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Odor-Aversion Learning in Neonatal Rats

Abstract. *Two-day-old rats were exposed to a novel odor and injected with an illness-inducing drug, lithium chloride. When tested at 8 days of age, these pups avoided pine shavings scented with the odor, whereas control pups did not. These results imply that rat pups are capable of associative learning at a much earlier age than was thought possible.*

Unlike some rodents such as the guinea pig, the rat's central nervous system (CNS) at birth is markedly underdeveloped and its sensory systems and behaviors are correspondingly primitive (*1*). The rat, however, matures rapidly. In about 4 weeks, its CNS approximates that of the adult, and its behavioral repertoire is rich (*1*). Consequently, by studying this mammal at various stages of development, one might discover important changes in its learning capabilities and identify neurological and neurochemical changes of functional significance to the learning process. An important step is to develop behavioral procedures that not only reveal learning in neonatal pups but also allow a systematic investigation of the variables likely to influence the learning process at various stages in the transition to adulthood.

To study the learning capabilities of the neonatal rat, however, the researcher must overcome a number of problems that are a direct consequence of the immaturity of its CNS. On the one hand, the neonate's sensory limitations restrict the nature of the stimulus experience about which it can be expected to learn, and, on the other, its limited behavioral repertoire makes it difficult to obtain a performance measure to identify the operation of associative-learning processes.

Perhaps because of such problems, there is little evidence of associative learning in pups less than 6 days of age, and we know almost nothing about the principal variables influencing the learning processes of the neonate nor about how these processes change as the organism matures. The purpose of this report is to describe several studies that have overcome some of these difficulties and have revealed evidence of associative learning in neonatal rats only 2 days old.

Several investigations of the neonatal rat's associative learning capabilities have employed Pavlovian conditioning procedures (*2, 3*). In these studies pups experienced vibrotactile stimulation (the conditioned stimulus, CS) paired with electrical shock (the unconditioned stimulus, UCS) to their forelegs. Reliable evidence of conditioned leg flexion has been reported for pups trained at least 6 days of age (*3*); results were mixed when the pups were less than 4 days old (*2, 3*). The possibility of associative learning at this early age thus remains to be determined.

We followed Pavlovian procedures that take advantage of recent developments in the study of the rat's responsivity to olfactory stimulation. (i) Shortly after birth, the rat pup is capable of discriminating among various odors (*4*). (ii) Adult rats acquire specific aversions to odors paired with an illness-inducing UCS (*5*). With these facts in mind, we attempted to induce aversions to olfactory stimulation in neonatal rats by pairing an olfactory CS with an illness-inducing UCS.

On the day of the odor-illness pairing, male and female rat pups were taken from the maternity cage and placed in a polyurethane bag (55 by 36 by 24 cm) containing fresh pine shavings scented with the odor CS. Approximately 5 minutes after being placed in this environment, pups were removed, injected intraperitoneally with the illness-inducing UCS (2 percent of body weight of a 0.15 *M* solution of lithium chloride) and then returned to the odor environment for an additional 30 minutes. When they were 8 days old, the pups were tested for aversion to the odor that had been paired with the illness. On each test they were placed in the center of a 30 by 20 by 10 cm compartment with a wire mesh floor. Beneath the floor were two 15 by 9 by 3 cm containers. One container was always filled with CS-scented pine shavings. The other container was filled with pine shavings either naturally scented or

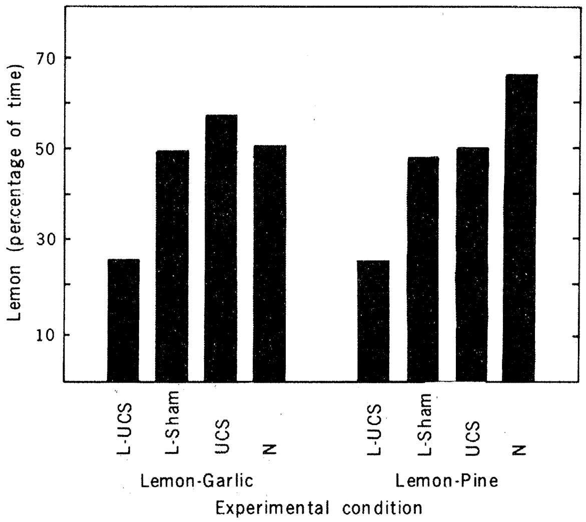

Fig. 1. Mean percentage of time spent over lemon-scented shavings. *L*, lemon; *UCS*, unconditional stimulus; and *N*, naive.

scented with a novel odor, depending on the test. Each test lasted 150 seconds. The dependent variable was the percentage of time the pup spent over the shavings scented with the odor previously paired with illness (that is, when its head and both forelegs were across a line dividing the two containers) (*6*). The experimenter did not know the treatment condition experienced by the pup being tested.

In the first study, 2-day-old pups in group L-UCS ($N = 13$) were given one pairing, as described above, of lemon (L) extract (McCormick) (2.5 ml in a volume of pine shavings sufficient to fill a 500-ml beaker) and the UCS. Three control conditions were included: (i) Animals in group L-sham ($N = 6$) were exposed to the lemon odor, but the injection was sham; (ii) group UCS ($N = 5$) experienced the illness while in the polyurethane bag, but olfactory stimulation was provided by shavings from the maternity cage; and (iii) the pups in group N ($N = 5$) were naive (N) at the time of testing and were simply placed for an equivalent time in a bag containing shavings from the maternity cage.

Each pup was tested twice. In one test, the lemon scent was pitted against garlic juice (McCormick) (2.5 ml in 500 ml of pine shavings); the other test pitted lemon-scented shavings against natural pine.

During both tests, the animals in group L-UCS spent significantly less time over the lemon scent than did those in the control groups (Fig. 1). An analysis of variance was performed on both the lemon versus garlic and the lemon versus natural-pine data [F (3, 25) = 4.30, $P < .025$; and F (3, 25) = 5.95, $P < .01$, respectively]. Subsequent individual comparisons using Tukey's (a) test revealed group L-UCS to differ from each of the control comparisons ($P < .05$).

These data imply that the experience of lemon paired with the UCS was necessary to produce the lemon aversion subsequently displayed by group L-UCS. The experience of neither the lemon scent alone nor the UCS alone was sufficient to produce this effect. Moreover, naive animals displayed either no preference (lemon versus garlic) or appeared to prefer lemon (lemon versus natural pine). This pattern of data thus suggests that the lemon aversion displayed by the pups in group L-UCS was a consequence of their having associated the lemon scent with illness. This conclusion could be further strengthened by a demonstration that this aversion to the lemon scent depended on the temporal proximity of the odor and illness.

In a second experiment, one group of 2-day-old pups ($N = 9$) were treated similarly to subjects in group L-UCS of the previous study. Subjects in two new conditions also experienced the lemon odor and illness but not in close temporal proximity. Pups in group L-60-UCS ($N = 10$) received their 35-minute exposure to the lemon scent 60 minutes before being injected with LiCl, and pups in group UCS-60-L ($N = 8$) were given the LiCl injection 60 minutes before being exposed to the lemon scent. When given a choice between lemon-scented and garlic-scented shavings at 8 days of age, the pups in group L-UCS displayed a pronounced aversion to the lemon scent ($\bar{X} = 16$ percent of the test time). In contrast, subjects in neither group L-60-UCS nor group UCS-60-L displayed this aversion (group L-60-UCS, $\bar{X} = 58$ percent; group UCS-60-L, $\bar{X} = 64$ percent). An analysis of variance revealed significant differences among the groups [F (3, 24) = 10.30, $P < .001$]; individual comparisons with Tukey's (a) test indicated that group L-UCS differed from group L-60-USC ($P < .01$) and from group UCS-60-L ($P < .01$).

The results of these two studies compel the conclusion that the 2-day-old rat pup has associative learning capabilities. In both experiments, the animals exposed to both the lemon odor and the LiCl injection in close temporal proximity (group L-UCS) displayed pronounced aversions to that odor. This aversion, however, was not displayed by animals in any of the five control conditions. The aversion of group L-UCS was thus a consequence of their having associated the lemon scent with the illness induced by LiCl.

Only a single exposure to the lemon-LiCl episode was necessary to modify the subsequent behavioral reaction of the pups to the lemon scent. The effect of the conditioning treatment was not transient; although the pups were conditioned when 2 days old, they were not tested until 8 days of age. Pups trained (as described above) at 2, 4, 8, and 14 days of age acquire equivalent aversions to the lemon scent.

The rat's ability to learn, at least when exposed to the training procedures of these studies, does not critically depend on the marked neurological and neurochemical changes in the CNS that take place during the first 2 weeks after birth. On the other hand, our more recent research has indicated that, as we have systematically investigated the training parameters of the odor-aversion learning task, important age-related differences emerge (*7*).

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