Open-field behavior of C3H mice: Effect of early handling, field illumination, and age at testing

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C3H mice, either handled or undisturbed at 1-7 days of age, were tested for later activity and defecation in the open field under low or high illumination at 50, 70, or 100 days of age. Activity scores indicated that handled Ss were more emotional at 50 and 70 days of age but less emotional at 100 days than controls. In contrast, defecation scores indicated that handled Ss were less emotional than controls at all ages. High illumination resulted in lower activity at all ages for both handled and control Ss but had differential effects upon defecation as a function of age and treatment. These results demonstrate the necessity of further research to clarify the relationship between activity and defecation as indices of emotionality in mice.

It has been pointed out by Goldman (1969), among others, that the literature concerning the effects of early stimulation upon later emotionality of rats and mice has supported all possible effects: increases, decreases, and no change at all in emotionality. King (1958) has suggested seven relevant parameters that could affect early experience studies which have been generally ignored. More recently, several of these parameters have been examined more systematically than in the past, and the results are rather striking. Henderson (1969), for example, has extensively examined the Treatment by Genetic interactions in experiments dealing with inbred mouse strains and various crosses between these strains. His results indicate that even strains within a species do not respond in similar ways to early treatments.

Generalization of results between strains, much less between species, is questionable without testing the strains or species involved. Indeed, even within strains of highly inbred animals, conflicting data have been reported that may be due to lack of control of other parameters. For instance, Ross et al (1966), McReynolds et al (1967), and Dixon & DeFries (1968) have reported that the open-field behavior of 30- to 40-day-old C57BL/6J mice is relatively unaffected by a wide range of illumination levels. However, Nagy & Glaser (1970) have demonstrated that illumination level affects the C57BL/6J mouse differentially as a function of age. These kinds of findings clearly indicate the need for more systematic investigation of the many parameters that could affect early stimulation results.

The purpose of the present experiment was to determine if the age at testing and the illumination level in the test situation would differentially affect activity and defecation in the open field of Ss that received either early handling or no handling.

SUBJECTS

The Ss were 120 male and 120 female C3H mice, born and reared in 19 x 11 x 5 in. polyethylene cages with wire-mesh tops, wood-chip shavings on the floor, and ad lib access to food and water. The Ss were maintained in the laboratory colony at 74 ± 2°F on a 12-h light-dark cycle.

APPARATUS

The test apparatus consisted of a 36-in.-square field, with 12-in.-high walls, constructed of unpainted Masonite. The floor was divided by black lines into 36 6-in. squares. A 150-W incandescent bulb, located 30 in. above the center of the field, was regulated by a Type 116B Powerstat variable transformer and provided two illumination levels of 100 fc (140 V) and 1 fc (40 V).

PROCEDURE

At birth, litters were randomly assigned to either handling or control conditions. Handling consisted of removing the mother from the home cage and then placing each S individually into 1-gal galvanized cans partially filled with wood-chip shavings for a 3-min period. The Ss were then returned to the nest and the mother replaced. This procedure was carried out once each day from 1 through 7 days of age. Controls were not disturbed until weaning. All Ss were weaned at 30 days of age and placed with four Ss of the same sex, age, and experimental group (handled or control) from other litters until the open-field tests.

Prior to testing, all Ss were earpunched for identification. At 50, 70, or 100 days of age, S was removed from the cage and placed in the center square of the field in an arbitrary direction. The number of squares entered and the number of boluses deposited by each S during a 2-min trial were recorded. Each S received one trial each day for 4 consecutive days.

At each of the ages, one-half of the handled and one-half of the control Ss were tested under high illumination, the remaining Ss under low illumination. Within postweaning living cages, three mice were tested under one illumination level on all days, the remaining two mice under the other level. No more than two Ss of either sex from any one litter were tested under the same experimental conditions, and each litter of the handled and control Ss was represented at every age. Each group consisted of 10 males and 10 females.

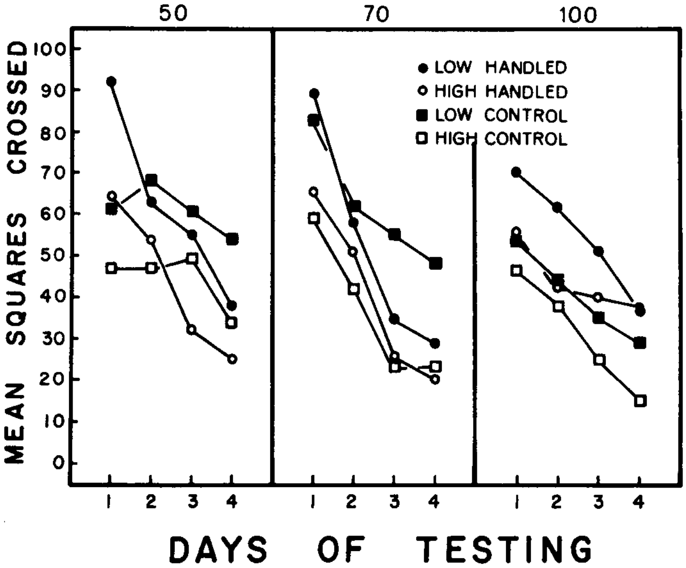
RESULTS

The activity data were analyzed by a five-way analysis of variance, with one repeated measure. The factors were age at testing, treatment (handled or control), illumination level, sex, and days of testing. Figure 1 presents the mean number of squares crossed over the 4 test days for all three ages as a function of prior treatment and illumination level of the test field. The main effect for sex was not reliable ($F = 0.21$, $df = 1/215$), and sexes have been combined in the figure.

The analysis showed a reliable age-at-testing effect ($F = 4.39$, $df = 2/215$, $p < .025$), with overall activity being greatest at 50 days of age and least at 100 days of age. Activity level was influenced by illumination level ($F = 30.73$, $df = 1/215$, $p < .0005$), with activity greater under the lower illumination level, and there was a decrease in activity for all ages over test days ($F = 106.82$, $df = 3/648$, $p < .0005$).

Although the main effect for handling was not reliable ($F = 1.83$, $df = 1/215$), a number of interactions with handling were significant: Age by Treatment ($F = 3.39$, $df = 2/215$, $p < .05$), Treatment by Days ($F = 8.08$, $df = 3/648$, $p < .0005$), Age by Treatment by Days ($F = 3.87$, $df = 6/648$, $p < .0005$), Treatment by Sex ($F = 5.08$, $df = 1/215$, $p < .025$). With the exception of the last, all of these effects can be seen in Fig. 1. Individual comparisons within the Age by Treatment by Days interaction showed that 50-day-handled Ss were reliably more active than controls on the first test day ($F = 14.70$, $df = 6/648$, $p < .0005$), but then decreased more rapidly than the controls and were reliably less active on Days 3 and 4 ($Fs > 3.16$, $df = 6/648$, $ps < .005$) than controls. A similar trend was found at 70 days of age, with handled Ss slightly more active on the first days but then becoming reliably less active than controls by the last day ($F = 3.04$, $df = 6/648$, $p < .01$). In contrast, handled Ss at 100 days were reliably more active than controls on all test days (all $Fs > 3.12$, $df = 6/648$, all

Fig. 1. Mean number of squares crossed over the 4 test days as a function of early handling, illumination of the field (high or low), and age at testing.



ps < .005). Because of the reversal in activity between handled and control groups over days for the 50- and 70-day Ss, overall activity differences between handled and control Ss at these ages were not reliable (Fs = 0.01 and 0.39, respectively, df = 2/215). Individual comparisons within handled and control groups across ages revealed that total activity for control groups was reliably less at 100 days than at either 70 days (F = 8.26, df = 2/215, p < .0005) or 50 days (F = 12.07, df = 2/215, p < .0005), while there was little difference between the 50- and 70-day controls (F = 0.36). In contrast, there were no reliable age differences between any of the handled groups (all Fs < 1.77, df = 2/215). Thus, it appears that the effect of early handling in this strain was not to increase activity per se, but rather to prevent the decrease in activity with increasing age, as displayed by the control groups.

Individual comparisons within the Treatment by Sex interaction showed that overall activity for male Ss did not reliably differ between handled Ss and controls (F = 0.41, df = 1/215, but female handled Ss were more active than control females (F = 6.50, df = 1/215, p < .01).

The defecation scores were transformed by a square root transformation, with 0.5 being added to each raw score prior to the transformation. The data were then analyzed by a five-way analysis of variance with one repeated measure, with age, treatment, illumination, sex, and days being the respective main factors. Reliable main effects showed that handled Ss defecated less than did controls (F = 4.43, df = 1/215, p < .05), females defecated more than did males (F = 59.36, df = 1/215, p < .0005), and defecation increased over test days (F = 69.89, df = 3/648, p < .0005). Reliable interactions were Age by Illumination (F = 3.41, df = 2/215, p < .05), Treatment by Sex (F = 4.38, df = 1/215, p < .05), and Age by Sex by Days (F = 2.27, df = 6/648, p < .05).

Individual comparisons within the Age by Illumination effect showed that at 100 days of age, Ss defecated more under high than under low illumination (F = 7.00, df = 2/215, p < .001), but illumination had no significant effect at earlier ages. Also, under high illumination, 100-day Ss defecated more than 50- or 70-day Ss (Fs > 5.51, df = 2/215, ps < .005), while under low illumination the differences between ages were not reliable (all Fs < 1.10, df = 2/215). Within the Treatment by Sex interaction, control males and females did not differ in defecation. Both male and female handled Ss defecated less than their respective controls, but only the male difference was reliable (F = 4.73, df = 1/215, p < .05).

DISCUSSION

In contrast to data demonstrating that illumination differentially affects the activity levels of C57BL/6J mice at various ages (Nagy & Glaser, 1970), the present study shows that the C3H strain is equally affected by illumination differences at the three ages tested, whether Ss experienced early handling or not. In all groups, high illumination depressed activity over the 4 test days.

The effects of early handling upon later activity of C3H mice is clearly dependent upon the age at testing. At 50 and 70 days of age, handled Ss were more active than controls on the first test day, but less active by the last test days. In contrast, at 100 days, handled Ss were more active than their respective controls on all days. Whimbey & Denenberg (1967) have interpreted high activity on Day 1 and low activity on Days 2-4 as indicative of high emotionality for rats, whereas low emotional Ss maintain a higher activity level over all test days. If this interpretation is also correct for mice, then handled mice show a reversal in emotionality as a function of age at testing, being more emotional than controls at 50 and 70 days of age, but less emotional at 100 days.

If emotionality is interpreted by defecation scores, however, one does not arrive at the same conclusion regarding emotionality. Handling resulted in decreased defecation at all ages in the present study, with males being more affected than females. If high defecation indicates high emotionality, as is usually interpreted with rat data (Denenberg, 1969), then one would conclude that handled Ss were less emotional at all ages than were the controls. The finding that handled Ss defecated less than controls is also in contradiction with most early-stimulation studies with mice, which usually reported higher defecation for early-stimulated Ss (Hall & Whiteman, 1951; Lindzey et al, 1960; Henderson, 1964). However, there are major differences in the present study and in earlier ones, including the strain of mice, type and duration of stimulation, and age at stimulation, which could probably account for these discrepancies.

The lack of consistency between activity and defecation scores as indices of emotionality in the present study, as well as the contradictions with previous early-stimulation studies with mice, indicates the necessity of further research to clarify the relationship between activity and defecation in mice. These behaviors must be examined under a variety of parameters, as suggested by King (1958), before general conclusions can be reached regarding early treatment effects.

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Fear level and rats' open-field activity and defecation[1]

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Two tests were made of Lester's suggestion that rats' open-field activity is an inverted U-shaped function of fear level. The first examined the relation between activity and defecation. The second test examined the relation between a possible low-level fear manipulation (estrus phase) and activity level. Both tests failed to support the suggestion.

EXPERIMENT 1

Lester (1967) has suggested that exploratory activity takes the form of an inverted U-shaped function of fear level. In one experiment designed to test this theory (Lester, 1968), he exposed male and female rats to an open field. In this study, defecation was assumed to be an index of fear level, and activity a measure of exploratory behavior. When divided into quartiles on the basis of their activity scores, he found that the two groups that were intermediate in terms of activity tended to have smaller numbers of defecators than the least active *and* the most active groups. Thus activity tended to increase to a maximum and then decrease as a function of increasing numbers of defecators per group.

This might appear surprising because several workers have reported an inverse relation between defecation and activity (Broadhurst, 1960). Such results, of course, could still be consistent with Lester's (1968) hypothesis of a nonmonotonic relation between activity and fear level if they are regarded as being derived from the higher fear level part of the function. This is plausible in the case of Broadhurst's (1960) studies, for he used an open field made more stressful by the addition of bright lights and loud noise. In his experiment, Lester (1968) appears to have minimized aversive stimulation. However, Lester (1968) used a rectangular field that was open on one side. With this type of arrangement, movements made by the E may affect the behavior being observed. For these reasons, it was decided to undertake an open-field study, using a fully screened arena, in which stressful stimulation was minimal.

Method

The Ss were 48 naive female hooded rats obtained from Animal Supplies (AS) and 34 naive female hooded rats obtained from the National Institute for Research in Dairying (NIRD). Both groups were approximately 130 days old. The open field consisted of a circular arena 33 in. in diam. The walls were 14 in. high. The inside surface area was painted a flat white, and the floor was divided by pencil lines into 25 sections roughly equal in size. No obvious stressful stimulation was added. The field was illuminated by a 25-W bulb suspended 5 ft above the center of the floor and was surrounded by a muslin screen. Each rat was carried to the experimental room, placed in the center of the field, and allowed to explore for 5 min. Two scores were taken—the number of sections through which a rat passed (activity) and the number of fecal boli deposited (defecation). An entry of all four feet (body, except for tail) was required before a unit of activity was scored. All animals were tested during the late afternoon.

Results and Discussion

Figure 1 shows that there was no suggestion of an inverted U relation between activity and defecation. Spearman rank correlations between the two variables gave values of −0.10 (ns) for the AS rats and −0.49 ($p < .01$) for the NIRD rats. Thirty-nine of the 48 AS rats and 14 of the 34 NIRD rats defecated. A fourfold comparison between defecators and nondefecators for both groups gave a value of $\chi^2 = 12.28$ ($df = 1$, $p < .001$), indicating that the tendency to defecate was greater in the AS rats ($\chi^2 = 18.75$, $df = 1$, $p < .001$) than in the NIRD rats ($\chi^2 = 1.06$, $df = 1$, ns). It is inconsistent with Lester's (1968) hypothesis that the group of rats who defecated more rarely (i.e., were presumably less fearful overall) should display a stronger *negative* correlation between the two variables than did the more fearful group.

EXPERIMENT 2

Another test of Lester's (1968) hypothesis might involve observing rats that one could reasonably argue, in an a priori fashion, were minimally fearful. These rats would then be subjected to a low-level fear manipulation—a level that would not elicit defecation—and their activity recorded. It might then be reasonable to assume that we would be observing activity patterns associated with low fear levels. One possible manipulation might be estrus cycle phase for it has been suggested that rats in estrus are less emotional (as defined by amount of defecation) than when in diestrus. Gray & Levine (1964) reported that rats in an induced estrus condition were more active and tended to defecate less in an open field. Burke & Broadhurst (1966) obtained similar, although less clear-cut, results using naturally occurring estrus. Although an inverse relation between fear level (in and out of estrus) and activity was preserved, Lester's (1968) hypothesis could still account for the results by postulating that the rats were too anxious in general. It is noteworthy that both experiments used the mildly stressful open field developed by Broadhurst (1960). For this reason, the second experiment attempted to examine the effect of naturally occurring estrus on the activity of a minimally fearful group of rats in the less stressful open field.

Method

This study began with 24 NIRD female hooded rats. At the time of testing they were about 7 months old. All rats had been regularly handled and had been used in three maze studies ending about 6 weeks