Suckling as Incentive to Instrumental Learning in Preweanling Rats

Abstract. *Neonatal rats as young as 7 days of age learned, reversed, and retained a left-right discrimination with a nonlactating nipple as the incentive. These results have implications for the ontogeny of associative processes and for their neurological and neurochemical mediators.*

Understanding the ontogeny of associative processes is accepted as an important goal by students of behavior (*1*). Ideally, to trace their major developmental paths, these processes should be identified in neonatal, altricial mammals whose nervous systems are relatively primitive and whose behavioral experiences and motoric repertoire are limited. Infant rats fulfill these requisites: in pups 7 days after birth, for example, whole brain weight is only 30 percent of adult brain weight (*2*); few if any cortical synapses have developed (*3*); and myelinization of cortex, thalamus, and midbrain has not yet proceeded (*4*). Also, central neurotransmitter systems are primitive (*2*). Not surprisingly, cortical electroencephalograms bear little resemblance to adult patterns (*5*). The demonstration that perinatal rats could learn an instrumental act, therefore, would be of considerable interest. It would facilitate analyses concerning age- and experience-related changes in associative capacities, and could provide a behavioral assay through which the functional significance of neurological and neurochemical development might be understood. Accordingly, we have exploited the preeminent natural behavior of neonatal rats, suckling, to demonstrate their capacity to learn an instrumental act. Even at 7 days of age, rats learn a discrimination task that is instrumental to providing them with a nonlactating nipple to suckle (*6*).

Sixty-four Sprague-Dawley rat pups, born in our colony, were assigned to age groups 7, 12, 17, and 21 days. All subjects were tested in a Y maze with the opportunity to suckle the mother's nipples as the reward. Before training initiation, pups were deprived of access to the dam for 24 hours. During this period, the 7- and 12-day-old pups were housed in nest-lined Styrofoam cups in a 41°C water bath (*7*). The 17- and 21-day-old pups, capable of thermoregulation, were deprived in mouse breeder cages with wood shavings lining the floor.

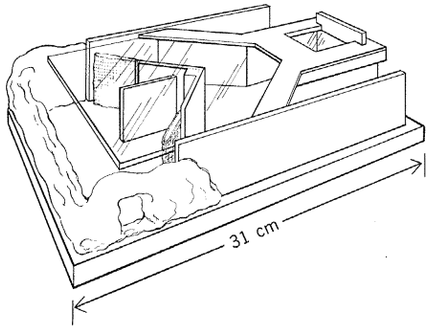

Fig. 1. Line drawing of the Y maze.

A rendering of the Y maze used to test spatial discrimination is shown (Fig. 1). Constructed of clay and plywood, the maze accommodated the anesthetized dam, who was positioned on her side so as to expose her ventrum and nipple rows to the pups as they approached from either arm of the maze. The clay into which the anesthetized mother could easily be placed was molded into a 17 by 9 by 7 cm bed. The maze itself was sealed to pup size, so that the start box (7 by 4 by 3 cm) for 7- and 12-day-old pups was situated 7 cm from the mother's ventral surface; the start box (9 by 6.5 by 6.6 cm) for 17- and 21-day pups was 14 cm from the mother's ventrum. A V-shaped partition divided the dam's surface into pectoral and inguinal goal compartments, each with two rows of three nipples. Nipple contact was thwarted in one compartment by wrapping that side of the mother with a double layer of thin gauze. This side was designated as nonrewarded (S−). The rewarded side (S+) was similarly treated except that the gauze had a 6 by 8 cm window cut in it to permit the pups to suckle. Thus olfactory cues, which are so critical in suckling elicitation (*8*), could not differentially guide choice behavior. In fact, other than suckling, there was little behavioral distinction between a pup on the S+ or S− side. In both cases vigorous rooting occurred. To insure that olfactory cues from a recently suckled nipple would not bias subsequent choices, a deprived control pup suckled an S− nipple while the test pup suckled an S+ nipple. Further, to eliminate the possibility that an odor trail was being followed, the maze floor was completely covered with a fresh piece of paper before each trial.

Each rat was allowed a 30-second exposure to both the S+ and S− compartments before the first acquisition trial. All acquisition and reversal trials were conducted on the same day until the criterion of eight correct responses out of ten was reached. Each trial was of 60-second maximum duration with an intertrial interval (ITI) of 30 seconds. After an incorrect choice the animal was confined to the S− compartment for 30 seconds. When a correct choice was made, suckling was allowed for 30 seconds. During the ITI the pups were placed in a plastic container on a warmer tray set at 36°C. When the criterion was attained on the acquisition trials, each rat was immediately tested on the reversal of the original problem by changing gauze position.

A second group of 48 Sprague-Dawley rats, 7, 12, 17, and 21 days old, were deprived for 24 hours and tested by the previously described procedure on both acquisition and reversal trials until the 80 percent criterion was reached. They were then returned to the mother for 24 hours, followed by a 24-hour period of removal from the mother. After this 2-day interval, the pups were tested for retention of the previously learned reversal. Specifically, the side of the maze designated as S+ for each subject was that which had been S+ on the previous set of reversal trials. Rats, now 9, 19, and 23 days old, were tested for retention in the same maze used for reversal training. However, the onset of vision (between 12 and 14 days) necessitated that rats 14 days old be tested in the larger maze, which prevented them from visually discriminating between S+ and S−.

Figure 2 demonstrates that preweanling rats acquired, reversed, and retained a left-right discrimination (*9*). A 2 × 2 analysis of variance revealed that neither age ($F = 1.78$; d.f. = 3, 60; $P > .10$), task (acquisition versus reversal: $F = 3.50$; d.f. = 1, 60; $P > .10$), nor the age-task interaction ($F = 0.74$;

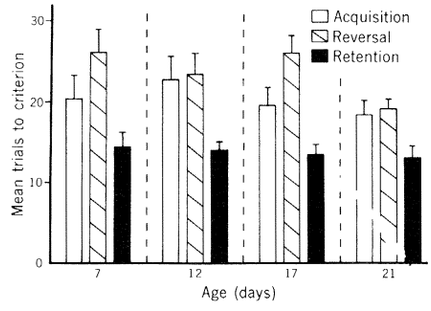

Fig. 2. Mean trials to criterion on acquisition, reversal, and retention tasks for rats 7, 12, 17, and 21 days old at the time of original learning. Acquisition and reversal data are from experiment 1, data on retention of the reversal are from experiment 2; standard errors are indicated.

d.f. = 3, 60; $P > .10$) was significant. We recognize that across-age comparisons are tenuous because of difficulties in equating motivational or activity consequences of a fixed period of deprivation. Nonetheless, even the youngest animals learned the tasks. Lack of age-related improvements does not equate the associative capacities of 7- and 21-day-old animals. Lack of improvement may be task-specific and differences may emerge with more complex tasks.

With regard to retention, all age groups (7, 12, 17, and 21 days) exhibited significantly fewer trials to criterion upon retesting after a 2-day interval. A repeated-measures analysis of variance showed that the task factor was highly significant ($F = 44.17$; d.f. = 2, 28; $P < .001$), but that neither age ($F = 1.20$; d.f. = 3, 88; $P > .10$) nor the age-task interaction ($F = 1.53$; d.f. = 6, 88; $P > .10$) was significant. Post hoc analyses indicated no significant difference in trials to criterion for acquisition versus reversal (Sheffé, all P's $> .10$) but that significantly fewer trials to criterion were required for retention as compared with either acquisition or reversal at each age (Sheffé, all P's $< .01$). Moreover, significantly fewer trials to criterion were required by rats 7, 12, 17, and 21 days old on retention as compared with acquisition or reversal tasks for all age groups (Sheffé, all P's $< .01$). Savings are therefore attributable to retention rather than maturational improvement in learning ability. As before, statements regarding age-related improvements are inappropriate. In this case the only retention interval used was 2 days. Others (*1*, *10*) have found that retention does improve during ontogeny.

These data are noteworthy in at least two respects. (i) The nonlactating nipple provides sufficient incentive for initiating and maintaining learned performance; milk letdown is not a necessary reinforcer. Accordingly, we may now determine when and how the appetitive stimuli that support learning will change during the course of ontogeny. (ii) From the point of view of learning and retention, these data make clear that neonatal rats can form and retain a simple left-right discrimination. Whether they are capable of forming other associations remains open, as does the development of complex discriminations during ontogeny.

These considerations are relevant for identifying the functional relationships between developing neurological and neurochemical systems and emerging associative behaviors. Despite recent advances in developmental neurology (*3–5*) and neurochemistry (*2*), the relationships between these systems and the learning capacities of very young animals have not been delineated—in part, because of difficulties in identifying neonatal learning abilities. The present report has provided a potentially powerful behavioral assay.

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References and Notes

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Prolactin-Like Immunoreactivity: Localization in Nerve Terminals of Rat Hypothalamus

Abstract. *Antibodies to rat prolactin were used in immunohistochemical studies of the hypothalamus and preoptic area of the rat. Evidence was obtained that a protein immunochemically related to prolactin was stored in networks of nerve terminals of many hypothalamic areas such as the arcuate nucleus, the dorsomedial hypothalamic nucleus, and periventricular regions of the hypothalamus and preoptic area. The neuronal storage of a prolactin-like protein in the hypothalamus was unaffected by hypophysectomy.*

Immunohistochemical studies have demonstrated the existence of luliberin-, thyrotropin-, and somatostatin-containing nerve terminals in various parts of the brain, particularly in the median eminence of the hypothalamus (*1*). The morphological studies support the view that these or closely related peptides may not only control the hormone secretion from the anterior pituitary but may act as transmitters or modulators in the central nervous system (*2*). It was therefore of interest to evaluate whether large proteins such as adenohypophyseal hormones could also be stored in nerve terminals in the central nervous system. Prolactin was chosen, since it has been identified in the cerebrospinal fluid of rabbits and rats (*3*). In the present investigation the hypothalamus was analyzed by the indirect immunofluorescence technique.

Male albino Sprague-Dawley rats (150 to 200 g) were used. In one experiment five rats were hypophysectomized 1 month earlier to remove all peripheral stores of prolactin. The rats were given Nembutal (60 mg per kilogram of body weight) intraperitoneally and were immediately perfused with 300 to 500 ml of ice-cold formalin through the aortic artery. The brains were removed and, after thorough rinsing, sections (10 μm thick) of the hypothalamus and preoptic area were cut on a cryostat. The immunohistochemical procedure was that described by Coons (*4*), including incubation with antibodies against rat prolactin [rat prolactin 5-A, diluted 1:16, supplied by the National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD)], rinsing, incubation with fluorescein isothiocyanate–conjugated sheep antibodies against rabbit antiserum (diluted 1:4), rinsing, mounting in a mixture of buffer and glycerin, and examination in a Zeiss Junior fluorescence microscope. Prolactin antiserum that had been treated with rat prolactin served as control serum. Ovine prolactin does not cross-react with rat prolactin, and in some experiments prolactin antiserum was first treated with ovine prolactin or synthetic corticotropin (1-24; 1-10; 4-10) as a specificity test. According to the NIAMDD, the antibodies against rat prolactin show no cross-reactivity with follitropin, lutropin, thyrotropin, or somatotropin. As a further test for specificity, sections were also incubated with antibodies against follitropin, lutropin, thyrotropin, and