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Long-Term Effects of Preweaning Isolation From Littermates in Rats[1]

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Male Wistar rats who were isolated from littermates on 12th postnatal day were weaned on 21st day and housed individually till adulthood. Comparison groups consisted of rats isolated only after weaning, and rats housed with littermates before and after weaning. In adulthood, reaction to handling, "social preference," resting heart rate, heart rate response to an "airblast," heart rate response to a strange rat introduced to home cage, and gastric ulceration after immobilization were tested. The "prewean isolates" were significantly different from the comparison groups in the reaction to handling, social preference, and in the immediate heart rate change following the introduction of an "intruder." The "prewean isolates" were more reactive to handling, preferred to be near a single adult rat, and showed less cardioacceleratory responses to "intrusion" than the comparison groups. The results indicate that the altered early social relationships produced by reduction of litter size to a single pup during the "socialization" period before weaning can have distinct long-term effects on behavior, social preference, and physiologic response in adult rats.

INTRODUCTION

Social isolation in animals produces lasting behavioral and physiologic alterations. Although many studies have been published concerning the effect of social isolation in rats after weaning (Hatch *et al.*, 1963; Gerall *et al.*, 1967;

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Ader, 1965), few focused on the effects of preweaning isolation from littermates in rats. Preweaning rats are usually found in a pile of 6-13 pups in constant contact before about 16 days of age, and are involved in frequent play behavior with the littermates until weaning. Thus, the littermates seem to be an important part of the preweaning environment of the developing rat.

In our previous study (Leigh and Hofer, 1973), we reported that removal of the littermates during the preweaning period resulted in profound changes in both the single remaining pup and its mother. When the isolation occurred before 10 days of age, almost all the singletons died after progressive inanition despite increased mother-pup contact in a nursing position. Isolation from littermates at 12 days of age resulted in a marked and prolonged increase in the amount of time the mother and pup spent together and in the stimulation each initiated toward the other. The normal decline in maternal behavior around 15 days of age (Rosenblatt, 1969) did not occur in the singleton-mother dyads. Increased active heart rates were also observed in the singleton prior to weaning.

This report concerns long-term effects of such altered mother-pup interaction following littermate removal on the 12th day of age on the single remaining pup. As marked changes in social relationships and heart rates had occurred in the preweaning pup after littermate removal, we decided to focus on these parameters in adulthood also.

METHOD

Animals and Isolation Procedure

A total of 36 pregnant Wistar females were housed in 21-liter terraria and delivered litters in our laboratory under conditions previously described (Leigh and Hofer, 1973). On the 12th day after delivery, 13 litters were chosen at random and all the pups except one male were removed from each cage (*prewean isolates*). All the other litters had similar handling on the 12th day. On the 21st day of age, all the remaining pups were weaned as follows. All the prewean isolates were housed individually. From ten of the remaining litters, one male pup was chosen randomly from each and housed individually (*postwean isolates*). The pups of the 13 remaining litters were weaned and 4 males from each litter were housed togerher (*grouped rats*). All these animals were then transferred to an air-conditioned animal room where the temperature was kept constant between 24 and 27°C, and humidity at 50%. They were not disturbed (except for routine change of food and water containers, etc.) until adulthood (120-250 days of age). One prewean isolate died at 49 days of age. A postwean isolate and a grouped rat were both found dead at 89 days of age. At autopsy, there were no gross significant findings except in the

grouped rat, whose stomach was filled with blood. This left the following number of experimental animals in adulthood: prewean isolates = 12, postwean isolates = 9, and grouped rats=12 (1 rat selected at random out of each of the 12 grouped litters was used as representative of the "grouped rats").

Adult Experiments

The animals were brought into the laboratory in new cages (glass aquaria 45 X 24 X 20 cm) and were allowed to rest for an hour before the first test.

The three experimental groups were, by and large, equally represented across the entire age range as the experiments were usually performed on three animals (one from each group) at one time.

(1) Gloved hand test. This test is a modified version of the Reaction to Handling test (Ader, 1965). This test was not originally planned but was incorporated into the experiment about midway as we become impressed by obvious differences in reactivity of the rats to handling. Thus, only 5 prewean isolates, 4 postwean isolates, and 7 grouped rats were tested in the standardized manner described. An experimenter who was "blind" as to which group the rat belonged attempted to pick up the adult rat with a gloved hand. We used a 0-3 point scale for the following reactions to the gloved hand: (a) jump, and (b) vocalization. 0-2 point scale was used for the "lift" category (0–impossible, 1–possible to lift but not easy, 2–easily picked up) and for overall "fierceness" (0–not fierce, no attempt to bite, 2–most fierce, actual biting). In addition, the number of boli left in the cage after testing was recorded.

(2) Social preference test. This test was done immediately after the "Gloved Hand" test. The rat was placed in the center of a specially constructed Plexiglas Y-maze. At the end of each arm of the Y was a compartment partitioned with clear Plexiglas with 5 holes 2.5 cm in diameter. Each compartment contained 0, 1, and 3 normal adult males. Thus, the experimental animal could choose to be near 3, 1, or 0 rats. During the first 3 min of the Y-maze, the experimenter recorded the position of the experimental rat every 15 sec. In addition, whether the rat interacted (sniffing or touching through the partition) with other rats was recorded.

Electrode Implantation

At the end of the Social Preference Test, the rats were all implanted with stainless steel electrodes (Plastic Products Inc.) under light ether anesthesia. The tips of the electrodes were attached to the skull with dentocement and a metal screw. Nine prewean isolates, seven postwean isolates, and eight grouped rats were successfully implanted. The electrodes were plugged into a flexible, wirebound, rotating cord connected to a Beckman Offner Dynograph

for recording EKG, cardiotachometer, impedence pneumograph, and EMG. Then, all the animals were housed individually in identical sound-shielded cubicles and allowed to rest 3 days.

(3) Resting heart rates. After 3 days, recordings of the heart rate were made for 3 min during rest. The recordings were made while the animals were awake but inactive without apparent awareness of the recording. The cardiotachometer reading was sampled every 10 sec.

(4) Airblast test. Following the resting baseline measurements, a thin transparent plastic tubing, 5 mm diameter, was introduced into the cage, which was connected to a compressed air source. It was inconspicuous, being near the clear glass wall of the cage. Approximately 30 min after the introduction of the tube, the air was turned on for 5 sec while the animal was at rest. The air pressure was approximately 20 psi, which caused a hissing sound and some disruption of the shavings on the floor of the cage. The heart rate during the airblast (5 sec rate) was recorded.

(5) Intruder test. Approximately 24 hr after the airblast test, a "strange intruder" (normal nonexperimental grouped male rat) was introduced into the home cage of the experimental animals for 5 min. We recorded the immediate heart rate (5 sec following "intrusion") as well as heart rates during the first 2 min, last 2 min, and 2 min after the intruder had been removed.

(6) Gastric ulcers after immobilization. Twenty-four hours after the Intruder Test, the rats were anesthetized by ether, then immobilized by being taped to a metal bar (approximately 2 cm in diam). The animals were put in a specially constructed sound attenuated cage for 21 hr. At the end of the 21 hr, the animals were sacrificed by etherization and the stomachs examined for evidence of ulcer under 3X magnification.

Data Analysis

Analysis of variance was used to test overall group differences, followed by individual comparisons between the experimental group (prewean isolates) and the two comparison groups (postwean isolates and grouped rats). Chi-square test was used for frequency data.

RESULTS

(1) Gloved hand test. Table 1 and Fig. 1 summarize the overall results. The group differences were significant at better than 5% level in four out of five categories. Individual comparisons revealed the following: The prewean isolates jumped significantly more than the grouped rats ($F(1,10) = 20.27$, $P < 0.01$). They also vocalized more than the grouped rats ($F(1,10) = 14.16$, $P < 0.01$). They were also harder to lift than the grouped rats

TABLE 1

Gloved Hand Test[a]

Category	*F*	*df*	*P*
Jump	6.44	2/13	0.05
Vocalication	6.21	2/13	0.05
Lift	8.67	2/13	0.01
Fierce	7.35	2/13	0.01
Bolus	2.76	2/13	0.10

[a]Overall test for the three groups; prewean isolates ($n = 5$), postwean isolates ($n = 4$), and grouped rats ($n = 7$).

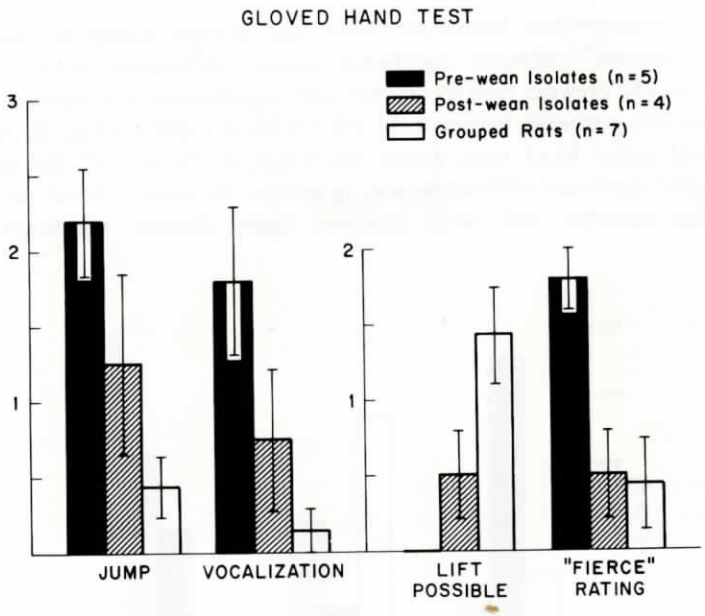

Fig. 1. Reaction to a "gloved hand" attempting to pick up the experimental animal. Mean points ± standard error of the mean.

($F(1,10) = 16.03$, $P < 0.01$). The prewean isolates tended to be more difficult to lift than the postwean isolates ($F(1,7) = 3.89$, $P < 0.10$), and were more "fierce" than either the postwean isolates ($F(1,7) = 14.60$, $P < 0.01$) or the grouped rats ($F(1,10) = 12.15$, $P < 0.01$). Prewean isolates left more boli as compared to the grouped rats ($F(1,10) = 3.87$, $P < 0.1$).

(2) Social Preference test. In general, most animals changed compartments at least once during observation. As Fig. 2 illustrates, the prewean isolates spent the least time next to the compartment containing three rats, while the grouped rats preferred it most. Prewean isolates preferred the compartment with only one rat most, grouped rats chose it second, and postwean isolates the least. The three groups were significantly differentiated by these measures ($\chi^2(6) = 65$, $P < 0.001$). 50% of the grouped rats were engaged in "interactions" as compared to 23% of postwean isolates and 8% of prewean isolates ($\chi^2(2) = 9.52$, $P < 0.05$).

(3) Resting heart rates. There was no significant difference between the prewean isolates and the comparison groups in resting heart rates.

(4) Effect of "airblast". All animals reacted with an increase in heart rate during the immobile period while the animals were "attending" to the airblast. There were no statistically significant differences among the groups tested.

(5) Intruder test. Immediate heart rate changes during the first 5 sec after "intrusion" showed significant group differences ($F(2,22) = 3.44$, $P < 0.05$). The prewean isolates reacted with significantly *less* increase in heart rate than the postwean isolates ($F(1,14) = 5.38$, $P < 0.05$). (Fig. 3). Although the actual active heart rates during the 5 min of "intrusion" did not show statistically significant differences among groups, the inactive heart rates 2 min after the intruder had been removed again showed significant group

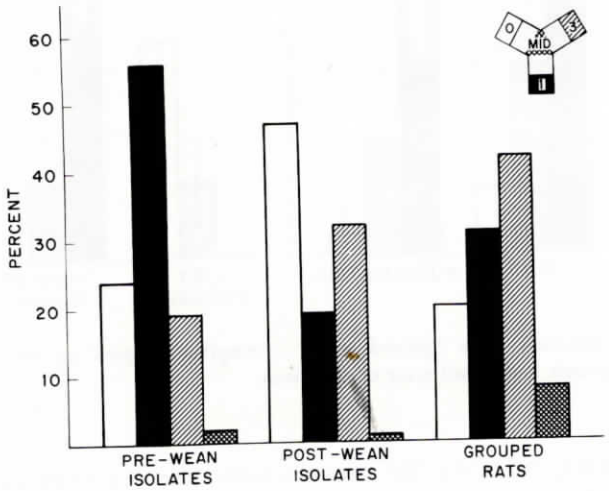

Fig. 2. Social Preference Test. The ordinate represents the percentage of frequency the experimental animal was observed next to the compartments containing 1, 3, or 0 rats, or in the middle part of the maze. The overall differences in frequency distribution were significant at $P < 0.001$ ($\chi^2(6) = 65$).

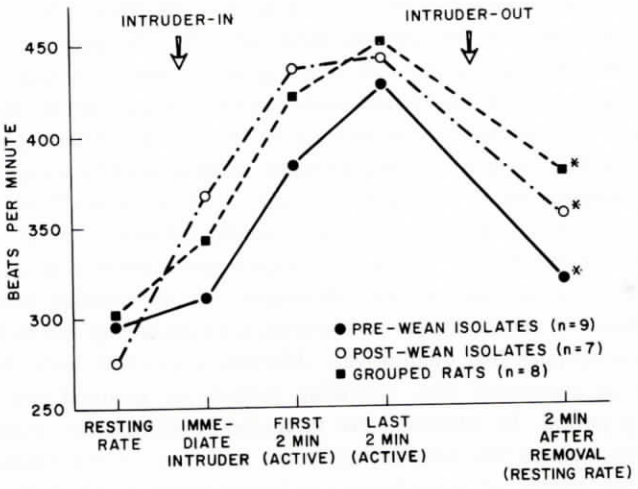

Fig. 3. Mean Heart Rates During Intruder Test. *Resting heart rates 2 min after the removal of the "intruder." During the "intrusion" the animals were all active.

differences ($F(2,13) = 7.98$, $P < 0.01$). Individual comparisons showed that the prewean isolates had significantly lower heart rates than the grouped rats ($F(1,10) = 14.72$, $P < 0.01$), and the postwean isolates ($F(1,9) = 5.20$, $P < 0.05$).

(6) Gastric ulcers after immobilization. The incidence of gastric ulceration was 44% in prewean isolates, 43% in postwean isolates, and 67% in the grouped rats. These differences did not reach statistical significance (Fisher exact test).

Body weight and age. There were no significant correlations or interaction effects among weight, age, and the various test results.

DISCUSSION

Isolated animals have generally been shown to be more "emotional," exploratory, and aggressive (Baenninger, 1967; Hatch *et al.*, 1963; Melzack, 1969). Our findings with the "Gloved Hand Test" show that the prewean isolates were, in one or more measures, more "emotional" and "fierce" than the grouped rats or postwean isolates.

Isolation from littermates during the preweaning period, however, does not appear to be simply a more prolonged type of postweaning social isolation. Consider the results of the "Social Preference Test" and the "Intruder Test." Although the prewean isolates preferred the compartment

with three rats least, followed by postwean isolates, the compartment containing a *single rat* was chosen most often by the prewean isolates and least by the postwean isolates. It is intriguing to speculate that perhaps the prewean isolates and grouped rats preferred the compartments resembling the environment of their first social interaction from 15 to 21 days postnatal age: grouped rats next to a group and prewean isolates next to a single rat. There is some evidence that, in mammals, the "critical period" for effects of stimulus deprivation might occur in "middle infancy," about the age of weaning (Bronfenbrenner, 1967). Our experiments show that an attempted stimulus deprivation (removal of littermates) before weaning resulted in an intensification of another type of stimulation (mother-pup interaction). Thus, our prewean isolates had qualitatively different interaction with their mothers and peers as compared with postwean isolates or grouped rats during the preweaning period. In addition, the intensity of stimulation experienced by the prewean isolates was probably different from that of the nonisolated pups although the length of stimulation (including interaction) might have been roughly comparable. Denenberg (1964) suggests that the amount of stimulus input in infancy acts to reduce emotional reactivity in rodents in a monotonic fashion. The attempted compensation on the mother's part for the lack of stimulation from littermates was, perhaps, not complete, resulting in long-term increased emotional reactivity in the prewean isolates as measured by the "Gloved Hand Test."

Quality and intensity of stimulation, age of the animal at the time of stimulation (or deprivation), and the organism's experience between stimulation and testing, among others, are considered to be important variables concerning critical periods for the effects of stimulation in infancy (Denenberg, 1968). Our experiments show that isolation from littermates at 12 days of age probably resulted in alterations in both the quality and quantity of the stimulation experienced by the pup through changes in the mother-pup interaction. The relationship between these alterations in the pattern of social interactions during the preweaning period and the different social and behavioral responses the prewean isolates show in adulthood should be studied further in future investigations.

The prewean isolates showed the least increment in heart rate upon "intrusion" by a strange rat, and the postwean isolates the most. Whether this selective effect of preweaning isolation on heart rate is related paradoxically to the increased active heart rate upon isolation in infancy (Leigh and Hofer, 1973) also requires further investigations into the underlying mechanisms.

The incidence of gastric ulceration from immobilization seems to be similar between the prewean isolates and postwean isolates but the small number of animals tested makes interpretation difficult.

Our findings demonstrate that isolation from littermates during the preweaning period has some different long-term effects from postweaning

isolation. Furthermore, some of the differential effects may be manifest only under specific conditions such as physiologic response to conspecific intruder or "fierceness" to the experimenter. Thus, we may conclude that the altered early social relationships produced by reduction of litter size to a single pup during the "socialization" period before weaning can have long-term effects on behavior, social preference, and physiological responses in adult rats.

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