Open-field behavior of C57BL/6J mice: Effect of illumination, age, and number of test days¹

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C57BL/6J mice were tested at either 50 or 100 days of age under one of two illumination levels in the open field for 10 consecutive days. Analysis of activity scores revealed a reliable Age by Illumination interaction, indicating that illumination had different effects at the two ages. Overall defecation scores formed an inverted U-shaped function over test days, with defecation increasing through the 6th test day, but then decreasing over the remaining days. The results of this study show the importance of testing Ss at several different ages and under several stimulus conditions before making general conclusions about the behavior of a particular strain.

Recent open-field studies with mice have shown that high illumination levels decrease activity and increase defecation scores in albino strains, but have little apparent effect on the pigmented C57BL/6J strain (Ross, Nagy, Kessler, & Scott, 1966; McReynolds, Weir, & DeFries, 1967; Dixon & DeFries, 1968b). This finding has been attributed to a genetic difference, with albinos being considered more photophobic than pigmented animals (Dixon & DeFries, 1968b). However, these studies utilized mice at only one age, 30 or 40 days of age, and for only a few trials. It seems imperative that Ss of several ages of a particular strain be tested more extensively before general statements are made about the behaviors of a particular strain.

The purpose of the present study was to determine if the illumination level of the open-field would have similar effects upon activity and defecation patterns of C57BL/6J mice at two ages and to examine the consistency of these behaviors over a greater number of trials than is commonly employed.

SUBJECTS

The Ss were 40 male and 40 female C57BL/6J 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 weaned at 30 days of age and placed with four other Ss of the same sex

and age in similar cages until testing. Half of the mice began testing at 50 days of age, the other half at 100 days of age. The Ss were maintained in the laboratory colony at $74 \pm 2^\circ\text{F}$ on a 12-h light-dark cycle.

APPARATUS

The open field was 36 in. square, with 12-in.-high walls of unpainted Masonite. In the center of the field, 30 in. above the floor, was a 150-W incandescent bulb, regulated by a Type 116B Powerstat variable transformer. The light was shielded by a silver-foil reflector to provide more even illumination in all parts of the field as determined by a light meter. Two illumination levels of 100 fc (140 V) and 1 fc (40 V) were provided by varying the transformer. Infrared filtered photoelectric cells were spaced 12 in. apart on the front and side walls of the field, dividing the floor into a 3 by 3 matrix of 12-in. squares. When S crossed any of the light beams, a count was automatically recorded in another room. An air-conditioner provided a steady background noise level of 63 dB during testing.

PROCEDURE

Prior to testing, Ss were earpunched for identification and assigned to experimental conditions according to a split-litter design. At either 50 or 100 days of age, each S was removed from the home cage and placed in a corner square of the field. The starting corners were varied in a random fashion for each S over days, as well as for between Ss on each day. The number of squares crossed and the number of boluses deposited by each S during a 3-min trial were recorded each day for 10 consecutive days.

Ten Ss of each sex at each age were tested under high illumination on all days, while the remaining Ss were tested under low illumination on all days.

RESULTS

To determine if an overall relationship existed between activity and defecation, a Pearson correlation coefficient was performed, using total squares crossed and total boluses deposited over the 10-day period for all Ss. The result was a reliable negative correlation, $r = -.538$ ($t = 3.94$, $df = 38$, $p < .001$), indicating that Ss that were highly active defecated less than Ss with low activity levels. To examine further this relationship as a function of age, illumination level, sex, and days of

testing, the 10 Ss of each group (one age, sex, and illumination level) were further divided into high-active and low-active Ss, based on their total activity scores over the 10 days. The five most active Ss of each group were designated high-active, the five remaining Ss as low-active.

The activity data were then analyzed by means of a five-way analysis of variance, with one repeated measure. The results were a significant age effect ($F = 23.70$, $df = 1/64$, $p < .001$), with 100-day Ss more active than 50-day Ss, no overall illumination effect ($F = 0.71$, $df = 1/64$), females more active than males ($F = 9.14$, $df = 1/64$, $p < .005$), a decrease in activity over test days ($F = 99.75$, $df = 9/576$, $p < .0005$), and, as expected, the high-active Ss more active than low-active Ss ($F = 94.54$, $df = 1/64$, $p < .0005$). Figure 1 shows the mean activity levels as a function of age, high- or low-active groups, and test days. It is clear that Ss placed into the high- or low-activity groups by means of total activity scores were fairly consistent in maintaining the activity differences over test days, although these differences tended to be less during the first and last days of testing, partially accounting for a significant Age by Activity Level by Test Days interaction ($F = 2.26$, $df = 9/576$, $p < .025$). The age difference in activity showed a similar trend over test days.

Although the main effect for illumination level was not reliable, two interactions with illumination level were significant. The Age by Illumination Level interaction ($F = 5.00$, $df = 1/64$, $p < .05$) revealed that under high illumination, there was no reliable activity difference due to age ($F = 1.73$, $df = 1/64$), while under low illumination, 100-day Ss were more active than 50-day Ss ($F = 12.62$, $df = 1/64$, $p < .001$). Thus, the main effect for age was due to activity differences under the low illumination condition rather than to a general age effect. The Illumination by Days interaction was also reliable ($F = 3.20$, $df = 9/576$, $p < .005$), due to Ss being less active under high than under low illumination on the first ($F = 5.49$, $df = 9/576$, $p < .0005$) and second ($F = 3.84$, $df = 9/576$, $p < .001$) test days, but on none of the following days. For the C57BL/6J strain, illumination effects appear rather short-lived and after 2 days of testing, the mice are no longer affected by the different levels used in this study.

The number of boluses deposited on each day served as the defecation score, which was transformed by a square root transformation, with 0.5 added to each raw score. These scores were then analyzed by a five-way analysis of variance with one repeated measure. The main factors were

Fig. 1. Mean activity levels as a function of age, activity level, and test days.

Fig. 2. Mean defecation scores (transformed) of high- and low-active groups over test days.

age, illumination level, sex, days of testing, and activity level. Results indicated that the only reliable main effects were due to activity level ($F = 34.90$, $df = 1/64$, $p < .0005$), with low-active Ss defecating more than high-active Ss, and days of testing ($F = 5.95$, $df = 9/576$, $p < .001$). The Activity Level by Days of Testing interaction was also reliable ($F = 2.76$, $df = 9/576$, $p < .005$) and is presented in Fig. 2. On Day 1, the difference in defecation between high- and low-active Ss was not reliable. However, on Day 2 and every following day, low-active Ss defecate significantly more than high-active Ss (all $F_s > 5.16$, $df = 9/576$, $ps < .0005$). Both low- and high-active groups showed an increase in defecation from the 1st to 2nd test day (both $ps < .001$). However, from the 2nd to 6th test days, low-active Ss increased defecation and then showed a general decrease to the last test day. By the last test day, the low-active group was still defecating more than on their 1st day ($F = 4.47$, $df = 9/576$, $p < .001$). In contrast, the high-active group decreased in defecation following the 2nd day and then showed slight changes from day to day thereafter. Only on Days 2 and 6 was defecation greater than on Day 1 (both $ps < .05$). The overall defecation score over days represents an inverted U-shaped curve, with the number of boluses increasing through the 6th day, followed by a general decrease over the last 4 days.

DISCUSSION

Several aspects of the activity data in the present study agree with previous research. The overall lack of activity differences due to illumination level is in general agreement with data reported by Ross et al (1966), McReynolds et al (1967), and Dixon &

DeFries (1968b) for the C57BL/6J strain, despite differences such as number of test days, distribution of trials, and absolute levels of illumination. Higher activity levels for naive Ss at 100 days of age than for Ss around 50 days of age has also been reported (DeFries & Weir, 1964; Dixon & DeFries, 1968a). However, in these previous studies, illumination level and age were not varied as factors within the same experiment. The reliable Age by Illumination Level interaction in the present study showed that 100-day Ss were reliably more active than 50-day Ss only under low illumination, suggesting that the apparent age effect occurs only under certain test conditions. In addition, the direction of activity differences under the two illumination levels was not the same at both ages. At 50 days of age, overall activity was greater under high ($\bar{X} = 326.4$) than low ($\bar{X} = 289.8$) illumination; at 100 days of age, activity was greater under low ($\bar{X} = 476.5$) than high ($\bar{X} = 395.6$) illumination. Although individual comparisons at each age were not reliable, the change in direction of activity resulted in the reliable interaction. The finding that 50-day Ss were more active under high than under low illumination agrees with the Ross et al (1966) study but contradicts the Dixon & DeFries (1968b) data. However, Dixon and DeFries used only 2 test days in contrast to 10 days in the present study and the massed trials in the Ross et al study. When activity under the two illumination levels was examined over the first 2 test days in the present study, 50-day Ss were more active under low ($\bar{X} = 135.5$) than under high ($\bar{X} = 125.0$) illumination and 100-day Ss were also more active under low ($\bar{X} = 196.1$) than

under high ($\bar{X} = 145.1$) illumination. Thus, the apparent contradiction in results is due to the number of test days employed in the respective studies.

The defecation data in the present study also agree with previous research by Collins (1966), who reported increasing levels of defecation over 6 test days for several strains, including C57BL/6J. The present data also show increasing defecation over the first 6 days, but then a reliable decrease over the remaining 4 days.

Although the differences were not reliable, Ss of both ages in the present study defecated more under low than under high illumination, in agreement with Ross et al (1966) and Dixon & DeFries (1968b) who reported similar nonsignificant differences due to illumination. Both studies also reported opposite results for albino strains, which defecated more under high than under low illumination. If it is assumed that a high level of illumination arouses greater fear in the open-field test than does low illumination, these results indicate that various strains of mice respond differently to fear, some strains showing increased defecation, others appearing to inhibit defecation.

The results of this experiment clearly show the importance of using more than a single age and test condition in assessing behavioral states, such as emotionality for mice. The need for more than 1 or 2 test days is also indicated by the changes in activity and defecation patterns over the 10 days. Recently, Whimbey & Denenberg (1967) provided evidence that activity in the open field for rats correlated positively with emotionality on Day 1, but negatively on Days 2-4. The increasing and decreasing

defecatory patterns found in this study also suggest that defecation and activity do not correlate in a simple manner for mice, and that more than 1 or 2 test days are needed to properly assess defecation patterns in mice.

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NOTE

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The Maudsley strains: The evaluation of a possible artifact¹

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Two experiments were carried out on the 31st and 32nd inbred generations of the Maudsley Reactive (MR) and Nonreactive (MNR) strains of rats to assess the contribution of the normal content of their digestive tract to their scores in the open-field test. It was found that the MR strain defecates a significantly greater weight of feces per 24 h than the MNR strain. The MNR strain, however, excretes a greater number of fecal boluses in the same period. Digestive transit time was then measured in the two strains. No differences emerged on this measure. The results together suggest that the differences in open-field defecation between the strains are the result of differential reactivity, rather than reflecting differences in the amount of fecal material in the rectum.

Rats drawn from the Maudsley Reactive (MR) strain defecate significantly more in the open-field test of emotionality compared with the Maudsley Nonreactive (MNR) strain. In addition, the extensive literature concerning their behavioral characteristics appears to suggest that the MR strain is more susceptible to the arousal of fear than the MNR strain (Eysenck & Broadhurst, 1964).

The strains differ markedly in other ways that, at present, do not appear to be

as readily related to emotionality. For the present context, it is notable that the MR strain is heavier than the MNR in adult body weight (Watson, 1960; in Eysenck & Broadhurst, 1964). It seems quite possible that such differences in body weight between the strains may indirectly contribute to the distinction between them in open-field defecation. Thus, the differences established in body weight between the strains may be correlated with differences in food intake and consequently the amount of fecal material available to excrete in a stress situation. It is the purpose of the following experiments to evaluate this possibility. Accordingly, it was decided to discover the relationship between home cage and open-field defecation in the two strains.

EXPERIMENT 1

Method

Males and females drawn from the 31st inbred generation of the MR and MNR³ strains were studied for 10 consecutive 24-h periods beginning on the 103rd day of age. The strains differed in the usual direction in adult weight. On Days 4-7 of the experiment, standard open-field tests (Broadhurst, 1960) were administered to all animals. Animals were housed one or two per cage for the duration of the experiment, a condition balanced as far as possible between sex and strain. Fecal boluses were removed from trays placed beneath the cages and weighed at approximately the same time each day.

Table 1 depicts the variation both in the number of fecal boluses excreted and their weight in the two strains.

Results

As can be seen, the MR strain excretes a significantly greater weight of fecal material than does the MNR strain ($U = 21$, $p < .05$). Conversely, if the number of fecal boluses excreted is taken as the criterion, then the difference between the strains is reversed. Animals in the MNR strain excrete a greater number of boluses compared with the MR strain ($U = 12$, $p < .02$). In the open-field test on Days 4-7, the MR strain defecated a mean number of 3.5 boluses, while the MNRs averaged .6. This is in accord with previous results established on the strains (Broadhurst, 1960). Open-field defecation also appears to have reduced the number of fecal boluses excreted on Days 5-8 in the home cage in the MR strain but not in the MNR.

Discussion

The differences in home-cage fecal weight between the strains are indeed in the direction expected if an explanation of the open-field defecation scores in terms of normally occurring differences in available fecal material was feasible. However, there are two main reasons why this does not appear to fit the data. First, the score taken in the open-field test is of the number of fecal boluses that are excreted in a 2-min period, regardless of their weight. If the home-cage data in Table 1 are considered again, it can be seen that, in fact, the MNR strain excretes more fecal boluses in the home cage than the MR strain, although the average weight of the feces is less. This is clearly evidence against the hypothesis that home-cage and open-field defecation are linked in the two strains in simple fashion.

Secondly, if the weight of fecal boluses excreted in the home cage is the major determining factor of open-field defecation, it is difficult to see how the differences depicted in Table 1 could account for the differences in open-field defecation. For example, if the amount of fecal material available at any one time is considered, then, to obtain an estimate of strain differences, it is necessary to apportion the total weight excreted over a 24-h period. Thus, if fecal material reaches the rectum regularly over a 24-h period, then, to obtain the amount available in a stress situation, the total amount should be divided by 24. If this is done, the hourly difference between the strains is .025 g. This amount is equivalent to less than a single normal bolus. As the difference in open-field defecation between the strains is in the order of 3-4 boluses, it is difficult to explain such a large difference in the above