, *Brain Res.* **119**, 476 (1977).
24. R. Simantov and S. H. Snyder, in *Opiates and Endogenous Opioids*, H. Kosterlitz, Ed. (Elsevier/North-Holland, Amsterdam, 1976), pp. 41–48.
25. J. S. Hong, H. Y. Yang, W. Fratta, E. Costa, *Brain Res.* **134**, 383 (1977); J. Rossier, T. M. Vargo, S. Minick, N. Ling, F. E. Bloom, R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5162 (1977); D. Wesche, V. Hollt, A. Herz, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **301**, 79 (1977).
26. H. Takagi, M. Satoh, A. Akaide, in *Characteristics and Function of Opioids*, J. van Ree and L. Terenius, Eds. (Elsevier/North-Holland, Amsterdam, in press).
27. E. F. Domino and A. Bartolini, *Neuropharmacology* **11**, 703 (1972); C. H. Hockman, R. G. Perrin, H. Kalant, *Science* **172**, 968 (1971); W. R. Klemm, *Nature (London)* **251**, 234 (1974); M. B. Wallach, E. Friedman, S. Gershon, *J. Pharmacol. Exp. Ther.* **182**, 145 (1972); M. B. Wallach and S. Gershon, *Neuropharmacology* **10**, 743 (1971).
28. We thank N. Ling for the gift of all the opioid peptides used in this study, Endo Laboratories for the gift of naloxone, B. J. Hoffer, S. J. Henriksen, and Q. Pittman for critical evaluation of this study, D. Games for technical assistance, N. Callahan for secretarial skills, and J. Simon for assistance with the artwork.

* Permanent address: Max-Planck-Institut für Psychiatrie, 8 Munchen 40, Kraepelinstrasse 2, Postfach 401240, Federal Republic of Germany.

8 December 1978; revised 27 April 1979

## Sensitization in *Aplysia*: Restoration of Transmission in Synapses Inactivated by Long-Term Habituation

Abstract. *Long-term habituation of a simple withdrawal reflex in* Aplysia *leads to an inactivation of synaptic transmission between identified sensory and gill motor neurons that persists for more than 3 weeks. A single sensitizing stimulus rapidly reactivates both the depressed behavioral response and the inactivated synaptic transmission. Thus sensitization, a simple competitive form of learning, provides a mechanism whereby changing environmental demands can rapidly override the long-term memory of habituation.*

Recent investigations of the neural mechanisms of memory in the marine mollusc *Aplysia californica* have shown that long-term habituation training of the defensive withdrawal reflex produces a profound depression of synaptic transmission leading to functional inactivation of the synapses between identified sensory neurons and gill motor neurons. This depression persists for more than 3 weeks (*1*). A variety of studies in both vertebrates in invertebrates indicate that sensitization, a simple competitive form of learning, can rapidly counteract the short-term memory for habituation (*2, 3*). We were therefore interested to know whether sensitization can override the long-term memory of habituation and if so, whether it leads to a functional reactivation of the synapses between the sensory and motor neurons.

Using a combined behavioral and cellular approach, we find that a single sensitizing stimulus reactivates both reflex function and synaptic transmission between sensory neurons and gill motor neurons. Thus, even long-term memory, which normally requires more than 3 weeks to recover, can be rapidly counteracted by a competitive short-term learning process.

We used 43 *Aplysia californica*, weighing 100 to 300 g (Pacific Bio Marine Co., Venice, California). All animals were housed individually in a 200-gallon aquarium for at least 5 days before an experiment.

In behavioral experiments, we first examined the effects of a single sensitizing stimulus on the reflex response of animals that had received long-term habituation training. Twenty-three animals were given ten trials of habituation training per day for 5 days (*4*). The animals exhibited a significantly habituated reflex response ($P < .01$) on day 5 compared to that on day 1 (*5*). This training procedure produces long-term habituation that persists unchanged for 1 week and is only partially recovered after 3 weeks (*6*). On day 5, the habituation scores of that day were ranked and the


0036-8075/79/0727-0417$00.50/0 

animals were assigned alternately to experimental (sensitized) ($N = 12$) or control (habituated) ($N = 11$) groups. Two hours after the training session, each experimental animal was placed in a separate small aquarium and was given a single strong sensitizing electrical stimulus to the neck region (20 mA, 60 Hz for 2 seconds, delivered through Ag-AgCl bipolar electrodes). The stimulus invariably triggered inking (*7*) and a pronounced, overall contraction ("balling up") of the animals. The animals were then returned to their home cages. Two hours later, both experimental and control animals were given a test habituation training session (ten stimuli) using a blind procedure (*1*, *6*). Experimental animals showed a significantly elevated reflex response during the test session compared to controls ($P < .001$) (Fig. 1A). Moreover, the control animals were still significantly habituated as indicated by comparison with their day 1 scores ($P < .01$), but scores of the experimental animals were no longer significantly different from those on day 1. Thus, the single noxious stimulus delivered to the long-term habituated animals produced a significant sensitization [dishabituation (*3*)] of the defensive withdrawal reflex; this indicates that the long-term depression of reflex responsiveness could be rapidly overridden.

We next examined whether the profound long-term synaptic depression that accompanies long-term habituation (*1*) could also be counteracted by the sensitizing stimulus. Twenty animals were given habituation training for at least 5 days before the cellular experiment (*8*). To assure a uniform baseline performance for both groups, only animals that showed at least 60 percent reduction of reflex responsiveness after 5 days of training were used. Approximately 70 percent of animals met this requirement. Each day, two animals (an experimental and a control) were matched on the basis of their habituation scores and studied. The experimental animal was given a single electrical stimulus to the neck region as in the first experiment, 2 hours after the training session. The pair of animals was then immediately coded and prepared for the cellular experiment (*9*) with their prior treatment unknown to the experimenters. The amplitude of the monosynaptic excitory postsynaptic potentials (EPSP's) between the sensory neurons and the gill motor neuron $L_7$ in both control and experimental animals was then measured (*9*). As in previous studies (*1*), we found that in control (habituated) animals ($N = 10$), the incidence of detectable synaptic connections was very low, $9 \pm 4$ percent (mean $\pm$ standard error of mean). However, in the experimental animals ($N = 10$), the incidence of such connections was high, $68 \pm 11$ percent. The difference between groups was significant ($t = 4.92$, $P < .005$) (Fig. 1B). The major difference between experimentals and controls was in the incidence of detectable connections, as evidenced by synaptic potentials of greater than 50 $\mu$V, the limit of our resolution in these experiments. The

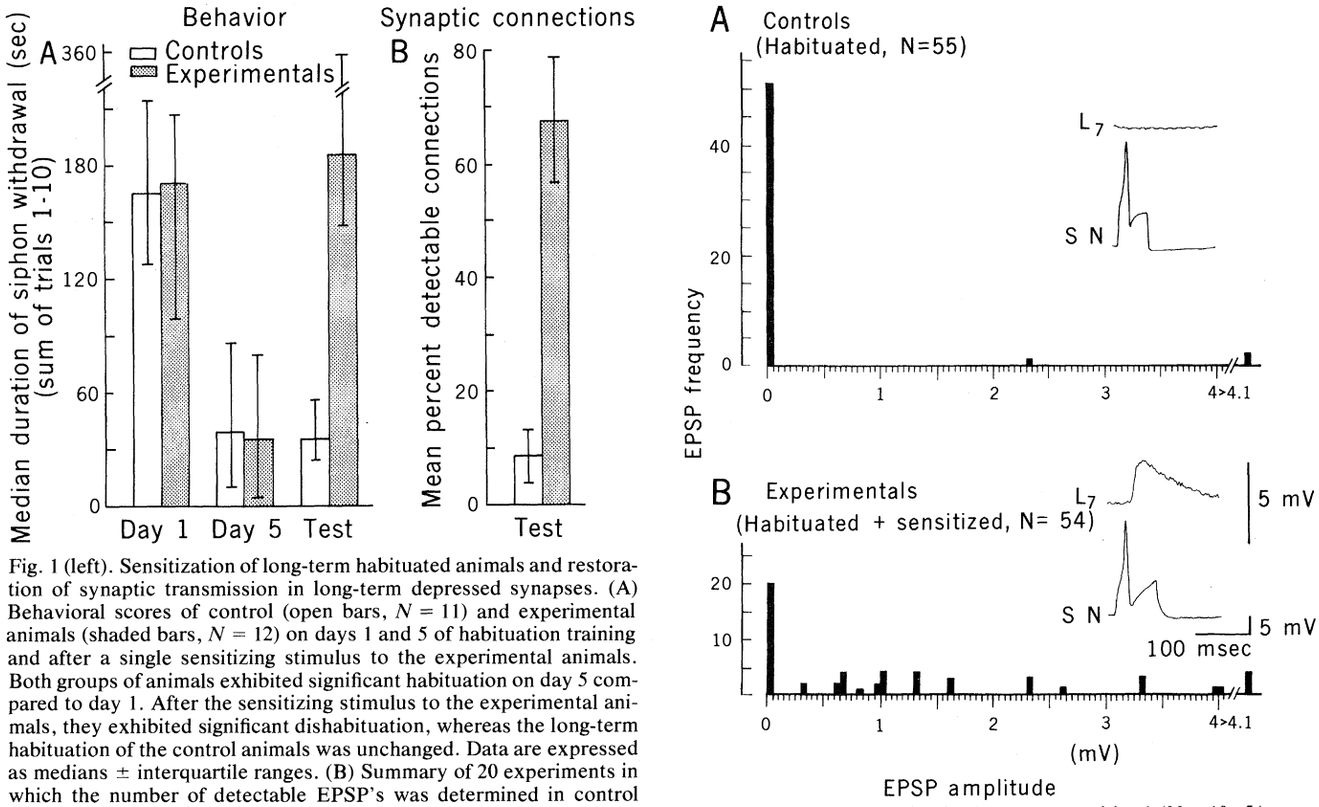


Fig. 1 (left). Sensitization of long-term habituated animals and restoration of synaptic transmission in long-term depressed synapses. (A) Behavioral scores of control (open bars, $N = 11$) and experimental animals (shaded bars, $N = 12$) on days 1 and 5 of habituation training and after a single sensitizing stimulus to the experimental animals. Both groups of animals exhibited significant habituation on day 5 compared to day 1. After the sensitizing stimulus to the experimental animals, they exhibited significant dishabituation, whereas the long-term habituation of the control animals was unchanged. Data are expressed as medians $\pm$ interquartile ranges. (B) Summary of 20 experiments in which the number of detectable EPSP's was determined in control (long-term habituated) animals ($N = 10$, 55 connections sampled) and in long-term habituated animals that were sensitized ($N = 10$, 54 connections sampled). Data were expressed as the ratio of the number of detectable EPSP's to the total number of connections sampled. Each animal contributed a single ratio. Sampling was obtained 1 to 2 hours after the experimental animals were given shocks. A blind procedure was used throughout the cellular experiments. Control and experimental animals were treated in exactly parallel fashion. Data are shown as the mean percentage ($\pm$ S.E.M.) of detectable connections. Fig. 2 (right). Histogram of EPSP amplitudes from control animals (long-term habituated, 55 cells from ten animals) and from experimental animals (long-term habituated and then sensitized, 54 cells from ten animals). All sampled connections of the experiment summarized in Fig. 1B are shown. Detectable EPSP's include all potentials above 50 $\mu$V. There is a clear trend toward detectable connections in animals receiving a sensitizing stimulus. The insets show a typical nondetectable EPSP from an habituated animal, and a typical detectable EPSP from a sensitized animal. The sensory neuron (*SN*) was depolarized intracellularly to trigger a single action potential and evoke a monosynaptic EPSP in the gill motor neuron $L_7$.

amplitude distribution of all sampled EPSP's (with nondetectable EPSP's included as 0 to 50 $\mu$V) (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.

THOMAS CAREW
VINCENT F. CASTELLUCCI
ERIC R. KANDEL
*Division of Neurobiology and Behavior, Departments of Physiology* and *Psychiatry, College of Physicians and Surgeons, Columbia University, New York 10032.*

### References and Notes

1. V. F. Castellucci, T. J. Carew, E. R. Kandel, *Science* **202**, 1306 (1978). "Inactivation" is used in this context to describe the end point of a continuum of progressively more depressed synaptic potentials, which finally cannot be resolved with our recordings.
2. W. A. Spencer, R. F. Thompson, D. R. Neilson, *J. Neurophysiol.* **29**, 221 (1966); R. F. Thompson and W. A. Spencer, *Psychol. Rev.* **173**, 16 (1966).
3. T. J. Carew, V. F. Castellucci, E. R. Kandel, *Int. J. Neurosci.* **2**, 79 (1971); P. B. Farel, *Brain Res.* **72**, 323 (1974).
4. Habituation training consisted of ten trials of water jet stimuli (800-msec duration) delivered to the siphon, with an interstimulus interval of 30 seconds. The duration of siphon withdrawal was measured for each trial. A single score was computed for every animal, the sum of trials 1 to 10 for each session (*1*, *6*).
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.
6. T. J. Carew, H. M. Pinsker, E. R. Kandel, *Science* **175**, 451 (1972).
7. T. J. Carew and E. R. Kandel, *J. Neurophysiol.* **40**, 692 (1977).
8. The median number of days of habituation training before cellular experiments was seven (range, five to nine).
9. Animals were anesthetized by injection of isotonic $MgCl_2$ (50 percent of body weight) by an independent experimenter who was not conducting the cellular experiments, in order to eliminate any postural cues before or during dissection (such as "balling up" in sensitized animals) that might compromise the blind procedure. Dissection procedures and the experimental protocol are described in detail (*1*). Basically, the connections of as many as possible sensory neurons to the gill motor neuron $L_7$ were sampled. A minimum of three sensory neurons was required to accept an experiment. Typically, five or six sensory neurons were sampled per experiment.
10. M. Klein and E. R. Kandel, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3512 (1978).
11. ———, in preparation.
12. Supported by PHS grants MH-26212, NINCDS-12744, and GM-23540, a grant from the McKnight Foundation, PHS career development award MH-00081 to T.J.C., and PHS career scientist award MH-18558 to E.R.K. We thank R. Hawkins, J. Koester, I. Kupfermann, and K. Weiss for comments on the manuscript, R. Hawkins and T. Walters for help with the blind procedures, and K. Hilten for the illustrations.

4 August 1978; revised 6 November 1978

## 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


0036-8075/79/0727-0419$00.50/0 