Severe maternal separation and adult emotional reactivity in BALB/c mice[1]

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BALB/c mice were subjected to severe maternal separation during the first 18 days of life, and data on adult emotional reactivity was later obtained. Mothers were removed from the pups for a period of 10 h out of every 12, beginning 24 h after birth until weaning at 18 days. After weaning, experimental and control groups were divided into isolate/nonisolate groups. At 45 days of age, all animals were tested for 3 consecutive days in an automated open field on three dependent measures: defecation, activity level, and quadrants traversed. The data obtained on activity level and quadrants traversed offer some evidence that severe maternal separation using intact litters may result in decreased emotional reactivity. The defecation data, however, were in the opposite direction of increased emotional reactivity. The implications of this and similar studies are discussed.

Although the primate deprivation syndrome (Mason, 1968) is now well documented, along with similar phenomena at lower phyletic levels (Igel & Calvin, 1960; Hersher, 1969), studies of maternal deprivation at the rodent level have been much less successful. Seitz's findings (1954) of increased emotionality in rats as a function of maternal deprivation cannot be taken as conclusive because of serious methodological shortcomings. All other more recent attempts to demonstrate deleterious effects of maternal deprivation in rats and mice have failed. Newell (1967), in a study of maternal separation in two strains of mice, failed to find any consistent treatment effects on emotional reactivity. Thoman & Arnold (1968b) studied the effects of rearing infant rats without the mother on adult emotionality measures and found no differences in emotional reactivity between maternally deprived rats and controls. Earlier, Thoman & Arnold (1968a) also found that rats reared with or without peers, in an incubator or with a mother, differed very little on directly observable maternal behaviors. LaBarba, Lutz, & White (1968) found no conclusive evidence that maternal deprivation in BALB/c mice produced differential patterns of adult emotional reactivity relative to controls.

The present study is a somewhat more elaborate and extended investigation of the effects of severe maternal separation on adult emotional reactivity in BALB/c mice and is based on the earlier report of LaBarba et al (1968).

SUBJECTS

Twelve litters of BALB/c mice were chosen randomly from a large number of litters of six or more pups. All 12 litters were culled to six pups each. Six of the 12 litters served as the experimental group and the remaining six litters served as the control group.

PROCEDURE

All animals were housed in standard polycarbonate mouse cages, with pine shavings for bedding, under typical laboratory conditions. Beginning approximately 24 h after birth, the animals in the experimental group were separated from the mother by removing the mother and placing her in a similar plastic cage for a period of 10 h. At the end of this 10-h period, the mother was returned to the infants for a 2-h period. After the 2-h period, the mother was again removed for 10 h. This procedure was followed for 18 days, at which time weaning took place. The infants were not handled by the E during this 18-day treatment. The mothers in the control group were removed from their pups at the same time as those of the experimental group but for a period of only 2 min. This was done to control for any maternal handling effects. Thus, the infants in the experimental group were separated from their mothers for 20 out of every 24 h, while those in the control group were separated for 4 min out of every 24-h period.

After weaning took place at 18 days of age, each litter in both the experimental and control groups was subdivided. In each litter, three of the animals were left as a group and housed together in one cage. The remaining three were separated and placed in individual cages. Thus, there were four groups of 18 animals each. Two groups, one from the control and one from the experimental, were isolated and the other two groups were nonisolated. After weaning, all animals were placed on an ad lib food and water schedule and were not further handled until testing began at 45 days of age.

The subdivision of the experimental and control groups into isolates and nonisolates was an attempt to obtain information about the influence of littermates on any maternal separation effects. The nonisolated litters were not left intact, even though such a procedure might have produced maximum differences between isolate and nonisolate conditions, because of possible effects due to overcrowded cage conditions. The cages used in this study can adequately house a maximum of four adult mice. Therefore, the nonisolates were divided into groups of three by the E.

At 45 days of age, all animals were tested in an automated open field for 3 consecutive days on three dependent measures: general activity level, quadrants traversed or locomotion, and defecation.

APPARATUS

The animals were tested in a Lehigh Valley Quadrant Activity Cage (Model 1497), which permitted two dependent measures, activity and quadrants traversed, to be obtained by electrical recording. This apparatus has been described elsewhere (LaBarba et al, 1968). The third index of emotional reactivity, defecation, was obtained by counting fecal boli collected under the metal grid floor. Test trials of 3 min were run on each animal for 3 consecutive days. The data for each of the three dependent variables were recorded daily for each animal. After each animal completed its 3-min test trial, the floor and walls of the open field were wiped with a vinegar solution to remove odors.

RESULTS AND DISCUSSION

The data on each of the dependent measures were analyzed by a mixed design analysis of variance. The results of the study are somewhat inconsistent with regard to the effects of maternal deprivation on emotional reactivity. Mean defecation for the maternal separation group was higher than that of the controls, 3.27 and 2.41, respectively ($F = 4.1$, $df = 1/68$, $p < .05$). The main effect of isolation indicated higher defecation rates for the nonisolates than for the isolated animals, 3.57 compared to 2.10 ($F = 11.8$, $df = 1/68$, $p < .005$). No other significant effects were found in the analysis of defecation. The defecation rates, then, would indicate that the maternally deprived animals are more emotional than controls. The data on activity level, however, revealed no significant main effects. Maternal separation, with or without isolation, produced no differences relative to controls. A significant days effect was found, with activity level decreasing on the 2nd day of testing and increasing on the 3rd day ($F = 4.6$, $df = 2/136$, $p < .025$). A significant Days by Maternal Separation interaction effect was also found ($F = 5.04$, $df = 2/136$, $p < .01$). This interaction effect was analyzed by making comparison tests

between the experimental and control groups for each day of testing.

The only significant difference between the groups was found to be on Day 3 activity level ($t = 2.69$, $df = 35$, $p < .05$). The mean activity level of the maternal separation group on Day 3 was 228, while that for the control group was 174. Thus, the interaction effect is apparently a function of Day 3 activity only. The dependent variable of activity level, then, does not converge with the defecation data. Interestingly enough, however, when the four groups in this study are collapsed into a maternal separation group and a control group, there can be seen a definite trend toward *decreased* emotional reactivity in the experimental group, with significant differences occurring on Day 3. On Day 2, the maternal separation groups were also more active on the average (205) than the controls (178), but the difference is not significant. When we look at the data in this way, the maternally deprived animals seem to be moving in the direction of being less emotional than the controls. This interpretation becomes all the more fascinating when we find that the quadrants traversed data reveal a significant main effect of maternal separation. The deprived animals traversed more quadrants ($\bar{X} = 27.61$) than the controls ($\bar{X} = 19.95$; $F = 12.67$, $df = 1/68$, $p < .001$). As these data are typically interpreted, the maternal separation groups were less emotional than the controls. A significant days effect was also found ($F = 6.04$, $df = 2/136$, $p < .005$). The experimental group showed little change between Day 1 (28.8) and Day 3 (27.6), while the controls dropped in quadrants traversed from 23.8 on Day 1 to 17.3 on Day 3. Pearson product-moment correlation coefficients between activity level and quadrants traversed among the groups yielded high positive correlations ranging from .85 to .94.

Although the defecation data reflect increased emotionality for maternally deprived animals, previous studies have pointed out the weaknesses in using defecation as a measure of emotionality (e.g., LaBarba et al, 1968). It is interesting to note that nonisolated mice defecated more than isolated ones, and to speculate on the notion that perhaps defecation as a territorial response is related to early experiential variables and not entirely a matter of innate mechanisms.

It is suggested that the data obtained on activity level and quadrants traversed in this study offer some evidence to hypothesize that maternal deprivation, if severe enough, in intact litters of mice may decrease adult emotional reactivity, and that such reduction is a function of increased stimulation input occurring during infancy as a function of the maternal deprivation. It is further suggested that when littermates of rats and mice are subjected to a maternal separation paradigm, as reported here, increases in stimulation input to the infants result as a function of temperature reductions, quantitative and qualitative changes in maternal behavior, hunger, and increases in general activity and intralitter stimulation resulting from visceral stimulation. Such effects would be consistent with Denenberg's theory of infantile stimulation (1964). The studies dealing with maternal separation in rodents have obtained negative results perhaps because of the compensatory effects of increased littermate stimulation, etc., resulting from the maternal separation treatment. The present study, along with others reported here, may perhaps be more accurately described as the effects of littermate stimulation rather than maternal deprivation.

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NOTE

1. This study is based on a Master's thesis submitted by the second author to the Department of Psychology, University of South Florida, in partial fulfillment of the requirements for the MA degree.

Paradoxical sleep in the rat: Comparison of early and late blinding

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Electrophysiological recordings of sleep in adult rats blinded prior to weaning were significantly different from those recorded in littermates blinded after weaning. Behavior on an open-field test failed to indicate any significant differences between these two groups. Thus, it appears that an early reduction of external sensory input can modify brain activity during sleep.

The functional significance of paradoxical sleep (PS), frequently termed rapid-eye-movement sleep (REM), is not clearly understood. Roffwarg, Munzio, & Dement (1966) suggest that it is required for the normal development of the central nervous system (CNS). They hypothesize that a reduction of exogenous inputs to the CNS will result in an increase of endogenous activity such as that associated with paradoxical sleep.

In our experiment, we attempted to investigate the interaction between neural feedback and sensory stimulation. We predicted that a reduction of external sensory input to the CNS of rats early in life would result in an increase of PS activity of adult rats. Specifically, the purpose of this study was to determine the effects of early vs late blinding on paradoxical sleep. We compared these measures with the behavior of the two groups with respect to ambulatory activity in an open-field apparatus.

APPARATUS AND PROCEDURE

Twenty-one (Wistar strain) rats, the result of matings in our own laboratory, were assigned randomly, using a split-litter