Early experience and the development of perseverative responding

W. JEANNE STREET and ROBERT L. ISAACSON
University of Florida, Gainesville, Florida 32611

Male and female rats from the same litters were subjected to the mild-to-moderate stress of being slowly shaken for 7 consecutive days prior to weaning. At about 2 months of age, the animals were tested for locomotor activity in the open field and for their ability to acquire a black-white discrimination problem in a Lashley jumping stand after exposure to an unsolvable problem. There were no differences in locomotor activity related to either sex or stress. All animals exhibited perseverative responding to one position of the jumping stand, and the stressed male animals took longer to overcome these perseverative tendencies than did nonstressed males when trained on the solvable discrimination problem. There were no effects of the stress on performance of the female subjects.

There are a wide variety of factors that can contribute to an animal's tendencies to establish perseverative types of responding. These include genetic factors, prenatal and postnatal environmental conditions, and specific learning experiences of both planned and accidental nature. One powerful factor contributing to the performance of unlearned and acquired reactions in adulthood is the amount of stimulation received by animals in the neonatal period, and at least some portion of the stimulation-induced changes seem to be mediated by activation of the pituitary-adrenal endocrine system (e.g., Dennenberg & Zarrow, 1971). For the most part, studies of the effects of early handling and stimulation have measured locomotor activity, stimulus-seeking behavior, or simple conditioning in adulthood. In the present study, we wished to extend this general approach by using a different type of behavioral measure: the evaluation of stereotyped, perseverative responding in a learning situation.

The establishment of persistent position habits in the Lashley jumping stand has been used as an index of frustration and emotional behavior (e.g., Maier, 1961). The work of Maier and his associates has been based on studies in which animals are given training on an unsolvable problem prior to training on a solvable discrimination task. The position habits that develop as a consequence of the unsolvable problem are more enduring and resistant to change than those learned by normal training procedures (Maier, Glaser, & Klee, 1940). However, the effects of stressful experiences before behavioral testing can lead to the development of rigid stereotyped reactions in many other kinds of situations, such as the water-filled maze (e.g., Mitchell & King, 1960; Obias & Stone, 1953).

In the present experiment, we undertook to determine the effects of a moderate-stress experience in

the neonatal period on the development of perseverative, stereotyped reactions in the jumping stand in adulthood. However, we undertook the experiment without a clear hypothesis as to the direction of any possible behavioral effects that might be found. If the early stress experiences produced a greater resistance to later stresses (e.g., Levine & Mullins, 1966), the animals might be less prone to the development of perseverative behaviors. On the other hand, others have found that early handling can produce an "infantilization" of behavior (Altman, Das, & Anderson, 1968) and a deficiency in spontaneous alternation behavior in adults (Douglas, 1975). In general, these latter authors would anticipate that early stress experiences produce less competent animals. The effects of early handling and stimulation could, therefore, be predicted to produce increased or decreased stereotypy in response to the unsolvable problem in the Lashley jumping stand.

METHOD

Two separate experiments were undertaken, the second being virtually a replication of the first. There were, however, some differences between the experiments that will be mentioned below. To simplify discussion, we will report the data as if two squads of animals (Groups A and B) were treated to the same experimental procedures but indicating the differences between the procedures given to them at appropriate places.

Subjects

Group A consisted of 12 Long-Evans hooded rats, 6 females and 6 males, born in the laboratory colony from stock obtained from the Charles River Breeding Laboratory. These animals were all littermates. Group B was also made up of 12 rats from the same source, 7 males and 5 females, all from the same litter.

Apparatus

Wooden microscope slide boxes, 9 × 17.5 cm, and a chemical shaker (115 V, 60 cy AC, Eberbach Corporation, Ann Arbor, Michigan) were used to stress the infant animals.

An automated open-field apparatus, 1 × 1 m, was used to

measure activity level of the rats. This automated maze recorded locomotor activity through the animals' interruption of photocell beams arranged along the sides of the maze. The arrangement of the beams divided the testing area into 16 equal squares (Lanier, Petit, & Isaacson, 1974). The apparatus was illuminated by a 25 W light over the center of the open field.

The Lashley jumping apparatus (1930) was used for the visual discriminating problems. The apparatus consists of a large vertical board, 91.5 cm wide $\times$ 151.5 cm high, with two square windows, 14.0 cm $\times$ 14.0 cm, about 91.5 cm above the base of the board. The windows are spaced 5.08 cm apart in the center of the board. A 71.1-cm-wide $\times$ 30.5-cm-deep $\times$ 20.3-cm-high "landing platform," parallel with the floor, is attached to the back of the vertical board, level with the bottom of the two windows. A metal shield projects at a 45° angle upwards and away from the vertical board beginning at the top of the windows. A moveable stand from which the rats jump is at the same height as the bottoms of the windows. The top of the jumping stand measures 12.7 $\times$ 20.3 cm. A net is stretched across the bottom of the vertical board to catch rats that fall. Reinforcement for successful jumps was sweetened, condensed milk in a petri dish set at the back of the landing platform.

One solid black and one solid white card were cut to fit behind the windows to serve as the discriminative stimuli for the visual discrimination problems. Two purple cards were used in preliminary training. Latches on both sides of the windows allowed the cards to be locked or unlocked as required by the experimental design.

Procedure

Group A. On Postnatal Day 15, three female and three male rats were placed separately in slide boxes and set in the chemical shaker. It was then activated for 2 min on low speed (spans 4-cm excursions approximately twice a second). This was done for 7 consecutive days.

The animals were individually tested in the open-field apparatus on Postnatal Days 49, 50, and 51 for 5 min each day, and the number of squares entered by the animals was counted. On Postnatal Day 66, training was begun on the jumpstand. Preliminary training was based on the method used by Maier et al. (1940). The rats were each placed on the stand with it against the vertical board. In this position, the animals could step from the stand, through the open windows, and onto the landing platform. The stand was gradually moved away from the vertical board until the rat jumped from the stand through the windows from 23.8 cm away. Initially, no cards were in the windows, but gradually the rats were trained to jump through the windows with the two purple cards covering the windows, but unlocked. The rats were guided to jump the same number of times to each side by preventing them from jumping to the undesired side while guiding them by the use of a card or hand to force them to jump to the correct window (e.g., Maier & Ellen, 1952).

By Postnatal Day 98, all of the animals had jumped at least 20 times to each window from 23.8 cm away, and training on the unsolvable visual discrimination problem was begun. In this problem, the black and white cards were presented on each trial. The color of the card that was unlocked, as well as the side left unlocked, was chosen randomly. An incorrect jump resulted in the rat's striking a locked card and falling to the net. A correct jump resulted in access to the milk and avoidance of falling to the net. The animals were given 10 trials a day for 8 days. On Postnatal Day 115, the animals were changed to a solvable problem for 10 days, with 10 trials given per day. In this problem, the side with the white card was always left unlocked; the side of the white card was chosen randomly.

Group B. On Postnatal Day 8, four of the male rats and three of the female rats were placed separately in slide boxes and placed in the shaker for 2 min on low speed (the same as Group A). This was done for 7 consecutive days. On Postnatal Days 51, 52, and 53, they were placed in the open field for 5 min a

day, just as was done with Group A. Training on the jumpstand was begun on Postnatal Day 91. On Postnatal Day 109, the animals began the unsolvable discrimination problem and were trained on this for 8 days, with 10 trials given a day. The solvable problem was begun on Postnatal Day 128 for 27 days, with 10 trials per day.

RESULTS

There were no differences between the mean open-field activity of the stressed and unstressed or male and female rats in either Group A or Group B.

All but one of the rats in Groups A and B formed position habits by the 8th day of training with the unsolvable problem. Females tended to form these habits before males. In Group A, four of the six females had formed position habits by the 3rd day of training in the unsolvable problem, whereas only one male had formed a consistent position habit at that time. In Group B, four of the five females had formed position habits on the 3rd day of training, but only one male had formed a consistent habit at that time.

In testing Group A, only 8 days of testing were given on the solvable problem, and only 4 animals learned the problem before the 8th day. In Group B, 25 days of training were given on the solvable problem and 10 of 12 animals learned the problem in this period. Three of the 4 animals of Group A that learned the problem in the 8 days of training were unstressed males. The 1 female animal that had learned the problem had been stressed. In Group B, all rats except 1 stressed female and 1 stressed male solved the problem on or before the 26th day of training. Overall, 54% of the stressed rats learned the problem while 70% of the unstressed rats learned the problem.

The effect of early stress can be evaluated by dividing all of the male and female animals, separately, at the median score relative to learning the solvable problem. By so doing, it is then possible to make a 2 by 2 chi-square table for stressed vs non-stressed animals and to determine whether the animal fell above or below the median performance of the male and female groups. Five unstressed males learned the solvable problem in fewer than the median number of trials for all males and none exceeded it, while five of the six stressed males exceeded the median and only one was below the median. This difference is significant at the $p < .01$ level by the exact chi-square test (Finney, Latscha, Bennett, & Hsu, 1963). A similar analysis for female rats revealed no difference among those receiving or not receiving stress early in life.

The latencies of each jump were recorded for Group B. There was no overall difference between mean latencies for stressed and unstressed rats on either the unsolvable or solvable problems. Mean

jump latencies for the trials on the days during training in the solvable problem but before the rats learned to solve the problem were compared between the two groups. At this time, all jumps were made to the one position, i.e., the right or the left windows, by all animals. Latencies of jumps to the correct card were compared to latencies of jumps to the incorrect card. The results of a two-way analysis of variance with one repeated measure indicated no main effect difference in latencies between the stressed and unstressed rats, but a significant difference between latencies of jumps to the correct (mean = 9.61) and incorrect cards (mean = 13.26) ($F = 33.14$, $df = 1/9$, $p < .01$). The same analysis comparing mean latencies of jumps to the preferred side to mean latencies of jumps to the nonpreferred side showed no main effect difference in latencies between stressed and unstressed rats, but did show a difference ($F = 15.64$, $df = 1/7$, $p < .01$) between latencies to the preferred side (mean = 7.27) as compared to the nonpreferred side (mean = 8.61), even though all jumps were to the correct card.

DISCUSSION

The early stress of being shaken by a standard laboratory shaker as done in the present study failed to affect locomotor activity as measured at about 50 days after birth. This result stands in contradiction to many studies that have found early handling and stress to increase locomotor activity (e.g., Denenberg & Morton, 1962; Denenberg & Whimbey, 1963), but the literature is far from being consistent in this matter (e.g., Lindzey, Winston, & Manosevitz, 1963).

The most suggestive aspect of the present results was the observation that early stress and handling may influence the perseverative position response induced by an unsolvable problem in male but not female rats. This is related to two issues: (1) differences between male and female animals and (2) the behavioral benefits or liabilities that result from early stress.

Several studies looking at stress and enrichment effects have found sex differences. In many cases, the results have supported the view that males are more susceptible to the effects of early environmental or chemical changes than females, although there are many exceptions to this "general rule." In mice, females are less affected by enrichment conditions than males both in weight gain and in locomotor activity measured at roughly 40 days of age (Manosevitz & Joel, 1973). Female rats were less affected by strychnine administration over the first 25 days of life than were males, as measured by an impairment in maze learning (Stein, 1971). Caul, Freeman, and Buchanan (1974) found that being raised in isolation produced greater heart-rate

decreases in a conditioned suppression paradigm than found in females. The magnitude of the heart-rate decrease in females was the same in the isolated and enriched animals. However, Wachs (1974) found that females were more influenced by early handling than were males in a test situation that measured responses that allowed the animals to observe changing stimulus conditions. Male mice become aggressive and show decreased serotonin levels after isolation, while females do not (Giacalone, Tansella, Valzelli, & Garatkini, 1968). Female rats treated with thiouracil early in life acquired bar-touching and barpressing tasks more rapidly than normal female rats (Davenport & Hennies, 1976), but this was not found in males. A superenrichment condition reduced the difference between the females produced by thiouracil (Davenport, 1976). Isolated females were slower than other females and treated and untreated males on the first trials of a maze problem and in an avoidance task.

Thus, at least some environmental stressors produce differential effects on male and female rats, but the specific effects found seem to be dependent on the conditions of early environmental treatments and the tasks used to measure performance in later life. In this study, males stressed early in life required significantly more trials to learn the discrimination problem than did unstressed males. Early stress, as produced by shaking, appears to cause males to persevere during a solvable task, but not females.

Many of the procedures used to manipulate animals early in life tend to stimulate secretions from the adrenal glands (Levine, 1962), and there is evidence that corticosterone release induced by a stressful situation plays an important role in altering behavior in the adult. When rats or mice are given a single, large dose of corticosterone in the first few days after birth, they evidence a polydipsia in most normal circumstances that is exaggerated when the animals are performing a VI operant task for food rewards even though their rates of response on the schedule are not different from controls (Howard, Olton, & Taylor, 1974). They are somewhat overactive during the initial phases of extinction in operant tasks and also more reactive on the DRL-20 schedule (Howard, 1973). Mice treated with corticosterone in infancy also showed reduced open-field activity in later life (Howard & Granoff, 1968).

Levine and his co-workers (e.g., Levine & Mullins, 1966) have argued that the stimulation of adrenal responses during infancy is probably useful for the animal in that it allows the animals to respond with moderate steroid release to novel situations in adulthood, whereas unstimulated animals tend to produce a maximal response to both novel and threatening situations. A similar position has been advocated by Denenberg (1967). However, as mentioned earlier, not all investigators share the view that early stimula-

tion produces beneficial results for the animals. The results of this study suggest that early stimulation may produce a deleterious effect on the ability of male animals to overcome perseverative responding in a discrimination problem. Naturally, this result should be extended to other behavioral problems in which performance is adversely affected by stereotyped or perseverative modes of responding.

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