EFFECTS OF CAGING ON THE BEHAVIOUR OF RATS IN THE OPEN FIELD

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CONTENTS

Abstract

Social and spatial characteristics of the cage environment interact to influence the behaviour of rats in the open field. Caging which does not allow adequate movement may impede maturational and social development in rats, with a resultant influence on their locomotor activity in the test situation. This implies that activity measures may not always be an accurate measure of emotional reactivity, or perhaps, that emotional reactivity may not be an appropriate description of all behaviour in the open field. The results are discussed with reference to psychopharmacological and developmental research, where activity measures are often used.

This study illustrates the prevalent ignorance and naivety of many experimenters with regard to both the caging and social behaviour of laboratory rats, and discusses a number of variables which are frequently neglected in the literature.

CHAPTER 1

INTRODUCTION

Over the past decade the social environment of the laboratory animal has caught the attention of many people concerned with the search for optimal cage conditions. "Experimental animals .. present a complex of varied problems. But in one respect they are all alike. Theirs is a man-made ecology. Their numbers, distribution, and environmental adventures are not an intrinsic problem, as those of wild animals remain to some extent, but a problem in human sociology; for they are determined by human needs and decisions." [33].

These human needs have dictated the emergence of large-scale industries which are devoted entirely to the breeding and caging of animals for human use. But "human" need not be confused with "humane"! Economising on space in the animal house seems to be the main criterion for determining cage dimensions. Most caging recommendations, for instance, state only that adequate movement of the animal must be allowed for.

Porter <u>et al</u> (1970), in a review of existing caging standards for rats and mice, found no reports of critical investigations of area requirements for either animal. But before this review several studies had indicated that social conditions within the cage environment could alter the behaviour of laboratory rats and mice in, for example, the open field.

The common practice of keeping one animal in a cage and isolating it from normal social contact with others was criticised, on both physiological and behavioural grounds, by such people as King, Puh Lee & Visscher (1955); Yen, Stanger & Millman (1958); Weltman <u>et al</u> (1962, 1968); and Hatch <u>et al</u> (1963, 1965), with such strong statements as: " - the routine practice of housing animals singly may readily nullify or modify anticipated experimental conclusions."[40]. Yet few studies, especially those concerned with developmental stimulation effects (Levine, 1967) state housing conditions as a possible artifact in their experimental results, while it has been established that the isolated animal behaves in a more "emotional" or "frightened" manner than does the group-housed animal.

Some studies on population density have also found that group-housed animals are less emotional than isolated animals (Ader, Kreutner & Jacobs, 1963;

Essman, 1966; Morrison, 1967; Thiessen, 1964), and it has been reported that animals tested in small groups show less emotionality than individual animals in the same situation (Davitz & Mason, 1955; Morrison & Hill, 1967). But, as Morrison & Thatcher (1969) point out, none of these studies have attempted to relate emotionality to population density in a systematic fashion. Their own study indicated that animals reared in varying degrees of population density reacted differently in response to being tested in the open field, and they claim that these differences indicate decreasing emotionality with increasing population density.

These results, though, may possibly be accounted for by the fact that cages of the same size were used for all groups. As Bell, Miller & Ordy (1971) show, in a recent study, this is an example of the crowded-group paradigm (CG) which, they say, confounds group size and crowding, while the density-group paradigm (DG) uses cage floor space that is proportional to group size.

Studies using CG typically report isolated animals to have heavier adrenals (Thiessen, 1964) and to be more active (Essman,1966) than aggregated animals, while studies using DG have found that isolated animals have lighter adrenals (Bailey, 1966) and are less active than aggregated animals (Christian, 1955). Bell <u>et al</u> thus claim that locomotor activity may be an interactive effect of group size with living space.

The study reported here investigates both the area requirements of the laboratory rat and possible interactions between group size and living space, using cages of three different sizes and testing the animals in both small and large open fields. Because of the diversity of studies relevant to this thesis part of the literature review is incorporated in the discussion of results.

CHAPTER 2

METHOD

Subjects

The subjects were 64 female 30 day old hooded rats caged as in Table 1. Food and water were provided ad lib. Throughout the study all subjects were handled by the E only.

Table 1.

Symbol	Cage size	Number of cages	Rats in each cage
S_1	small	6	1
S_2	"	3	2
S_3	"	2	3
S_4	"	1	4
M_1	medium	6	1
M_2	"	3	2
M_3	"	2	3
M_c	"	1	18
L_1	large	6	1

Apparatus

As indicated in Table 1, cages of three sizes were used. The small cages measured 7 x 7 x 7 in.; the medium cages 18 x 14 x 12 in.; and the large cages 24 x 24 x 24 inches. The small and medium cages were constructed of iron and painted grey and white respectively, while the large cages were constructed of wood and painted brown.

Lighting was cycled with 15 hours of light and 9 hours of darkness. Because of the differing cage construction light was slightly greater in the large cages (with wire-netting over the top) than in the iron cages, which were more enclosed. Although it may be argued that brightness differences between the three cage conditions could have influenced later behaviour, this seemed unlikely; the construction of the iron cages was such as to minimise these differences.

The test apparatus consisted of a small glass-fronted box measuring 12.75 x 9.8 x 10 in. with a wire gauze lid, the floor of the apparatus being divided into four areas of 4.75 x 6.25 in.; and a large open field measuring 24 x 24 x 10 in., the floor of which was divided into 16 squares, each of area 6 x 6 inches. Both fields were painted black with the lines dividing the field painted white. Further discussion of the apparatus is included later in the text.

Illumination of each structure during testing was provided by a 40W lamp situated 22 in. above the centre of the field. After each subject had been observed, all faecal boli were removed and the floor of the apparatus wiped with a damp cloth. Room temperature was maintained between 15 and 20^{o}C as far as possible, though this was higher in the two crowded cages. White masking noise of approximately 40-50db was used throughout testing.

Procedure

A time-sample method was used (Bindra & Blond, 1958) with each subject being left in the field for one minute and then observed, using a buzzer in an electric timer, at 5 second intervals over a 5 minute period. By a comparison of data collected by the E and an independent observer on the performance of two consecutive subjects under test conditions, inter-observer reliability of less than 5% error was established.

Twenty-one days after being placed in their selective cage conditions the rats were tested in the small field. Samples of 6 rats were selected from the medium cage containing 18 animals. The behaviour observed at the sound of the buzzer was of four categories:

1. Ambulation (a) was recorded if all four limbs of the animal were moving on the floor of the field when the buzzer sounded.

2. Rearing (r) was recorded if both front limbs of the animal were off the floor of the field when the buzzer sounded.

3. Immobility (i) was recorded if the animal was moving no limbs when the buzzer sounded.

4. Grooming (g) was recorded if the animal was rubbing or scratching any part of its body with the two front limbs, when the buzzer sounded.

The number of squares crossed over the whole period (N) and defaecation (the number of faecal boli deposited) were also recorded.

After 48 days the animals were tested again in the small field and 9 days later they were tested in the large field. Seven days later the animals were tested again in the large field, this time in the presence of another female rat (chosen randomly from the main animal house) which had been left in the field for half an hour to habituate it to the environment. Reasons for retesting in the same test condition will be discussed in the first section of the results, which considers constancy of behavioural indices in the field situation.

CHAPTER 3

RESULTS

Probabilities obtained, using the Kruskal-Wallis one-way analysis of variance by ranks to determine whether the nine independent samples (cage groups $S_1 - M_c$) were drawn from different populations, are shown in Table 2.

Table 2.

Test Conditions	Number of squares crossed	Ambulation	Immobility	Rearing
SMALL FIELD (1)	0.05	N.S.	N.S.	N.S.
SMALL FIELD (2)	0.001	0.001	0.02	0.01
LARGE FIELD	0.05	0.01	N.S.	N.S.
LARGE FIELD + ANOTHER RAT	0.05	N.S.	0.01	0.001

Very little grooming was observed during the testing so this index was not analysed. Defaecation, also, seldom occurred during the testing sessions. The significance level adopted throughout this study is $p = 0.05$. Probabilities greater than 0.05 are quoted in all results as N.S.

Using the Mann-Whitney U test, frequencies of the number of squares crossed, ambulation, immobility and rearing obtained in the four testing conditions were compared. From Table A in the Appendix one can, for example, find the probability that the frequency of ambulation observed for the small cage containing one animal (S_1) was stochastically larger than that for the medium cage containing one animal (M_1) in the small field and second testing session equals 0.001. The table of results in the Appendix contains all results (including those found not significant in Table 2) to facilitate references further on in the text.

These two tables (Table 2 and Table A in the Appendix) form the basis for the following grouping of results, concerned with the effects of:

1. The constancy of the behavioural indices (number

of squares crossed, ambulation, immobility and rearing) in the test situation, over time.

2. Isolation

3. Crowding

4. The crowded-group paradigm

5. The density-group paradigm.

1. The constancy of behavioural indices (number of squares crossed, ambulation, immobility and rearing) in the test situation, over time.

The Sign Test, for two related samples, was used to determine whether there was any significant difference in the behaviour of the nine groups of animals between the two testings in the small field on the four behavioural indices: number of squares crossed, ambulation, immobility, and rearing. Animals reared alone in the small, medium, and large cages were the only subjects to show any significant difference in behaviour upon retesting in the small field after 48 days.

The median value for the number of squares crossed, for the isolated animals reared in small cages changed from 35, on the first test, to 28 on the second, and the significance of this change, measured by the Sign Test, was $p = 0.03$. Similarly, the median value for immobility scores changed from 19 to 15; the significance of this change being $p = 0.01$.

Considering the isolated animals reared in medium cages, the median value for ambulation scores decreased from 20 to 12.5, the significance of this change being $p = 0.01$, and the median value for rearing scores increased from 22.5 to 30.5, with the same significance value. Animals isolated in the large cages also changed in their behaviour on the rearing index, the median value for this index rising from a median value of 13.5 to 26.5, with a significance of $p = 0.01$.

These results indicate that either the selective caging of the isolated animals was having some behavioural effect over time, while animals in the other cage conditions showed little such effect; or that there were differential rates of adaptation to the test situation by the different groups of animals.

But the results do not support the validity of comparing median values obtained for the different cage groups on the behavioural indices in the two test conditions without regard for the distribution of the scores contributing to these measures of central tendency. All cages were marked in this study so that each subject functioned as its own control. Use of the Sign Test enables a direct comparison of these distributions.

2. Isolation

Reference to Table B, in the Appendix, indicates significant differences in the behaviour of animals, in the open field, which were reared in cages of different spatial dimensions.

Animals reared in small cages, alone, showed the greatest number of significant differences (10) on all behavioural indices. With respect to the number of squares crossed in the large field when another animal was present, the isolated animals reared in small cages crossed significantly less squares (median = 85) than did both the isolated rats reared in medium cages (median = 112) and the isolated rats reared in large cages (median = 113). Considering the ambulation scores obtained, the isolated rats reared in small cages and tested in the small field (second test) ambulated significantly more (median = 24) than did both the isolated animals reared in medium cages (median = 19.5) and in large cages (median = 19,5).

Looking again at the immobility scores obtained in the large open field in the presence of another animal, the isolated animals reared in small cages were significantly more immobile (median = 23.5) than were both the isolated animals reared in medium cages (median = 15) and isolated animals reared in large cages (median = 16.5). In the small field, the animals isolated in small cages reared significantly less (median = 27.5) than did the animals isolated in medium cages (median = 30.5), and in the large open field they reared significantly less (median = 20) than both animals isolated in medium cages (median = 30) and in large cages (median = 29).

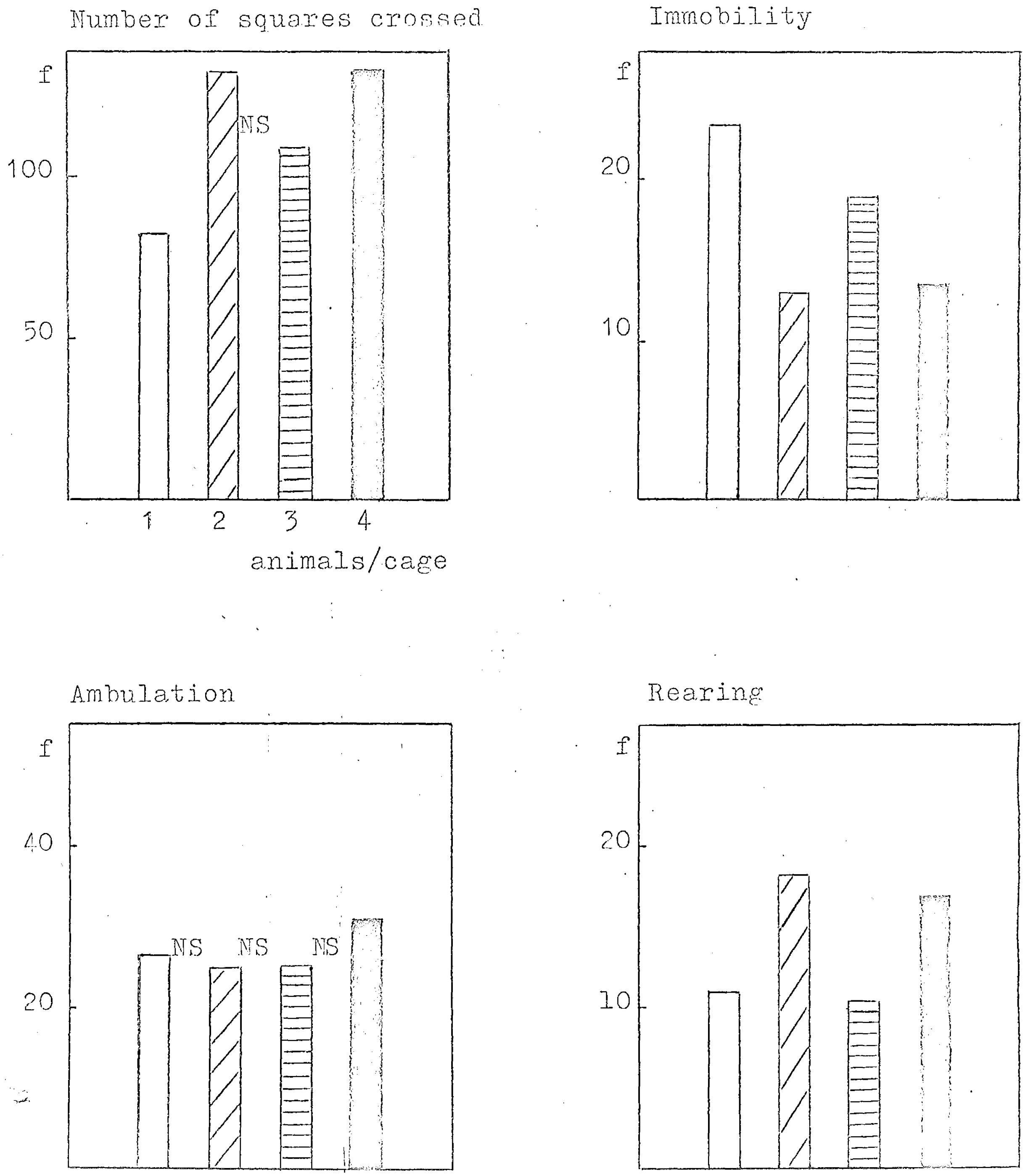
As may be seen from the figures quoted, no significant difference occurred between the behaviour of animals reared in the medium and large cages. This is substantiated by the contents of Table B in the Appendix. Looking at the results for isolated animals, overall, in terms of the conventional indices of "emotionality" there was no clear difference between the three groups of animals.

Similarly, no definite trend occurred in the activity of isolated animals, when the number of squares crossed and ambulation scores obtained by these animals are compared with the scores obtained on the same indices by animals reared in groups. An exception occurred in the behaviour of the different groups of animals in the large open field in the presence of another animal.

Figure 1 illustrates the changing pattern of behaviour of animals housed according to the crowded-group paradigm in the small cages. It can be seen that both the isolated animals and those housed three to a cage show a pattern of behaviour characterised by a low number of squares crossed, high immobility, and little rearing. All the differences between groups in Figure 1 are significant, except for that between the groups containing two and three animals on the index of number of squares crossed, and between all groups on the index for ambulation. An interpretation of Figure 1 will be given later in the text.

Figure 1.

Median frequencies obtained by animals reared in small cages in groups of 1, 2, 3 and 4 animals on four behavioural indices in the large open field in the presence of another animal.



3. Crowding

A number of enquiries were sent to major animal breeding and cage manufacturing centres in this country, the United States, and Britain, asking two questions:

i) What did they consider to be the "ideal" space requirement of the laboratory rat?

ii) What criteria were used to determine such a requirement?

The replies received varied considerably in their answers to the first question, with areas per rat ranging from 16 square in. to 140 square inches. Criteria used for determining such areas were legislation (U.S.), intuition, and the provision of adequate movement for the animal. No answers considered the social requirements of the animal; as did none of the animal care manuals and journals referred to.

Crowding, in this study, refers to a violation of the optimal living space of the animal. Because nobody seems to know what this optimal living space is, the number of rats placed in the small and medium cages were determined by the amount of space the animal had to move around in. The area allocated per animal in the small cage containing four animals was 12.25 square in., and the area allocated per animal in the medium crowded cage was 14 square inches. The small cage containing three animals provided 16 square in. per animal, which is the minimum area allocation stated by the Animal Welfare Institute, in New York.

As with the isolated animals, differences occurred in the behaviour of animals, reared in crowded conditions in cages of different size, in the test situation. The most striking difference occurred in the behaviour of the two groups of animals in the small field, where the crowded animals in the small cage showed behaviour which might be described as "hyperactive" (median number of squares crossed = 40) while the crowded animals in the medium cage exhibited the pattern of behaviour usually associated with high "emotionality" (median number of squares crossed = 19).

Table 3.

The position of the small crowded and medium crowded cage groups in a ranking of the nine groups of subjects on the behavioural indices: number of squares crossed, ambulation, immobility and rearing, where 1 = lowest rank for the lowest median value and 9 = highest rank.

		Number of squares crossed	Ambulation	Immobility	Rearing
SMALL FIELD	S_4	9	9	1	4
	M_c	1	2	7	2
LARGE FIELD	S_4	8	6	1	3
	M_c	7	1	5	9
LARGE FIELD + rat	S_4	8	9	4	5
	M_c	9	1	2	9

S_4 : small crowded cage (4 rats)

M_c : medium crowded cage (18 rats)

The results obtained from a ranking of the nine groups of Ss (lowest rank = 1, highest rank = 9) on the different behavioural indices are shown in Table 3. Some interesting questions raised by these figures in the table will be discussed later in the text.

In the first two test conditions (the small and large fields) the crowded animals reared in a small cage differed significantly from the crowded animals reared in a medium cage on all behavioural indices, suggesting that some sort of caging spatial effect is operating. In the third test condition (large open field with another animal present) there were no significant differences in the behaviour of the two crowded groups.

4. The crowded-group paradigm

The small cages, containing one, two, three and four animals respectively, are an example of what Bell *et al* (1971) call the crowded-group paradigm; where group size is increased within a fixed area. With the medium cages, also, this situation existed with one, two and three animals per cage, providing a comparison between the same groups of animals (for example S_3 and M_3) reared in cages of differing area.

Comparing the median values obtained by the seven cage groups (S_1 - S_4 and M_1 - M_3) on the number of squares crossed and ambulation in the small and large fields, in no case was the activity (the two indices considered together) of isolated animals significantly greater than that for other groups. Bell et al's (1971) study claims that isolated animals caged in this manner should be more active than grouped animals.

Considering the number of squares crossed in the small field, the isolated animals in small cages (median = 28) differ significantly from the animals in the small crowded cage (median = 40) as do both the animals reared two to a cage (median = 32) and three to a cage (median = 27.5). Animals reared alone in the medium cages were significantly more active (median = 31.5) than those reared alone in the small cages.

Ambulation scores obtained in the small field show similar differences among animals reared in small cages. Results obtained in the large open field are not so clear, but still, in no case was the activity of the isolated animals significantly greater than that of the grouped animals. But the animals in Bell's study were caged in groups of 1, 4, 8, 16, and 32 so the effects cited may not be showing up clearly with groups of up to 4 animals only. Their study also used male mice while the present study used female rats; the degree of behavioural correspondence between the two animals in the sort of test situation employed in this study has not been established.

5. The density-group paradigm

The study discussing the density-group paradigm (Bell *et al*, 1971) was published after the present study was completed. However, consideration of the area per animal in the small crowded cage (12.25 square in.) and the medium crowded cage (14 square in.) shows that the requirements of this paradigm are very nearly met in the present study, in that the animals in the two cage conditions have approximately the same area per animal to move around in.

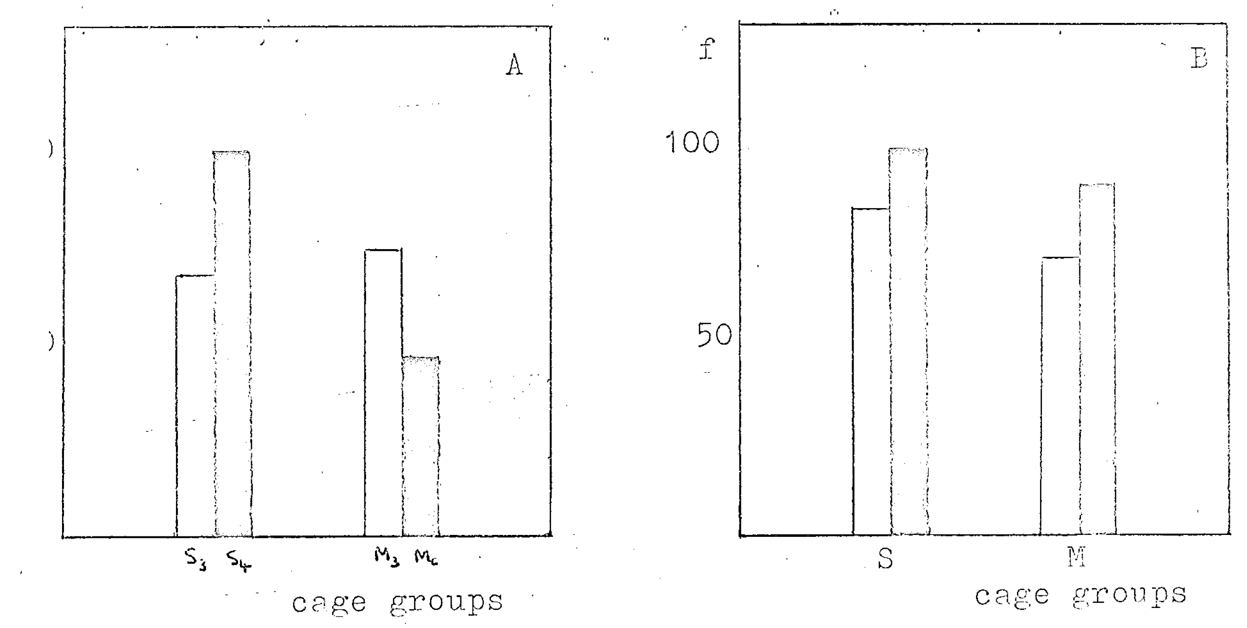
Figure 2 compares the performance of three animals in a small cage (16 square in./animal) with that of four animals reared in a small cage (12.25 square in./animal); also the performance of three animals reared in a medium cage (84 square in./animal) and eighteen animals reared in a medium cage (14 square in./animal), on the two behavioural indices of locomotor activity: number of squares crossed and ambulation.

Significant differences occurred between the two groups in similar cage conditions in all cases (S_3 and S_4, M_3 and M_c). Significant differences also occurred in the performance of the crowded animals from small and medium cages in all cases, in that the number of squares crossed and ambulation decreased between the two conditions. No significant differences occurred in the performance of the other two cage groups, though the results indicate increasing activity.

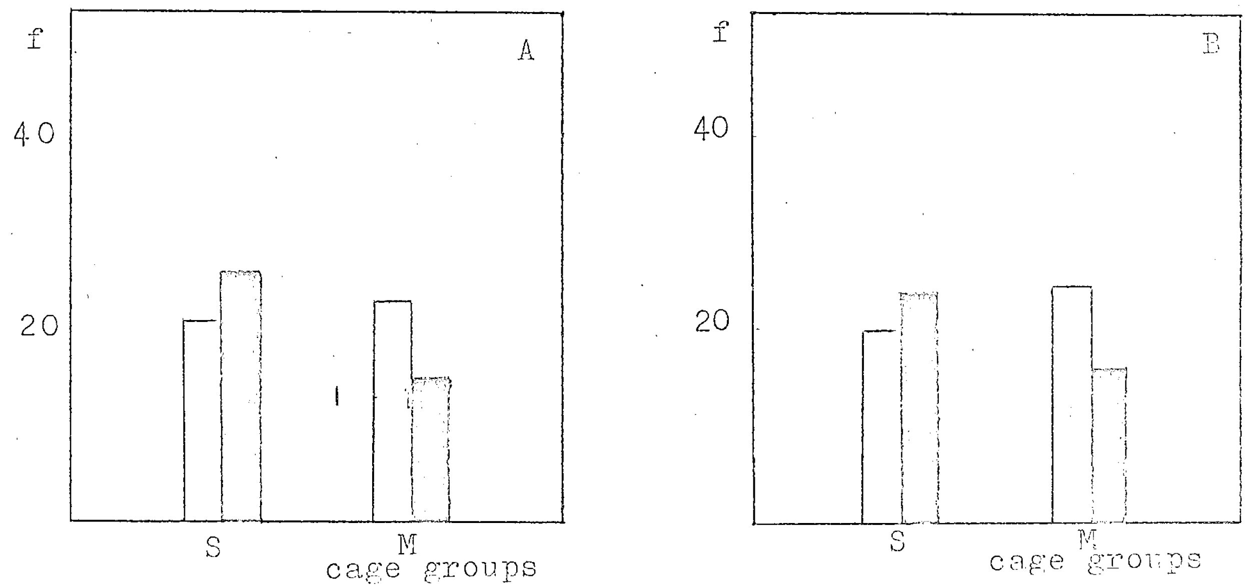
ure 2.

dian frequencies obtained in the small (A) and large (B) fields four groups of animals (S_3, S_4 and M_3, M_c) reared in small and dium cages.

mber of squares crossed



Ambulation



cages containing 3 animals

crowded cages

CHAPTER 4

DISCUSSION

An overall view of the results

The values obtained, using the Kruskal-Wallis one-way analysis of variance, showed that there were a number of significant differences appearing between the different cage groups on the various behavioural indices.

Although it was not originally intended to compare related groups over the same testing conditions, the figures obtained in Table 2, which showed that more significant values were obtained in the second testing in the small field, prompted a closer look at the constancy of these groups' behaviour in the test situation, over time.

It might be expected, from the literature on such social conditions as isolation, that differences between these isolated animals and grouped animals on the behavioural indices considered would increase over time, as the animals in isolation became increasingly "stressed". This appears to be so, looking at the results in Table 2, but further investigation using the Sign Test revealed that, within these significant values only the isolated animals differed significantly in their behaviour in the same test conditions over time. These animals crossed fewer squares, were immobile less, ambulated less and reared more; but all of these changes may be attributed to maturation, since no clear pattern of behavioural change emerged.

Isolation

The isolated caging of animals, as utilised in the present study, had a significant, if ambiguous, effect on behaviour exhibited in the open field. Animals reared alone in small cages showed the greatest difference from all groups on the behavioural indices considered.

It is interesting that no definite trend occurred in the activity of isolated animals, when the number of squares crossed and ambulation scores obtained by these animals are compared with the scores obtained on the same indices by animals reared in groups. Studies using a crowded-group paradigm, in which different size groups are placed in cages of constant size, have usually found that isolated animals were more active than those reared in groups. In no case was such a difference found to occur in the present study, although, considering the medium cage conditions, animals reared two to a cage were significantly more active in the small field than those reared in isolation.

Studies using a density-group paradigm have found that isolated animals are less active than those reared in groups. This design was not incorporated in the present study, although a discussion of both paradigms, as classified by Bell, Miller & Ordy (1971) is presented elsewhere.

A complication arises with the discovery that animals reared alone in small cages act differently in the test situation from animals reared alone in both the medium and large cages. Bell et al, with the observation that living space per se does not significantly alter exploratory behaviour (on slender evidence) do not offer a direct comparison of the behaviour of isolated animals from the two conditions, CG and DG. The present study indicates that this deceptively simple spatial effect warrants further investigation. Results for the two crowded groups support this contention.

Animals isolated in small cages ambulated more and reared less, in both small and large fields, than both the isolated animals in medium and large cages. A plausible explanation for this could be that the small cages offered so little movement to the animals confined in them that maturational processes were impeded. Noting that similar, but generally nonsignificant, differences occurred between the animals kept in pairs in the small and medium cages, it could be that the isolation syndrome accentuated this developmental set-back.

The only conclusion one can make about these results is that the area is not as straight-forward as most of the studies would have us believe. Possible confounding variables such as weaning age and litter size are discussed separately.

Crowding: the use of activity as a measure of emotional reactivity

Reference to Table 3 in the Appendix shows that there is, generally, a lack of correspondence between the number of squares crossed and the ambulation scores obtained by a typical group of rats. The most extreme example of this is seen in the behaviour of crowded rats which, as noted in the results, exhibited "hyperactive" behaviour.

The lack of correspondence could possibly be attributed to the time-sample method used in that it was possible for an animal to run around the perimeter of the field in the 5 second period between the buzzer and thus be stationary when the observation was made (no ambulation; 12 squares crossed). A large number of squares could also be crossed when the animal was rearing and walking on the hind legs; which happened more as the animals matured. The early studies with the crowded animals showed them to be running very quickly with most of the weight on the back legs and the front legs barely touching the floor. Movement was of a darting quality, with the animal covering a large area in a very short time. This was especially marked with the crowded animals from the small cage.

A movement explanation may be appropriate here, in that although, theoretically, the crowded animals in the two cage conditions (small and medium) had approximately the same area per animal to move around in, this was not the case in practice. Crowded rodents, especially when young, tend to aggregate in one corner of the cage, piling one on top of another. "If large numbers are put into one cage, however capacious, they will huddle together, and those underneath will be suffocated." (Worden & Lane-Petter, 1957) Using a large cage this situation leaves much of the cage area free for a few animals to move around in; a situation which never occurred in the small cage where the animals were, from the start, in close proximity. This may explain why there were such clear differences between the two crowded groups early on in the study, in their behaviour in the small field.

Reference to Table 3 shows that, in the small field, the animals reared in crowded conditions in the small cage crossed the highest number of squares, produced the highest ambulation scores, and the lowest immobility scores for all groups. This hyperactivity could have been the result of either spatial restrictions imposed by the cage environment or an attempt to compensate for the heat loss consequent upon removal from physical contact with the other animals (Hatch et al, 1965). On the other hand, the animals from the medium crowded cage exhibited behaviour associated with high emotional reactivity, in that they crossed the lowest number of squares, had the second lowest ambulation score, and a high immobility score as compared with all other groups.

According to existing information (Christian, 1961; Thiessen, 1964; Morrison & Thatcher, 1969) the two groups of crowded animals (defined in terms of unit space per animal) should exhibit similar physiological and behavioural abnormalities. Only Bell, Miller & Ordy (1971), in their formulation of the density-group paradigm have discussed the possibility of dissimilarity in the behaviour of the two groups, though their study incorporates no such comparison as that in the present study between the animals crowded in the small and medium cages.

Over time, perhaps, behavioural similarity may occur (see Table 3 in the Results) when one looks at the ranks obtained by the two groups in the large field. Both groups show a high number of squares crossed, though the ambulation scores for the crowded animals in the medium cage are still less, as are the immobility scores for the animals from the small cage. The animals from the medium cage now show the highest rearing score, which may be an effect of greater physical maturation. The rise in the number of squares crossed may be explained, as earlier, by the animals rearing freely and walking on the hind legs; another result of greater physical maturation encouraged by greater movement opportunities in the cage environment.

Obviously these measures of "activity" do not result only from emotional reactivity. As discussed with reference to litter size, group size, and cage dimensions, many factors can distort the emotionality criterion, especially in developmental studies where these factors have the greatest impact on the immature animal. One cannot assume that a manic person is not emotional. Similarly, a rat reared in crowded conditions and deprived of movement, which subsequently behaves abnormally in the test situation cannot be assumed to be emotionally unreactive.

It would be interesting to investigate further Bell _et al_'s DG paradigm in the light of their criticism of CG to see more precisely, using cages of the correct dimensions to allow equal area per animal, what the relationship is between crowding in different spatial conditions. As discussed below, the present study suggests that DG may hold for crowded groups; but it questions the alleged confounding of group size and crowding. It does give theoretical support to the idea that relative space is an important variable to be considered in the study of the "sardine syndrome" (Russell & Russell, 1970). In effect, the caging of animals in zoos presents another aspect of the problem.

The density-group paradigm

Criticisms of Bell, Miller & Ordy's conception of the density-group paradigm have been presented elsewhere in this discussion. However, in the present study an interesting, if approximate, variation on the paradigm yields results which agree, in some respects, with theirs.

Bell <u>et al</u> found that, for subjects (mice) in the DG housing, activity decreased as group size increased 1 - 16, and then increased with further increases in group size. The present study, comparing the small (4 animals) and medium (18 animals) crowded cages, which approximately fit the DG paradigm, found that the activity of the animals in both small and large fields was consistently less for the animals from the medium cage than that of the animals from the small cage.

But results gained in the present study do not conform with Bell <u>et al</u>'s conception of the CG paradigm, where the animals are supposed to show decreasing activity with increasing group size. Comparing the results for animals housed 3 and 4 to a small cage, it is seen that the rats from the latter group were considerably more active in the test situation than were the rats housed 3 to a cage.

<u>Gregarious behaviour in the large open field</u>

The presence of an unfamiliar stimulus rat has been found to have a selective effect on the behaviour of animals, reared in different social conditions, in the open field (Latané, Cappell & Joy, 1970). Reference to Figure 1 shows that this may have been the case in the present study.

Here, gregariousness was tentatively supposed to be indicated if a group of rats was immobile longer, crossed fewer squares/ambulated less, and reared less than other groups tested in the same way. The median value obtained on these indices was taken to represent the behaviour of the group, as a whole. A tentative comparison was also made between the performance of groups in the large open field in the two conditions; with and without the presence of the unfamiliar animal.

An obvious criticism of the present study is that the possibility of familiarisation, in the open field, was not controlled for. This is true. If the observations made in this part of the study were to make any statistical predictions about the gregarious behaviour of the different groups of animals it should have included a retest session in the field after the testing with the unfamiliar animal had been carried out, to ascertain whether or not constancy in the behavioural indices observed was maintained over time. In this respect the only justification the present study gives is that constancy was found to exist in the small field over time over the first two test conditions. Any conclusions drawn, then, about the effects of housing density on gregarious behaviour are tentative ones only. The following discussion is, in the opinion of this author, valuable only in the incentives it may provide for future work in this area.

When another stimulus rat was present in the test situation the animals reared alone in small cages crossed significantly less squares than did the animals reared alone in both the medium and large cages. This is an interesting occurrence because it might be expected that all these animals, which had had no physical contact with another animal since being placed in isolation at 30 days of age, would have shown similar changes in behaviour upon being placed in this test condition. A possible explanation may be that the small cages afforded little opportunity for the animals to see others, while the rats in the medium and large cages could either claw their way up the metal grill at the front of the cage (medium) or cling to the wire-netting roof of the cage (large) and thus, perhaps, have greater contact with other animals in the room. As Salazar (1968) found, when investigating the gregariousness of young rats, isolation prior to testing considerably affected their sociability!

Stern <u>et al</u> (1960) claim that: "If group housing of animals is considered the 'normal' habitat then the individually housed animals were subject to sensory deprivation". In this context, it was noted during the present study that all the isolated animals "harassed" the stimulus rat in the field, climbing all over her and following her if she tried to move away. Dimond (1970) has observed that animals reared in isolation never experience defeat by another animal, with the result that they never learn submission, are unsocialized, and "aggression occurs at a high level". The behaviour described above, with reference to the present study, might have been aggressive or it might have been due only to excessive curiosity about the other animal. In no case did the isolated animal lead the stimulus rat around the field, whereas with the animals reared in groups this was a frequent occurrence.

Morrison & Thatcher (1969) claim that, in the open field test condition, " - the presence of another has a calming effect for rats housed 1 and 4 per cage, and an arousing or energizing effect on rats housed 16 and 32 per cage." Reference to Table A in the Appendix shows that all animals except those isolated in small cages crossed a higher number of squares, ambulated more, and reared less in the presence of the unfamiliar rat. All of the groups from small cages were immobile longer, while the doubles, trios and crowded animals in the medium cages were less immobile. The present study showed an "energizing effect" only, insofar as the comparison of bulk median values is valid over the two test conditions. There was certainly no indication of Morrison et al's selective results.

As Latané, Cappell & Joy (1970) point out, the presence of an unfamiliar or familiar animal should influence results in this area. "In previous experiments, animals housed in pairs were tested with cagemates and isolated animals were tested with an unfamiliar partner. It is possible that the results reflected attraction to an unfamiliar testing partner, rather than a general increase in social attraction." In their study, as in the present one, all rats were tested with an unfamiliar partner and still differences between isolated and group housed conditions appeared.

Another explanation given by these authors for the differences observed in the gregariousness of isolated versus group housed animals is concerned with the development of social repulsion through crowding. If animals are forced to compete with each other for food, water and space in their home cage, they may develop antagonisms and lowered social attraction; increased crowding would lead to increased competition and thus decreased attraction. In their experiment no differences were found in gregariousness among animals housed under a wide range of densities. Isolation, they claim, was the significant variable.

Figure 1 shows that there was a significant difference in the behaviour of animals housed 3 and 4 to a small cage, in their behaviour in the large open field in the presence of an unfamiliar animal. Both of these cage conditions may be regarded as high density housing and both would present competition for water. Food was scattered ad lib on the floor of the cage, so there would be no competition for this. On the other hand, there was only one food and water supply in the medium cage which housed 18 animals, yet no appreciable difference was observed in the behaviour of these animals and those housed 4 to a small cage in the test condition. An interesting variation on Latané <u>et al</u>'s study might be to have a medium cage with 18 animals which received water and food just before testing only so that competitive behaviour would be accentuated in the test situation. If Latané's interpretation is correct one would expect that animals from this condition would show less gregarious behaviour towards the unfamiliar rat.

CHAPTER 5

VARIABLES AFFECTING CAGING AND EXPERIMENTAL RESULTS

Weaning age

Many studies do not state the precise age of weaned rats (for example, Hatch _et al_, 1963 & 1965). Thus, the studies investigating the "isolation syndrome", for instance, are not as useful as they might be because weaning age possibly influences the susceptibility of young rats to individual housing.

Of 38 rats weaned at 21 days of age (15 males and 23 females), 4 males and 8 females died within 3 days of the animals being placed, individually, in small cages. No definite physiological reason was discovered to account for these deaths (Wild, 1971). These animals were taken from a later breeding session of the same mothers of the animals used in the present study.

Of the 64 females weaned at 30 days of age in the present study no animals died. This is interesting because females show a more marked physiological response to isolation stress (Hatch et al, 1965).

From these observations one could tentatively conclude that weaning age affects susceptibility to isolation, though further study of the problem is necessary. It may have been that the earlier weaned rats were more susceptible to water deprivation effects in the Wild study, in that it is known that some difficulty was had with faulty water bottles, but the same bottles were used in the present study and no mortalities occurred.

Sex variations

This study considered female rats only because the ultimate aim of the investigation was to determine the feasibility of using socially-induced stress in later studies on prenatal influences. Only one study (Keeley, 1962), using mice, has investigated prenatal stress induced by crowding and its effects on offspring behaviour. Another study (Sackler et al, 1969), also using mice, looked at isolation effects on the behaviour and endocrine function of offspring. No studies in this area have used rats.

Sex segregation, in the caging of laboratory animals, is a common occurrence, for in this way only can controlled breeding programs be carried out. In the future a similar study to the present one, preferably using more subjects, should be made using male rats so a comparison of results will be possible. It has been known for some time, for instance, that male rats are more susceptible to crowding stress (Christian & Lemunyan, 1957). Gregariousness in rats may also differ when different sexes are used. This possibility is not discussed in any of the studies cited under this topic.

Litter size

A subjective observation by Wild (1971) that weanling rats from large litters seemed more susceptible to post-weaning mortality concurs with the results of a number of previous studies. This variable was not controlled for in the present study, but LaBarba & White (1971) have confirmed the results of previous investigations which indicated that rats from small litters were significantly less emotional than those from large litters. King (1969), for instance, reported strong litter effects on several weight and maturational variables in Sprague-Dawley rats, demonstrating that "uncontrolled litter effects might account for the early experience effects that have been so frequently reported in the literature."

LaBarba & White (1971) showed that mice reared in large litters exhibited significantly greater emotional reactivity (measured in an automated open field with three dependent measures: activity level, quadrants traversed, and defaecation). The effects were found to be consistent across all three indices of emotionality. Consequently, the authors suggested that the results strongly implicated litter size variations as a "confounding variable in developmental research with rodents and could weaken those studies which have failed to control for this variable."

With reference to the present study and that of Wild (1971), if rats reared until weaning in litters of different size can differ in their "emotional" behaviour in the open field, it might be possible that these animals have shown different susceptibility to caging conditions and thus different behaviour in subsequent testing sessions. LaBarba & White weaned their animals (from selective litter sizes) and housed them individually. The subjects were not handled until testing began.

Of the mortalities previously discussed (Wild, 1971), 3 of the 4 male weanlings had been handled since birth, as had all of the females. According to Levine (1957) such early stimulation enables the animal to adapt more successfully to stresses later in life. Animals that receive no stimulation early in life grow up to be timid and deviant in behaviour (Levine, 1967). The reason for these differences lies in the differing response of the pituitary-adrenal hormonal system to stress. As adults, the animals stimulated in infancy show a prompt and effective hormonal response to stress, while the unstimulated animals respond slowly and ineffectively.

Thus one would expect that handled animals would respond more quickly to the stress imposed by individual housing. Combined with this, the animals from large litters would have to make compensatory heat adjustments when placed suddenly in isolation, resulting in a higher level of circulating steriods and growth retardation (Hatch *et al*, 1965), which would result in greater susceptibility to such factors as water deprivation or lack of social communication.

Litter size, as such, may not be the vital factor in overt differences in emotionality. Not only did LaBarba & White place their animals in isolation at weaning; they also did not handle them between this time and testing. The housing variable may be the critical one, and further work in this area seems promising.

Group size

While many studies describe the behaviour of animals reared in "groups" there is little consistency in the size of the "groups" considered. It may consist of two animals (Ruegamer & Silverman, 1956) or twelve animals (Winokur, Stern & Taylor, 1959). Although it is possible that animals kept in pairs may differ from those kept in large groups in their behaviour, no mention is made of this in the literature.

Other studies, considering the effects of increasing population density, house animals in groups of 1, 4, 8, 16 and 32; assuming that nothing is "happening" between the isolated animals and those housed in groups of four. The present study investigates this first social "interval", using groups of 1, 2, 3 and 4 respectively, housed in small cages.

Bell, Miller & Ordy (1971) claim that this crowded-group paradigm confounds group size with crowding. To overcome this they use a density-group paradigm, which involves increasing the cage area as the number of animals used increases.

But the very word "crowding" implies the existence of a fixed spatial arrangement with increasing numbers enclosed within it. To crowd, according to the Oxford Dictionary, is "to force (people, things) <u>into</u> space or receptacle", a definition which implies a spatial restriction of some sort. While the density-group paradigm may be investigating optimal group size, it does not concern crowding, and the contended confounding of group size and crowding is, to this author, invalid.

Comparability of studies using mice and rats

A continuing difficulty arises in the interpretation of the studies referred to throughout this discussion in that some use mice and others, rats. There is little information in the literature as to the extent to which one may generalise from the former studies to the latter, both on physiological and behavioural indices.

Mice, for example, show greater susceptibility to isolation stress, in that they quickly become aggressive and difficult to handle. As an adjunct to the present study 4 weaned white mice were placed in isolation for two days. After this short period of time the effects of the caging showed up clearly; much more quickly than with rats of the same weaning age. Both rats and mice had some pre-weaning handling, though the rats were easier to catch and handle at all times.

Little appears to be known, also, about how far one can compare densities of living conditions of mice with those of rats. For example, mice are more susceptible to crowding stress than are rats. Christian & Lemunyan (1957) considered that 40 mice in a cage of area 234 square in. (5.85 square in./mouse) constituted experimental crowding. Male mice succumbed to crowding stress in greater numbers than female mice. Fighting ceased after the first two days of crowding and there was no competition for food or water. Morrison & Thatcher (1969) incorporated groups of 32 rats of both sexes in a study using crowded rats, where the area per rat was approximately 6 square in. and reported no deaths for either sex. Care needs to be taken, then, in any comparison of the effects of social stress on the two animals.

Use of the open field

The open field was chosen as a testing device not because of any deep belief in its validity, but rather for the opportunities for comparison of results from a wide range of other studies which it offered. Because differences among the nine groups on the various behavioural indices were the main object of interest, rather than the absolute scores obtained by any one group, the validity of testing the animals in the same apparatus, over time, was assumed (Ivinskis, 1968).

It may be argued that the two apparati were dissimilar in that the small field had a wire gauze lid and was not truly "open", but cage-like or enclosed. It also had one glass side, which the large field did not. But as far as this E was concerned since differences between groups were of interest and the spatial characteristics of the small field were definitely less than those of the large field, the apparati served the function intended.

Spatial Interactions

Limitations in the numbers of subjects and cage conditions available for the present study preclude any statistical generalisations about the interaction between cage area and the spatial character of the open fields. Two groups of subjects, caged similarly, are necessary if both groups are to be tested at the same time in the different spatial conditions. This was not possible, and the only comparison gained was that between the same groups tested in the different fields at different periods of time.

If spatial effects are not significant in altering behaviour in the open field, using a time-sample method of observation and the orthodox criterion of emotional reactivity, one would expect the behavioural indices to be constant for the two groups caged similarly and tested in different sized fields; ignoring the fact that the large field may offer greater opportunities for such activities as exploration).

In the present study a comparison of the performance of isolated animals caged in small and large areas revealed that differences did appear in the behaviour of the two groups in the two fields. The number of squares crossed is ignored in this comparison because this index was not subject to the time-sample method of frequency notation. It is postulated that these differences in the frequency of ambulation, immobility and rearing may have been due to the operation of a spatial effect because the differences in these indices, between the two cage groups, were not constant over the two test conditions.

Animals isolated in small cages showed constant immobility scores for both small and large fields; ambulation was greater in the large field, while rearing frequency was less. Animals isolated in the large cages showed a much lower median frequency for immobility in the large field than in the small field, a higher ambulation frequency in the large field, and a relatively constant rearing frequency for both fields.

Comparing the performance of the two cage groups in the small field, the animals from the large cages showed a higher median frequency for immobility than the animals from the small cages, while in the large field they showed a slightly lower median frequency on this index than the animals from small cages. In both fields the animals from large cages ambulated less than animals from small cages. With respect to the rearing index, animals from large cages reared slightly less in the small field than animals from small cages. In the large field, however, animals from large cages reared considerably more than those from small cages.

A tentative explanation for these results, as given elsewhere in the text, may be that the small cages offered less opportunity for exercise to the rats and thus impeded maturational processes. This could explain the difference in rearing scores for the two groups. An exploration hypothesis may account for the difference in ambulation scores. In fact there are a large number of possible explanations for the differences that occurred, but this is not so important as the fact that they did occur and existing experimental practices do not consider such possible spatial interactions. As Bell, Miller & Ordy (1971) point out: "It may be that previous studies which have concluded that group size, but not space, influences autonomic development have either failed to vary group size and space across sufficient ranges or have failed to adequately investigate the role of unit space per subject in the environments over a sufficiently prolonged period of time." Just before this statement they illustrate " - the need for parametric research in population density studies."

But the results gained in the present study are tentative ones only. Even so, with the refinements previously discussed along with many others yet to be discovered, the elucidation of spatial interaction of the sort envisaged promises to provide an interesting area for future research, and perhaps subsequent standardisation of experimental methods.

CHAPTER 6

BAD CAGING

Physiological and behavioural correlates

Both isolation (Hatch *et al*, 1963) and crowding (Christian, 1958) promote a stress response in caged rodents; that is, pituitary-adrenocortical stimulation.

A survey of the literature on the housing of laboratory animals revealed that no consensus of opinion exists as to the spatial and social characteristics of the "ideal" cage environment. The two principal criteria stated for the determination of such an environment were:

1. Professional judgement
2. The provision of adequate space for movement, growth and reproduction.

Caging recommendations generally state that adequate movement is necessary for the animal's welfare, but give no reasons for this, or information about the effects of <u>not</u> allowing adequate movement. As stated in the introduction, little reference is made to social conditions within the cage environment.

Provision of optimal caging is a " - problem in human sociology" (Russell & Burch, 1959), but surprisingly enough little work has been done in this area until recently.

As stated elsewhere in this discussion activity measures serve as a useful technique for studying emotional reactivity (Hall, 1936) particularly in such areas as developmental psychology and psychopharmacology. It seems logical that caging, which does not allow a full range of movement and/or social interaction to the young rat, may influence measures of activity, and thus emotionality, as measured in the open field.

The optimal mix of spatial and social factors, with respect to conditions in the animal house, should be determined and standardised if activity measures, for example, are to reflect the functioning of comparable experimental situations.

A spatial effect does seem to interact with locomotor activity, as Bell et al (1971) suggest, but not always in the direction they propose. This thesis postulates a movement explanation to account for the differences in locomotor activity in the open field, of animals reared in cages of different dimensions and in different social groups. This movement explanation is incorporated with some of the known physiological and behavioural facts on caged animals to provide a tentative theoretical approach to the caging of rats for use in the laboratory for both behavioural and pharmacological purposes.

Psychopharmacological and methodological implications

Chance (1956), in his distinction between the proximate and developmental environment, seems to have been one of the first to realise the importance of caging and experimental procedures in determining results in drug studies. The developmental environment directly interacts with genetic factors, while the proximate environment acts upon the combined system of phenotype and dramatype; the latter being the pattern of performance in a single physiological response of short duration relative to the animal's life-time; for example, the reaction of the whole organism to a drug. Therefore, in order to fully control the variability of physiological responses, one should first control the phenotype by breeding methods together with influencing the environmental conditions in which the animals are reared; and second, control the environmental conditions in which the animals are tested. Chance thus challenged the prevalent assumption that, provided conditions are kept constant (and are not excessively unhealthy) it does not matter what the conditions are; the physiological responses of the animals will tend to be uniform, because they are in a uniform environment.

As early as 1953 Lane-Petter complained about our ignorance of laboratory animal behaviour, and warned of the serious consequences this could have in experimentation. In fact there was a tendency to disregard this factor altogether. "According to this fallacy, if the animal does not grow the diet is at fault; if it does not breed there is an endocrine disorder; if it will not keep still while it is being inoculated it must be forcibly restrained. Such paralogism is not possible if the animal is regarded as having its own innate behaviour pattern, representing one of the links between the physical environment and the physical response of the animal." Even if one disagrees with the concept of an innate behaviour pattern, it is hard to fault the sentiment expressed in this quotation.

The systematic study of the social behaviour of the more common laboratory animals has hardly begun (Chance, 1957; Dimond, 1971; Crook, 1970; Baenninger, 1970). But there is a resurgence of interest in this area, as it affects the caging of such animals, both in its own right and with respect to psychopharmacological research (Yen, Stanger & Millman, 1959; Consolo et al, 1965; Welch & Welch, 1966; Baumel, DeFeo & Lal, 1969). Perhaps, eventually, a rationale will exist for the optimal caging of laboratory animals with a consequent refinement and re-evaluation of popular experimental techniques.

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APPENDIX

Table A.

SMALL FIELD (1) Number of squares crossed

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	35	37	36	42.5	38	34	41.5	25	34
S_1	-								
S_2	0.2	-							
S_3	0.3	0.4	-						
S_4	0.02	0.08	0.1	-					
M_1	0.1	0.5	0.5	0.08	-				
M_2	0.2	0.09	0.1	0.005	0.09	-			
M_3	0.04	0.2	0.1	0.4	0.09	0.01	-		
M_c	0.002	0.001	0.004	0.005	0.004	0.01	0.001	-	
L_1	0.3	0.1	0.5	0.4	0.2	0.4	0.04	0.09	-

K.-W. $p = 0.05$

SMALL FIELD (1) Ambulation

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	19.5	17	21	17.5	20	22	22.5	15.5	16
S_1	-								
S_2	0.3	-							
S_3	0.5	0.5	-						
S_4	0.3	0.4	0.3	-					
M_1	0.2	0.3	0.5	0.4	-				
M_2	0.2	0.1	0.4	0.2	0.4	-			
M_3	0.2	0.4	0.3	0.3	0.4	0.3	-		
M_c	0.09	0.2	0.4	0.4	0.03	0.04	0.1	-	
L_1	0.06	0.1	0.4	0.4	0.03	0.03	0.1	0.5	-

Kr-W. $p = 0.5$

SMALL FIELD (1) <u>Immobility</u>

medians	19	17.5	14.5	13	12.5	17.5	14	21	28
	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
S_1	-								
S_2	0.2	-							
S_3	0.2	0.5	-						
S_4	0.2	0.4	0.2	-					
M_1	0.06	0.2	0.5	0.3	-				
M_2	0.2	0.5	0.5	0.4	0.3	-			
M_3	0.2	0.3	0.5	0.5	0.3	0.5	-		
M_c	0.4	0.3	0.5	0.4	0.5	0.5	0.5	-	
L_1	0.4	0.1	0.06	0.05	0.09	0.09	0.03	0.09	-

Kr-W. $p = 0.5$

SMALL FIELD (1) Rearing

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	19	19.5	22	23.5	22.5	13.5	23.5	14.5	15.5
S_1	-								
S_2	0.2	-							
S_3	0.01	0.2	-						
S_4	0.05	0.2	0.3	-					
M_1	0.1	0.2	0.5	0.4	-				
M_2	0.2	0.1	0.04	0.08	0.1	-			
M_3	0.09	0.4	0.5	0.4	0.4	0.1	-		
M_c	0.2	0.1	0.03	0.05	0.06	0.3	0.1	-	
L_1	0.4	0.1	0.09	0.1	0.09	0.3	0.2	0.5	-

Kr-W. $p = 0.5$

SMALL FIELD (2) <u>Number of squares crossed</u>

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	28	32	27.5	40	31.5	37.5	30	19	29
S_1	-								
S_2	0.3	-							
S_3	0.5	0.2	-						
S_4	0.01	0.03	0.005	-					
M_1	0.2	0.5	0.1	0.01	-				
M_2	0.01	0.03	0.002	0.4	0.02	-			
M_3	0.3	0.3	0.3	0.005	0.2	0.004	-		
M_c	0.004	0.03	0.008	0.05	0.002	0.001	0.02	-	
L_1	0.5	0.2	0.2	0.005	0.2	0.002	0.3	0.008	-

Kr-W. $p = 0.001$

SMALL FIELD (2) <u>Ambulation</u>

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	18	20	21.5	26	12.5	22.5	23.5	15.5	14.5
S_1	–								
S_2	0.5	–							
S_3	0.03	0.1	–						
S_4	0.1	0.05	0.3	–					
M_1	0.001	0.03	0.001	0.005	–				
M_2	0.3	0.2	0.3	0.1	0.001	–			
M_3	0.03	0.03	0.2	0.1	0.001	0.5	–		
M_c	0.03	0.1	0.01	0.01	0.008	0.04	0.004	-1	
L_1	0.008	0.04	0.001	0.01	0.5	0.01	0.001	0.1	–

Kr-W. $p = 0.001$

SMALL FIELD (2) Immobility

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	15	11	23	7	15	12	11	18.5	20.5
S_1	-								
S_2	0.3	-							
S_3	0.06	0.2	-						
S_4	0.01	0.2	0.005	-					
M_1	0.4	0.3	0.03	0.005	-				
M_2	0.1	0.5	0.002	0.05	0.1	-			
M_3	0.06	0.3	0.001	0.05	0.1	0.5	-		
M_c	0.3	0.3	0.3	0.05	0.3	0.1	0.1	-	
L_1	0.09	0.2	0.3	0.005	0.1	0.008	0.004	0.4	-

Kr-W. $p = 0.02$

SMALL FIELD (2) Rearing

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	27.5	26	13.5	23	30.5	24	22	17	26.5
S_1	-								
S_2	0.3	-							
S_3	0.03	0.1	-						
S_4	0.2	0.4	0.01	-					
M_1	0.04	0.2	0.002	0.05	-				
M_2	0.3	0.5	0.008	0.3	0.1	-			
M_3	0.04	0.3	0.1	0.08	0.01	0.1	-		
M_c	0.2	0.1	0.4	0.05	0.03	0.1	0.4	-	
L_1	0.5	0.3	0.004	0.4	0.09	0.4	0.09	0.09	-

Kr-W. $p = 0.01$

LARGE FIELD **Number of squares crossed**

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	86	110	85	101	88	78	72.5	91.5	84
S_1	-								
S_2	0.1	-							
S_3	0.3	0.1	-						
S_4	0.05	0.5	0.05	-					
M_1	0.3	0.06	0.5	0.005	-				
M_2	0.2	0.06	0.3	0.01	0.3	-			
M_3	0.04	0.01	0.09	0.005	0.09	0.3	-		
M_c	0.3	0.04	0.3	0.005	0.3	0.3	0.1	-	
L_1	0.3	0.06	0.5	0.005	0.5	0.3	0.09	0.5	-

Kr-W. $p = 0.05$

LARGE FIELD **Ambulation**

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	24	25	20	24	19.5	17.5	24.5	16	19.5
S_1	-								
S_2	0.5	-							
S_3	0.03	0.2	-						
S_4	0.5	0.4	0.05	-					
M_1	0.004	0.06	0.1	0.005	-				
M_2	0.004	0.03	0.1	0.005	0.4	-			
M_3	0.5	0.4	0.09	0.5	0.02	0.01	-		
M_c	0.002	0.01	0.02	0.005	0.09	0.06	0.002	-	
L_1	0.004	0.06	0.2	0.005	0.5	0.2	0.02	0.06	-

Kr-W. $p = 0.01$

LARGE FIELD   Immobility

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	13.5	9	11.5	7.5	10.5	10.5	14	11.5	11
S_1	-								
S_2	0.06	-							
S_3	0.3	0.06	-						
S_4	0.03	0.4	0.005	-					
M_1	0.3	0.1	0.1	0.02	-				
M_2	0.3	0.2	0.4	0.1	0.4	-			
M_3	0.3	0.06	0.3	0.05	0.2	0.2	-		
M_c	0.4	0.2	0.5	0.02	0.3	0.4	0.2	-	
L_1	0.1	0.2	0.2	0.08	0.4	0.5	0.2	0.3	-

Kr-W. $p = 0.3$

LARGE FIELD — Rearing

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	20	26.5	26	25.5	30	29.5	16	30	29
S_1	-								
S_2	0.01	-							
S_3	0.03	0.4	-						
S_4	0.01	0.4	0.06	-					
M_1	0.002	0.1	0.06	0.05	-				
M_2	0.02	0.1	0.3	0.2	0.3	-			
M_3	0.09	0.004	0.004	0.01	0.002	0.02	-		
M_c	0.02	0.1	0.09	0.08	0.5	0.5	0.008	-	
L_1	0.001	0.09	0.1	0.08	0.5	0.5	0.002	0.4	-

Kr-W. $p = 0.2$

LARGE FIELD + ANIMAL   <u>Number of squares crossed</u>

medians	83	132	110	134	112	113.5	107	138	113
	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
S_1	-								
S_2	0.01	-							
S_3	0.09	0.1	-						
S_4	0.02	0.3	0.05	-					
M_1	0.03	0.1	0.5	0.1	-				
M_2	0.002	0.3	0.3	0.3	0.3	-			
M_3	0.02	0.2	0.3	0.1	0.4	0.3	-		
M_c	0.002	0.2	0.02	0.3	0.03	0.09	0.6	-	
L_1	0.008	0.1	0.2	0.3	0.4	0.4	0.4	0.1	-

Kr-W. $p = 0.05$

LARGE FIELD + ANIMAL <u>Ambulation</u>

	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
medians	27	25.5	25.5	31.5	27	27	29	25	28
S_1	-								
S_2	0.5	-							
S_3	0.5	0.4	-						
S_4	0.1	0.2	0.2	-					
M_1	0.4	0.5	0.4	0.1	-				
M_2	0.4	0.4	0.3	0.1	0.5	-			
M_3	0.1	0.04	0.2	0.3	0.1	0.1	-		
M_c	0.4	0.3	0.3	0.08	0.5	0.2	0.03	-	
L_1	0.4	0.3	0.4	0.1	0.4	0.5	0.2	0.1	-

Kr-W. $p = 0.8$

LARGE FIELD + ANIMAL <u>Immobility</u>

medians	23.5	13	19	13.5	15	9.5	9	11	16.5
	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
S_1	-								
S_2	0.03	-							
S_3	0.3	0.03	-						
S_4	0.02	0.3	0.03	-					
M_1	0.2	0.2	0.2	0.1	-				
M_2	0.002	0.09	0.004	0.2	0.03	-			
M_3	0.001	0.05	0.002	0.08	0.008	0.3	-		
M_c	0.004	0.1	0.004	0.2	0.03	0.3	0.1	-	
L_1	0.04	0.1	0.09	0.01	0.5	0.002	0.001	0.001	-

Kr-W. $p = 0.01$

LARGE FIELD + ANIMAL <u>Rearing</u>

medians	11	18.5	10.5	17	12	18.5	17	22	15.5
	S_1	S_2	S_3	S_4	M_1	M_2	M_3	M_c	L_1
S_1	-								
S_2	0.001	-							
S_3	0.5	0.008	-						
S_4	0.02	0.2	0.02	-					
M_1	0.2	0.01	0.1	0.05	-				
M_2	0.001	0.3	0.008	0.1	0.01	-			
M_3	0.01	0.1	0.06	0.3	0.06	0.2	-		
M_c	0.004	0.2	0.002	0.1	0.008	0.3	0.1	-	
L_1	0.06	0.04	0.02	0.1	0.1	0.01	0.2	0.01	-

Kr-W. $p = 0.001$

APPENDIX

Table B.

All probabilities used for this table were obtained with the Mann Whitney U Test of frequency differences between two groups of Ss, with 6 Ss per group. Significant differences ($p \leq 0.05$) are shown by the symbol: X. Nonsignificant differences are not marked

		N			a			i			r		
FIELD TEST	CAGE SIZE	S	M	L	S	M	L	S	M	L	S	M	L
	S	–			–			–			–		
SMALL (2)	M		–		X	–			–		X	–	
	L			–	X		–			–			–
	S	–			–			–			–		
LARGE	M		–		X	–			–		X	–	
	L			–	X		–			–	X		–
	S	–			–			–			–		
LARGE + rat	M	X	–			–			–			–	
	L	X		–			–	X		–			–

Symbols used:

N – number of squares crossed

a – ambulation

i – immobility

r – rearing

S – small cage

M – medium cage

L – large cage