Spatial interaction between cage and test environments: Position preferences of young isolated and pair-housed rats

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This study further investigates the relationship between cage size and activity in the open field. Male hooded rats were housed at weaning either individually or in pairs in small, medium, or large cages and observed after 15 days in an apparatus providing a choice between three environments of identical construction and dimensions to the different home cages. Three experimental conditions were employed. Both isolated and pair-housed rats were tested alone in the unfamiliar apparatus and some isolated rats were first habituated to the apparatus. Time spent in each size apparatus component was recorded. The results failed to explain earlier findings of a positive relationship between open-field behavior and cage size in isolated rats, in terms of spatial preference. Isolated component preferences were inversely related to cage size, while pair-housed preferences were positively related to cage size. Component preference was not related to component area or perimeter distance. The implications of this spatial behavior for exploration and emotionality theories are discussed.

The search continues for an acceptable theoretical model of rodent behavior in the open field (e.g., Archer, 1973, 1975); one possible approach to the problem is to relate home cage variables to behavior in this setting.

Although the influence of caging on the behavior of laboratory rodents has been acknowledged, investigations have centered on the social parameter (Morrison & Hill, 1967; Moyer & Korn, 1965; Thiessen, 1964). Besides gross manipulations to distinguish between drive or related theories of motivation (Bronfenbrenner, 1968; Lore, 1968; Syme, 1975), environmental aspects of the cage have been ignored.

Some evidence suggests that cage floor area can affect open-field behavior of rats. Morrison (1968) showed that rats housed alone in large cages (45.7 × 36.8 × 22.8 cm) tended to be less "emotional" (i.e., moved around the field more) than animals housed alone in small cages (17.0 × 24.0 × 17.0 cm). The difference, however, was not significant. Syme and Hughes (1972), in a further investigation of this effect, tested rats housed alone in small (15 × 15 × 10 cm), medium (30 × 30 × 20 cm), and large (60 × 60 × 40 cm) cages in an open field of identical dimensions and construction to the large cages. A highly significant positive relationship was found between cage size and movement. In a later study (Syme, 1973), this effect did not occur for pair-housed rats. Manosevitz and Pryor (1975) have,

however, found a positive relationship between cage size and the activity of mice.

While these findings have considerable relevance for the interpretation of open-field data in general, a more specific theoretical question is why different cage dimensions alter activity in the open field. Three possible alternatives can be advanced.

Interpretation 1. Animals in smaller cages have more corner area and therefore become more thigmotaxic. Early research on thigmotaxis (e.g., Fredericson, 1953) would interpret these results in terms of the enhancement of a thigmotaxic or corner-sitting response following exposure to the greater proportion of corner area in the smaller cages. Syme and Hughes (1972) reported more corner-sitting behavior with decreased home cage size.

Interpretation 2. Animals become more "emotional" as their home cage size decreases. This is consistent with the finding that both ambulation and rearing scores were directly related to cage size (Manosevitz & Pryor, 1975; Syme & Hughes, 1972).

Interpretation 3. During early development, the occupants of smaller cages become accustomed to restricted movement and thus move less in the open field. This interpretation would be consistent with Kuo's theory of behavior potentials (Kuo, 1967). Zajonc's mere exposure hypothesis (Zajonc, 1971) could take this logic further by suggesting that animals come to prefer the area to which they have been exposed from weaning. That is, given a choice, animals housed in small cages will prefer small areas and rats housed in large cages will prefer large areas.

The first view is indirectly supported by our earlier finding (Syme, 1973) that pair-housed rats did not show this effect. When housed in pairs, a rat's

activity may be socially rather than solely spatially mediated within the simple cage environment; i.e., in the social condition, much activity is provoked by the imitation of, and response to conspecifics.

The experiment described here was designed to investigate each of these three alternatives, and also to provide some much needed information about the effects of spatial manipulation on the development of behavior in the rat. Subjects were allowed a simultaneous choice of small, medium, and large environments after being housed in either small, medium, or large cages (see Syme & Hughes, 1972). There were three experimental groups: (1) Individually housed rats tested in a novel choice environment. (2) Individually housed rats tested in a familiar choice environment. (3) Pair-housed rats tested individually in a novel choice environment.

The experimental prediction for each alternative was as follows.

Interpretation 1. Thigmotaxis. If the thigmotaxic suggestion is appropriate, all individually "small"-housed rats should show a greater preference for the smallest choice area (which also had the lowest roof) than those animals housed in the medium and large cages. Theoretically, the rats housed in the medium cages should have a greater preference for the smallest area than the rats housed in the largest cage.

Interpretation 2. "Emotionality." If the emotionality hypothesis is correct, animals housed alone in the small cages should not exhibit any particular spatial preference in the novel conditions; their behavior, being primarily governed by "fearfulness," would involve considerable time spent in motionless corner sitting. Since each square component provides a choice of four sitting corners, the random distribution of these isolates would be disturbed only by a bias towards the smallest space, the low roof of which may act as an additional thigmotaxic stimulus.

For the habituated isolates, however, one would expect a decrease in "emotional reactivity" to the test environment with a decrease in corner-sitting behavior. In fact, corner frequency should cease to be the governing factor with greater movement in the test setting; this role would be assumed by floor area, or perimeter distances. Thus habituated isolates from small cages should spend more time in the larger areas than their nonhabituated counterparts.

Interpretation 3. Behavior potentials or mere exposure. If Kuo's and Zajonc's theories are at least partially appropriate, one would expect the animals to prefer the area which provides about the usual degree of activity, or which they have come to prefer through mere exposure. For example, the rats housed in medium cages should prefer to remain in the medium apparatus component longer than those animals housed in large or small cages. This

response should be independent of novelty effects and, therefore, should occur for both the habituated and nonhabituated individually housed rats. However, since the activity of the pair-housed rats is primarily socially mediated, these animals should not show the effect.

METHOD

Subjects and Apparatus

The subjects were 60 male hooded rats (N.Z.B.W.S.) weaned and housed differentially at 25 days of age in small (S), medium (M), and large (L) cages (Syme & Hughes, 1972). Fourteen subjects were placed alone in small cages, and three pairs were similarly housed. The same procedure was followed for rats in the medium and large cages. Food and water were freely available and the animals were maintained on a reversed light schedule. Cages were cleaned once a week. The subjects were not otherwise handled. The apparatus consisted of three boxes of identical size, construction, and color as the cages housing the subjects (S, small; M, medium; L, large). However, in opposite walls of each box, a 5 × 5 cm square was cut out in the middle of the bottom edge, such that a rat could run through adjacent boxes. The squares of wood removed were retained so that the boxes could be fitted together in three combinations: A (S-M-L), B (S-L-M), and C (M-S-L). These combinations are shown in Figure 1. Wire gauze lids were placed over the tops of these boxes during testing. These were painted white underneath and black on top to minimize external distraction. Two 22-W fluorescent lamps were suspended 1 m above the apparatus to illuminate the three boxes equally. The observer sat on a high stool looking over and down on the apparatus and manipulated two stop clocks, one for each of the outside boxes. Time spent in the middle box was obtained from the total time registered on each clock and the time left in the 600-sec test period.

Procedure

Before testing began, each animal in one individually housed group was habituated for 10 min in the three combinations of the apparatus: A, B, and C. Thus the three treatment groups in this study were: (1) individually housed and habituated to the apparatus, (2) individually housed but not habituated to the apparatus, and (3) pair-housed and not habituated to the apparatus. A fourth possible experimental condition, pair-housed rats habituated to the test environment, was not included since it did not appear to assist in differentiating between the three theoretical alternatives. (In view of the surprising nature of the results of this experiment, however, future studies should incorporate this group within the experimental design.) The order of testing was arranged in an incomplete block design (Cox, 1958). This ensured that, over each of the 3 testing days, each animal was tested in a different apparatus combination.

When the animals were 40 days old, they were each observed, as described above, in each condition for a 10-min test period. Nine "time" measures were thus obtained for each subject, three for
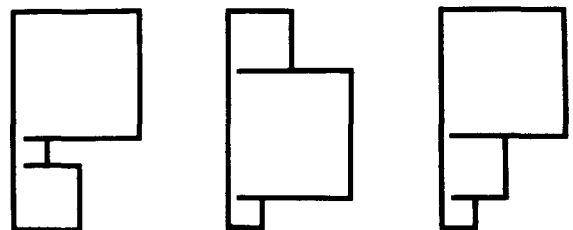

Figure 1. A diagrammatic representation of the three combinations of the box components of the spatial choice apparatus.

Table 1
Means and Standard Deviations of the Time Spent (Total Possible 1,800 sec) Over the Three Testing Sessions
for Each Animal in Each Condition

Test Conditions		Isolated						Pairs		
		Pretest Experience			Pretest Experience			Pretest Experience		
		Nonhabituated Cage Size			Habituated Cage Size			Nonhabituated Cage Size		
		Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
Small	Mean	620.7	523.1	656.2	596.4	424.0	779.6	704.3	480.2	615.5
	S.D.	94.6	47.6	68.5	244.3	98.1	250.5	244.8	46.3	242.3
Medium	Mean	627.6	470.0	702.4	714.5	451.9	633.6	684.2	491.8	624.0
	S.D.	72.4	57.8	65.4	249.9	96.8	242.7	98.9	39.4	238.6
Large	Mean	715.7	490.6	593.7	654.9	495.3	649.8	494.7	555.5	749.8
	S.D.	103.6	41.1	91.6	245.5	457.3	317.7	69.7	73.4	72.8

each box size in each order. The subjects were immediately placed from their home cage into the middle box of the apparatus for all testing. All animals were tested individually.

RESULTS

The means and standard deviations of the times spent by each group in each area summed for the three testing periods are shown in Table 1. It is immediately apparent that there were large differences in variability both between conditions for the isolated subjects and between groups for the paired subjects. For example, the standard deviations for the S and L scores for the habituated isolates were always more than twice those of the corresponding nonhabituated rats. Because of these large differences, a nonparametric analysis was used. All probabilities are two-tailed.

Interpretation 1

The hypothesis that animals housed alone in S cages should show a greater preference for the smallest apparatus component, or testing area, was investigated as suggested in the introduction. The results for the S, M, and L rats were compared by pooling the scores from both isolated groups and calculating a Kruskal-Wallis one-way analysis of variance. The resultant value (H = 8.97, p < .02, MdnS = 603 sec, MdnM = 642.5 sec, MdnL = 685 sec) was significant. However, as the medians show, contrary to Interpretation 1, the animals housed in larger cages tended to stay longer in the smallest enclosure.

Interpretation 2

The "emotionality" hypothesis, that the habituated S rats should spend more time in the large arena than naive S rats, was tested by comparing the two groups with a Mann-Whitney U test. The value obtained was not significant (U = 14, p > .05, MdnH = 708 sec, MdnNH = 680 sec) even though the medians differed in the appropriate direction.

Interpretation 3

The third hypothesis predicts a similar outcome to the first. But while the first hypothesis demanded only that rats housed in S cages acquire a greater preference for the small arena, the present hypothesis requires that each housing dimension result in a preference for that size test arena. In attempting to evaluate Interpretation 1, we compared small arena preferences over the three housing conditions for both isolated groups combined. Although significant effects were found, these were in the opposite direction to those expected.

A second suggestion was, however, that these effects should not occur for the pair-housed rats whose movement patterns are socially rather than solely environmentally determined. The preference times for the small test arena were therefore compared for S, M, and L pair-housed rats using a Kruskal-Wallis one-way analysis of variance. This was significant (H = 7.87, p < .02, MdnS = 572 sec, MdnM = 640.5 sec, MdnL = 489 sec); on this occasion, however, the L group spent significantly less time in the small arena than either of the other two groups (Mann-Whitney U L/S = 5, p < .05, U L/M = 2, p < .01). Caging therefore had opposite effects on spatial preference for pair-housed and isolated animals.

To establish whether this difference could be demonstrated absolutely, a Mann-Whitney U test was calculated between the 14 isolate scores and the six pair scores for smallest arena preference of L rats (U = 12, p < .02).

The relationship between cage size and preference for the large test arena was investigated for the isolates as for the small arena scores. Like these preferences, the Kruskal-Wallis one-way analysis of variance was significant (H = 10.51, p < .01, MdnS = 701 sec, MdnM = 687 sec, MdnL = 581 sec); also, the isolates housed in the S and M cages spent more time in the large arena than did those housed in the L cages. There was no signifi-

cant difference between conditions for the pair-housed rats (H = 2.68, p > .05, MdnS = 714 sec, MdnM = 643 sec, MdnL = 740 sec). The data for the M arena choices were not analyzed because of their ipsative relationship with the S and L choices.

General

Current theories of exploratory behavior might predict that absolute times spent by naive rats in each arena would be proportional to either floor area or perimeter distance, since rats spent most time at the perimeter of an open field. A comparison between the means of the M and L arenas, however, showed no such relationship, although L was consistently occupied more frequently than M. Thirty-three of the 39 subjects spent more time in L than in M (χ^2 = 18.69, df = 1, p < .001). But the differences between the means were nowhere near those expected either from an area hypothesis (L = 4M) or the perimeter hypothesis (L = 2M). The best model from the present data would predict a relationship of L = 1.31M.

The S cage data was omitted from this analysis owing to the confounding with thigmotaxic behavior.

DISCUSSION

The results do not clarify the antecedents of the positive relationship reported earlier between cage size and the open field activity of isolated rats (Syme & Hughes, 1972). It appears that this relationship is not caused by the spatial dimensions of the cage producing an environmental size preference. Also, the rejection of Interpretation 2 in this experiment suggests that increased "emotionality" is not responsible for the lesser activity of the rats housed in small cages. However, replication of these findings under a variety of experimental conditions would be desirable.

It is possible that the cage size/activity relationship is merely a nonspecific one. That is, isolated animals become habituated to a low degree of movement if consistently restricted by their cage environment and thus move less in the open field. This hypothesis concurs with an earlier study (Syme, 1975) where the environmental restriction of a specific response in rats (rearing on the hind legs) resulted in a decreased level of rearing in the unrestricted open field.

As well as providing information on the particular problem which prompted this work, the present study has generated interesting basic data concerning the spatial behavior of rats. First, in contrast to most behavior of laboratory rodents, the spatial preferences of our rats showed *greater* interindividual variation *after* habituation. This may reflect a relatively consistent effect of novelty-induced arousal, which dissipates to reveal individual differences in spatial preference once familiarity is achieved. It is significant, though, that there was a large difference in variability within conditions for the pair-housed rats, which means that this explanation cannot be universally adopted. This variability difference warrants further investigation.

Secondly, the isolated rats housed in small cages preferred the largest test arena more than their large-housed counterparts. Alternatively, the isolates from large cages preferred the small arena more than those housed in small cages. These results may simply demonstrate a preference for novel space, i.e., novelty of spatial dimensions produces a similar response to that for other novel stimuli. But, as shown previously (Syme & Hughes, 1972), this does not imply that the isolates from small cages prefer the large arena because they can move around it as may be suggested by activity-drive theories (Baumeister, Hawkins, & Cromwell, 1964).

The pair-housed rats provide a surprising contrast to the isolates in that they showed evidence of a positive relationship between their own cage-size arena and their spatial choice. This was most clearly demonstrated in the small-arena preference times. In discussing the third interpretation, we suggested that this could provide some "spatial" support for the mere exposure hypothesis (Zajonc, 1971). But since this was significant only for the small test arena for the pair-housed rats, the first hypothesis (thigmotaxis) is equally acceptable.

However, the major point of interest is the opposite response for the isolated and pair-housed animals. This may reflect isolation stress (Stern et al., 1960) due to lack of social stimulation with a resultant choice for a contrasting environment. Alternatively, the pair-housed rats may be better housed in that they are socially stimulated and thus do not seek environmental novelty. The confined state in the small cages could well accentuate such opposing influences.

Finally, the relatively short time spent by non-habituated rats in the large test arena is notable, since many theories of exploration seem to predict a stronger relationship between arena and time spent "exploring" than observed in the present study (e.g., Broadhurst, 1957). Clearly, the relationship observed in the present study should provoke further research on this phenomenon. Perhaps the rats habituated quickly to the apparatus, making any area/exploration interaction transitory. However, even assuming a random distribution of occupation per area only, there was a remarkably low occupancy of the large test arena.

Despite our failure to discover an unequivocal solution to the problems which motivated this study, the results have raised a number of interesting

questions. Further investigation of individual differences in the spatial behavior of laboratory rodents may even promote a reevaluation of the popular concepts of exploration and emotionality. While the uniform test settings used to investigate "emotionality" and "exploration" have the advantage of producing controlled data, they may be oversimplistic in spatial terms. Research in this area should concentrate more on the spatial origins of subjects and be prepared to employ increasingly diverse environments.

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