Open field behavior of C57BL/6J mice as a function of age, experience, and prenatal maternal stress¹

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Abstract

In order to assess the role of age, prior test experience and prenatal maternal stress on open-field behavior, both stressed (treated) and control litters were divided into two groups. Group 1 was tested in the open field at both 40 and 120 days of age, and Group 2 at only 120 days. Comparison of activity level and location of activity in the open field of naive Ss at 40 days of age with those of naive Ss at 120 days of age demonstrated significant age effects. Similarly, comparisons of these behaviors of experienced Ss at 120 days with those of naive Ss at the same age, indicated effects of prior test experience. In addition, possible interactions of both age and prior test experience with the effects of prenatal stress were indicated. The importance of these findings for the design of studies in which the effects of experimental variables are examined at several different ages is discussed.

Problem

That prenatal maternal stress may influence offspring behavior of both rats and mice has been demonstrated (cf. Thompson et al, 1962; Weir & DeFries, in press, for review of pertinent literature). Several of these studies included tests of offspring at more than one age (e. g., Thompson, 1957; Hockman, 1961; Ader & Belfer, 1962), and differential responses of Ss at the two test periods were suggested. Since all offspring were tested at both ages, these results may have been due to differences in age, prior test experience, or both. The primary objective of the present study was to assess the role of age and prior test experience on the open-field behavior of mice and their possible interactions with the effects of prenatal maternal stress.

Method

Female C57BL/6J mice were caged individually with males and checked daily for the presence of vaginal plugs. One-half of the 26 females which subsequently produced viable litters were exposed to three daily physical stress conditions, which were begun one week after mating was indicated and were terminated upon littering; the remainder served as controls. The daily stress conditions employed in the present study included being placed in groups of four for 3 min. each morning in a swim tank, 3 min. each afternoon in a lighted tilt-box in which a loud tone occurred at random intervals for about 50% of the stress period, and 3 min. each evening individually in a brightly lighted circular open field.

These stress conditions are described in detail elsewhere (Weir & DeFries, in press).

At 40 days of age, offspring were removed from their home cages and segregated according to sex. The average litter size of the 13 treated and 13 control litters at this age was 5.4 and 5.8, respectively. Each litter was split into two groups: Group 1 (n=36 and 38, treated and control) was tested in the open field on each of 5 successive nights, beginning at 40 days of age and then again at 120 days of age. Group 2 (n=34 and 37, treated and control) was tested during only the later test period. These subclasses were balanced as well as possible according to sex of offspring and were subsequently reduced to 34 animals each for ease of statistical analysis.

The open-field behaviors examined in the present study include activity level and location of activity in the open field. Each S was placed in the center of the field at the start of the test, and its progress during a 2 min. test period was traced on a scale map of the field. A map measurer was then used to trace the path and the ambulation data were recorded in map units (1 map unit = 2.1 in). Distance traveled toward the center of the field, after S had first gone to the edge, was also determined and expressed as a % of the total distance traveled to indicate the location of the activity in the open field. For both measures, individual 5-day totals were computed and served as each S's score.

Results

The mean activity level scores of the treated and control Ss representing Groups 1 and 2 are presented in Table 1. In order to assess the effects of prenatal stress and age, independent of prior test experience, data from Group 1 at 40 days of age and Group 2 at 120 days of age were analyzed in a 2 x 2 analysis of variance. The main effect due to prenatal stress was not significant, $F(1,132) < 1.0$. The main effect due to age, however, was highly significant, $F(1,132) = 9.70$; $p < .005$, and the interaction of age and prenatal stress approached significance, $F(1,132) = 3.47$; $.05 < p < .10$. The age main effect is the result of a much higher activity level of naive Ss at 120 days of age ($\bar{X} = 970.0$) than at 40 days of age ($\bar{X} = 809.2$). From Table 1, it may be seen that prenatal stress results in a decreased activity level when naive Ss are tested at 40 days of age and a slightly increased activity level when tested at 120 days of age, which accounts for the marginally

Table 1. Mean Open Field Activity Level and Center Scores of C57BL/6J Mice as a Function of Age, Experience and Prenatal Maternal Stress

Stress		Activity Level		Center Scores	
Group	Condition	40 Days of Age	120 Days of Age	40 Days of Age	120 Days of Age
1	Treated	739.2	505.4	95.1	104.7
	Control	879.2	661.4	107.8	122.6
2	Treated	-----	996.2	-----	193.8
	Control	-----	943.8	-----	181.8

significant interaction between age and prenatal stress.

In order to assess the effects of prenatal stress and prior test experience, independent of age, data from Group 1 at 120 days of age and Group 2 at 120 days of age were also analyzed in a 2 x 2 analysis of variance. The main effect of stress was again nonsignificant, $F(1,132) = 1.09$. The effect due to prior testing experience, however, was significant, $F(1,132) = 60.88$; $p < .001$, as was the interaction between the effects of prior testing experience and prenatal stress, $F(1,132) = 4.42$; $p < .05$. The effect due to prior test experience is accounted for by the much higher activity level of Ss tested for the first time at 120 days of age ($X = 970.0$), as compared with those tested for the second time at 120 days of age ($X = 583.4$). The interaction between prior test experience and prenatal stress may be observed by comparing the 120-day means presented in Table 1. Treated Group 1 Ss (experienced) continued to show a lower level of activity than controls, whereas Group 2 Ss (naive) showed a slight effect in the opposite direction.

The mean center scores of the experimental and control Ss representing Groups 1 and 2 are also presented in Table 1. The data from which these means were obtained were subjected to analyses of variance identical to those described above for activity level. The main effects due to age and prior test experience were again highly significant, $F(1,132) = 48.21$ and 34.71 , respectively, $p < .001$, and were in the same direction as activity level. However, in neither analysis was the main effect due to prenatal stress or its interaction with age or experience found to be statistically significant.

Discussion

If, as in Group 1 of this study, all animals had been tested at both ages, two conclusions might have been drawn: (1) Age or prior test experience or both result in a decreased activity level at 120 days of age, and (2) the effects of prenatal stress on activity remain permanent through 120 days of age. The inclusion of Group 2 animals in this study, however, yields important additional evidence which leads instead to the following conclusions. By comparing the activity of naive Ss

at 120 days of age with that of Ss at 40 days of age, it is clear that age, independently of prior test experience, results in an increase in open-field activity. However, by comparing naive Ss at 120 days of age with experienced Ss at the same age, it may be seen that prior test experience results in a decrease of activity. The finding of the significant interaction between prenatal stress and prior test experience indicates that the effects of prenatal stress are manifested at 120 days of age, only if the animals have undergone previous test experience in the open field. It is possible that stress resulting from open field testing at 40 days of age enhances the effects of prenatal stress so that they continue to be expressed at 120 days of age.

The results of this experiment clearly indicate the importance of designing longitudinal studies in which the effect of some experimental variable is examined at several different ages in such a way that the role of age and previous test experience may be separately assessed.

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