Open-field behavior of C3H mice: Effect of size and illumination of field

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Open-field activity and defecation scores were obtained from C3H mice tested for 4 days in fields of three different sizes and two illumination levels. Overall activity was greatest in the largest field and decreased as the fields decreased in size. High illumination reliably decreased activity in the larger fields, but not in the smallest field. Size of field affected defecation scores for male, but not for female, Ss; illumination level had no reliable effect for either sex on any of the three field sizes. The results suggest that contradictory results reported in the open-field literature may be due in part to lack of standardization of the open-field test rather than to differential effects of prior treatments.

Since the early studies of Calvin S. Hall (1934, 1938), the open-field test has become a widely used measure of the emotional states of a variety of species, ranging from mice to monkeys (e.g., Candland & Nagy, 1969). One of the more obvious reasons for the widespread use of the open-field technique is the ease with which animals of various sizes and species can be placed into an environment not previously experienced, and some basic behaviors measured. Despite the simplicity of the technique, open-field data have not always been consistent. In the literature concerning the effects of early stimulation, the open field has been reported to show increased emotionality, decreased emotionality, or no change at all (Goldman, 1969). Although it may be argued that the early treatments differed in many respects, one possible reason for these disparate results is the lack of standardization of the open-field apparatus in different laboratories. For example, the size of the field has varied from a 7-ft circular arena (Hall, 1934; Candland & Campbell, 1962) for rats to a 9-in. square field for mice (Whittier & McReynolds, 1965). The majority of other studies employed fields with sizes somewhere between these extremes. A second parameter that has been extremely inconsistent is the illumination level of the field, ranging from fields lighted by flood lamps to completely dark fields (Candland & Nagy, 1969). Although recent studies have demonstrated that illumination level

has an effect on open-field behaviors of certain animals (McClern, 1960; Ross, Nagy, Kessler, & Scott, 1966; DeFries, Hegmann, & Weir, 1966; McReynolds, Weir, & DeFries, 1967; Dixon & DeFries, 1968), most Es report illumination levels inadequately and make replication difficult.

The purpose of the present experiment was to determine the effects of varying both the size and illumination level of the open field upon activity and defecation in mice.

METHOD

The Ss were 60 male and 60 female C3H mice born and reared in 19 x 11 x 5 in. polyethylene cages with wire-mesh tops, woodchip 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 but different litter in similar cages until testing. All Ss were maintained in the laboratory colony at $74^{\circ} \pm 2^{\circ}\text{F}$, $50\% \pm 2\%$ humidity, and on a 12-h light-dark cycle.

The apparatus consisted of a 36-in., a 24-in., and a 12-in. square field with walls 12 in. high. All fields were constructed of unpainted Masonite, with the floors of each field divided by black lines into 6-in. squares. Thus, the 36-in. field contained 36 squares, the 24-in. field, 16 squares, and the 12-in. field, 4 squares. In the center of each field, 30 in. above the floor, was a 150-W incandescent bulb regulated by a Type 116B Powerstat variable transformer. The transformer was varied to provide two levels of illumination, 100 fc (140 V) and 1 fc (40 V). A silver-foil-covered reflector was placed above the light source to provide more even illumination in all parts of the field. An air-conditioner provided a steady background noise level of 63 dB.

Prior to testing, Ss were earpunched for identification. The Ss were assigned to experimental treatments according to a modified split-litter design, with no more than two Ss from any single cage or from the same litter receiving the same treatment. Twenty male and 20 female Ss were tested on one of the three sizes of fields; half of each sex were tested under high illumination, the remaining half under low illumination. Beginning at 50 days of age, each S was removed from the home cage and placed in the center square of one of the fields in an arbitrary direction. The number of squares entered and the number of boluses deposited during a 2-min trial were recorded on each day for 4

consecutive days. The field was cleaned between each S tested.

RESULTS

Figure 1 depicts the mean number of squares crossed for each test day as a function of illumination level and size of the field. A four-way analysis of variance with one repeated measure revealed a reliable decrease in total activity as the size of the field decreased ($F = 60.98$; $df = 2/108$; $p < .0005$), less activity under high illumination than under low ($F = 22.22$; $df = 1/108$; $p < .0005$), and a decrease in activity over test days ($F = 27.53$; $df = 3/324$; $p < .0005$). There was no difference in activity between sexes ($F = 0.08$; $df = 1/108$), and no interaction with sex approached significance. Individual comparisons showed that total activity was greater in the 3-ft field than in either the 2- or 1-ft fields, and greater in the 2-ft than in the 1-ft field (all $ps < .01$). Of primary interest in this study was the effect of the size of the field upon differences in activity due to illumination level. Individual comparisons revealed that in the 3- and 2-ft fields, activity was greater under low illumination on each of the 4 test days than under high illumination (all $Fs > 3.44$; $df = 6/324$; all $ps < .005$), while in the 1-ft field, activity differences due to illumination were not reliable on any test day (all $Fs < 1.66$; $df = 6/324$).

The defecation scores were expressed as square-root transformations, with 0.5 being added to each raw score prior to transformation. The mean transformed defecation scores are presented in Fig. 2 as a function of size of field and sex over the 4 test days. The transformed data were analyzed by a four-way analysis of variance with one repeated measure. The results showed a reliable main effect for size of field ($F = 3.09$; $df = 2/108$; $p < .05$), that females defecated more than males ($F = 13.37$; $df = 1/108$; $p < .0005$), and that defecation increased over test days ($F = 39.75$; $df = 3/324$; $p < .0005$). Illumination level had little effect upon defecation ($F = 1.09$; $df = 1/108$). Inspection of the left panel of Fig. 2 indicates that the decreased defecation score in the 3-ft field is due largely to male Ss. Individual comparisons showed that males defecated less in the 3-ft field than in either the 2- or 1-ft fields ($Fs > 5.88$; $df = 2/108$; $ps < .005$), while defecation did not differ between the 1- and 2-ft fields ($F = 0.27$; $df = 2/108$). Females did not differ in defecation between any of the three fields (all $Fs < 0.13$; $df = 2/108$), although they defecated more than males in all fields (all $Fs > 6.86$; $df = 2/108$; all $ps < .005$).

DISCUSSION

The results clearly support the

Fig. 1. Mean number of squares crossed over the 4 test days as a function of the size and illumination level of the field.

Fig. 2. Mean defecation scores (transformed) over the 4 test days as a function of size of field and sex of Ss.

hypothesis that differences in the illumination level and size of the open field may cause differences in activity and defecation levels unrelated to prior treatments. In the present study, Ss received similar treatment prior to testing, yet overall activity was found to decrease as the size of the field decreased and was greater under low than under high illumination. Of greater importance is the finding that the activity difference due to illumination level was not reliable in the 1-ft field, although highly significant in the 2- and 3-ft fields. These findings suggest that the effects of early stimulation may be reflected when Ss are tested in a field or apparatus of a certain size and illumination (or some variation of other parameters), but not in others. Thus, many discrepancies reported in the early stimulation literature may be due to differences at the time of testing, i.e., apparatus differences in various laboratories, rather than to differential effects of early treatments. A similar argument has been put forth by Henderson (1969) to account for disparate results of prior-treatment experiments by showing the importance of treatment-test and treatment-genetic interactions, which are often ignored.

The differences in overall activity in the three-sized fields also suggest that activity levels do not simply reflect "emotionality," but may also be reflecting exploratory behavior as suggested in open-field studies with rats (Whimbey & Denenberg, 1967; Denenberg, 1969). Presumably the open-field situation increases emotionality in animals because it presents an unfamiliar environment, one that is usually much larger and more brightly lighted than the quarters in which the animals were previously housed. When tested in this situation, the emotion² animal, at least the rat, is defined as the one with low activity and high defecation

(Denenberg, 1969). In the present study, there appears to be a confounding of emotional reactivity and exploration. On the one hand, the mice were less active under the higher illumination, implying that they were more emotional than Ss tested under low illumination, a level similar to the one under which they had been maintained. However, when tested in the 1-ft field, which was only slightly larger than their home cages, Ss were less active than those tested in the larger fields, which, because of their greater size, might be expected to arouse greater fear and, hence, less activity. In terms of exploratory behavior, one might expect greater activity in the larger fields since there was a greater area to explore, and, since the size of the squares used to measure activity was the same in all fields, locomotion from one corner to another in the 3-ft field required five squares to be crossed, whereas the same locomotion from one corner to another in the 1-ft field required only one square to be crossed. Similarly, the decrease in activity over test days could be interpreted as a decrease in exploratory drive with adaptation to the environment rather than to an increase in emotionality over days. From these data, it is clear that other types of measures are needed to differentiate between emotional and exploratory activity. Several Es have begun using physiological measures and correlating these with open-field activity and defecation (e.g., Levine, Haltmeyer, Karas, & Denenberg, 1967).

The defecation data in the present study are also of interest. For all groups, defecation increased from the first to the last test days, in contrast to rat data that usually show decreased defecation with repeated testing, and is interpreted as reflecting decreased emotionality. However, increasing defecation upon repeated exposure to novel environments has been reported for mice of other strains

and ages (Bruell, 1963; Collins, 1966; Candland & Nagy, 1969) and appears to be a major species difference between rats and mice. If the mice were defecating as an emotional response, one would infer that the mice were becoming more emotional over test sessions, despite the decrease in activity, which might be regarded as an index of adaptation and decreased emotionality. Bruell (1963; 1969) has suggested that defecation in mice may be a territorial marking response to indicate certain territories as "off-limits" to other mice. If it is assumed that mice do become less emotional with repeated exposure to an environment, this would imply that they begin to mark their territory as they become more familiar with it and less fearful. If this were the case, an increased emotional state would be expected to suppress defecation. Some evidence for this hypothesis is provided by the males in the 3-ft field defecating significantly less than males in either the 2- or 1-ft fields. However, since the open field is generally considered a mildly noxious situation, these findings do not rule out increased defecation as an emotionality response to more highly noxious situations. It is possible that the defecatory response is bimodal for mice. Under high levels of fear, high defecation may represent high emotionality. At intermediate levels of fear, defecation may be low, as shown on the first days of testing in the present study. With further decreases in fear, as might be expected with repeated exposure to the field, defecation may begin to increase, but now reflecting a territorial-marking response rather than an emotional response.

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NOTE

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The effects of vagotomy on the water intake of the white rat*

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Unilateral, cervical vagotomy was found to reduce significantly the water intake of rats under conditions of ad lib food and water intake and during food deprivation. Vagotomy had no effect on hypovolemic drinking produced by polyethylene glycol injection. In addition, animals with right cervical vagotomies showed a significantly greater urine output than did controls or animals with left cervical vagotomies.

It has become increasingly apparent in recent years that no single physiological mechanism can account for drinking

behavior under all of the varied conditions that give rise to an increased water intake. For example, Cannon's (1918) xerostomic, or dry mouth, theory of thirst was based largely on the observation that desalivate dogs show an excessive water intake when

placed in a warm environment. It is probably true that an excessively dry mouth serves as a stimulus for water intake, but this appears only under special conditions. Cannon's result is complicated by the fact that the profuse salivation of dogs under heat stress, coupled with hypernatremia, serves as a thermoregulatory function and thus may be totally unrelated to mechanisms regulating water balance. Also, salivarectomized dogs (Fehr, 1862; Montgomery, 1931) evidence no excessive water intake over periods of weeks at normal room temperature.

Desalivation in rats, on the other hand, does lead to an excessive water intake when the animals are feeding on a dry diet (Epstein et al, 1964; Vance, 1965). In this case, the increased "thirst" is associated with an exaggerated prandial drinking, the animals using large amounts of drinking water to facilitate the swallowing of dry food.

Quite apart from these oral factors is the elicitation of drinking by osmotic stimuli, notably, hypertonic sodium-chloride solutions. Whether injected peripherally (Gilman, 1937) or centrally (Andersson, 1953; Andersson & McCann, 1955), hypertonic sodium chloride elicits a prompt and prodigious intake of water. Recently, however, it has been possible to separate this sodium, or hypernatremic, thirst from other dipsogenic conditions. There is an abolition of sodium thirst in animals with bilaterally-transected olfactory bulbs, but no disturbance of water intake in ad lib feeding, during food deprivation, or during hypovolemia produced by sodium depletion (Vance, 1967). Blass (1968) reported a similar absence of sodium thirst in animals with frontal-pole lesions, but no disruption of normal water intake or hypovolemic drinking produced by polyethylene-glycol injection. While Blass found no change in sodium thirst with olfactory-bulb section (there is thus conflicting evidence as to the exact anatomical structures involved), it is clear that the integrity of some anterior forebrain structure is necessary for sodium thirst but dispensable for water intake in response to normal dehydration and hypovolemic thirst. Since the "osmoreceptor" mechanism of hypernatremic thirst is evidently not involved in these last two cases, it is of interest to consider alternative mechanisms mediating "normal" and hypovolemic thirst.

At present, one possible candidate is some type of volumetric mechanism. It is well known that reduction of the extracellular volume by bleeding (Fitzsimons, 1961), by sodium depletion (Holmes & Cizek, 1951), or by

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