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Effects of Crowding and Intermittent Isolation on Gerbils (*Meriones unguiculatus*) [1]

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HULL, E. M., E. CHAPIN AND C. KASTANIOTIS. *Effects of crowding and intermittent isolation on gerbils* (Meriones unguiculatus). PHYSIOL. BEHAV. 13(6) 723–727, 1974. – Gerbils were reared from weaning to adulthood in mixed-sex paired, crowded or isolate-crowded conditions, the latter consisting of placing crowded animals into individual boxes for 2 hr per day. Paired males exhibited more ventral marking and nonaggressive contact in social interaction tests; paired females were far more successful reproductively than either crowded group. Isolate-crowded males were more aggressive in the home cage and in social interaction tests, and isolate-crowded females were somewhat less successful reproductively than crowded females. The only anatomical or biochemical differences among groups were heavier testes in paired males and heavier body weights in crowded males. Reproductive failure and decreases in behavioral measures were not mediated by adrenal steroids. The isolate-crowded conditions aggravated the effects of crowding, rather than decreasing them.

Crowding Gerbils Aggression Reproduction Adrenal gland Cortisol Scent marking

THERE is considerable evidence that population density influences the behavior and physiology of animals [2, 3, 15, 19]. Generally, one or more aspects of reproduction are depressed, and frequently a physiological stress reaction occurs with overproduction of adrenal corticoids. These reactions occur whether or not there is overt fighting and wounding, and have therefore been attributed to "psychological" stress rather than to any physical damage [2, 19]. Furthermore, in gerbils, social interactions with strange animals are less sought out and ventral gland marking is severely depressed [9,20].

Effects of crowding on reproduction may be manifested in any of several ways. Males may produce fewer or lower quality sperm and/or copulate less frequently. Females may not come into estrus as frequently, may resorb embryos after implantation, may produce too little milk after birth, or may not care adequately for the offspring. Furthermore, maturation may be slowed for both sexes by the stress of crowding. All of these effects probably occur to greater or lesser degrees in various groups of animals.

Most of the above studies have involved constant conditions of grouping throughout the duration of the experiment. However, Goldberg and Welch [7] found increases in adrenal activity and weights in response to intermittent grouping (15 min per day for 7 to 10 days) in male mice. Southwick [16] obtained similar results with intermittent grouping for 1 hr per day, but found that mice which were transferred to new cages for 1 hr per day or were merely handled, exhibited either temporary increases or no increases, respectively, in adrenal activity. Thus, it is grouping per se, and not the concommitant physical disturbance, which is most important in altering adrenal activity. Finally, decreases in density have been shown to produce decreases in adrenal weight [4,13], indicating that population reduction is not stressful and that it allows the animals to return to a more normal level of adrenal activity.

The present experiment was designed to determine whether daily periods of social isolation in a physically enriched environment would decrease the stressful effects of crowding. Gerbils were used as subjects partly because their territorial marking has been fairly extensively studied [21,22] and partly because both marking and social interactions of gerbils have been shown to be affected by population density [8, 9, 20]. Furthermore, gerbils possess a relatively natural repertoire of behavior, compared with most common laboratory animals. Exploratory activity, ventral gland marking and social interaction measures were examined for the males, as well as reproductive success and body and endocrine gland weights for all animals. Since cortisol is one of the two major hormones produced by the

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adrenal cortex of gerbils (the other being 19-hydroxy-11-deoxycortisol [11]) fluorometric analysis of cortisol levels was made for males.

METHOD

Animals

The animals were 50 female and 46 male Mongolian gerbils *(Meriones unguiculatus)*, obtained at weaning (4 weeks of age) from Tumblebrook Farms, Inc. They were divided into three categories, paired (P), crowded (C), and isolate-crowded (I-C). The P group consisted of 16 male/female pairs, each of which was placed into a clear plastic cage, approximately 23 X 45 cm. The C group consisted of two groups of 16 animals each, 8 females and 8 males in one and 9 females and 7 males in the other. They were placed in 10 gal aquariums, approximately 27 X 51 cm. The I-C group also consisted of two groups of 16 animals each, distributed and caged exactly as the C animals. The daily isolation procedure was carried out for the I-C groups between the ages of 4 and 18 weeks. Behavioral testing of the males was carried out during the last 5 weeks of that period, after which they were all sacrificed within a 3 day period. Food, water, and a wooden block for gnawing were available ad lib throughout the experiment for all animals. Cage floors were covered with wood shavings, and cloth and paper for shredding and nest building were supplied.

Apparatus

The 16 enriched environments for the I-C group measured 61 X 61 X 46 cm and were made of plywood painted gray. They contained various objects including a plastic house or hollow log, an activity wheel, cloth, sticks, and regularly replaced small cardboard boxes. Floors were covered with wood shavings. Hardware cloth, framed by 1.3 X 2.5 cm boards, served as removable tops.

The open field apparatus measured 1 m^2, was painted gray, and was lined off into 16 squares of equal size. Pegs made of plastic dowels 2 cm high were inserted into holes at the corners of each square except along the boundary of the field. The sides were 45 cm high, and were hinged at the middle.

Procedure

Upon arrival, the animals were separated into their various categories, anesthetized and earmarked for identification. The I-C animals were placed individually in the enriched environments every day for 2 hr. All animals in each aquarium were placed into the environmental boxes at the same time, and each animal was always placed into the same box. Thus, every day each box was inhabited first by an assigned animal from one I-C group and next by an assigned animal from the other I-C group. Order of placement of the two groups was varied.

After 10 weeks in experimental conditions, behavioral testing was begun on the males. Females were not tested because of possible disruptive effects on pregnancy, lactation, or maternal behavior and also because previous work [9] indicated smaller effects of crowding on female than on male behavior. Daily isolation periods for the I-C groups continued throughout the testing period, with at least 1 hr between isolation and testing on any day. Each male was first tested individually in the open field apparatus for 10 min. Number of lines crossed with all 4 feet, number of ventral rubs on either pegs or floor, and number of fecal boluses and urine pools were tallied. Only those responses which included perceptible lowering of the back were counted as ventral rubs. After each trial the apparatus was cleaned with a 20% solution of alcohol.

Next, each male was allowed to interact in the same open field apparatus in 3 separate tests with 3 other males, one from each of the density conditions. Order of confrontation was completely counterbalanced, and no animal was tested with a cagemate. The 2 animals for each trial were marked on their tails with different colored felt-tipped pens for identification. One experimenter watched the animals and quietly called the scores to a second experimenter who recorded and kept time. All but a few trials, for which only one experimenter was available, were run blind, with the scorer selecting and marking the animals without the judge's knowledge of animal conditions. Each time an animal initiated sniffing or body contact, ventral marking, or aggressive behavior, the appropriate score was recorded. The sniff or contact category included any nose or body contact with the other animal which did not appear to be aggressive. Ventral marking was defined as above. Aggressive behavior included fighting, chasing, pushing, biting, and crawling over. Aggression was scored conservatively; when there was doubt as to the aggressive intent of the animal, the interaction was scored as a contact rather than aggressive behavior. Again, the apparatus was cleaned after each trial. All testing was done during the light half of a 12 hr light/12 hr dark cycle, with lights on and an air circulation system providing a constant background noise. The testing room was adjacent to the room in which the animals were housed.

At the termination of behavioral testing, all male animals were sacrificed within a 3 day period by rapid decapitation, and blood samples were collected, Serum samples were quantitatively analyzed for cortisol using the fluorometric method developed by Clark and Rubin [5]. The ventral glands, adrenal glands, and testes were weighed to the nearest milligram. One adrenal gland from each animal was stained with Oil Red 0 and examined microscopically.

Until the time of sacrifice of the males, any female producing a litter was removed with her litter to a separate cage. Pups were weighed at 5 and at 30 days of age. The mother was then weighed and sacrificed, and ventral gland, adrenal glands, uterus, and ovaries were weighed to the nearest milligram. The uterus was also examined for scars of implantation. Discrepancy between number of scars and number of pups born was taken as probable evidence of resorption of embryos.

RESULTS

Behavioral Tests

There were no significant differences in activity in the open field tests. All animals were active and crossed an average of about 300 lines during the 10-min tests. There was, however, a very large difference in open field marking scores, with paired animals marking almost 6 times as frequently as animals in the two crowded conditions, $F(2,41) = 21.28$, $p<0.0001$. (See Table 1 for results of all behavioral tests.) Paired males in the social interaction tests again marked about 6 times as frequently as animals in the two crowded conditions, $F(2,41) = 29.66$, $p<0.0001$.

Paired animals also initiated more nonaggressive sniff and contact behavior, $F(2,41) = 3.24$, $p<0.05$. However, it was the I-C males which initiated the most aggressive encounters, $F(2,41) = 3.83$, $p<0.03$. Defecation and urination scores showed no significant differences among groups.

Anatomical Measures

Body weights were greatest in crowded males, $F(2,41) = 7.58$, $p<0.01$. (See Table 2 for results of anatomical measures.) Relative testes weights were considerably greater in paired males than those from both crowded groups, $F(2,41) = 17.86$, $p<0.0001$; however, absolute testes weights of paired and crowded animals were similar, while those of isolate-crowded animals were smaller, $F(2,41) = 7.89$, $p<0.01$. Other anatomical measures did not show significant differences, though the decline in relative ventral gland weight in the two crowded groups approached significance, $F(2,41) = 2.60$, $0.1>p>0.05$. Among the females, analysis of variance on anatomical measures showed no statistically significant differences due to experimental variables.

Reproductive Success

Paired females had almost twice as many pups as did crowded females and three-and-one-half times as many pups as did isolate-crowded females (total pups = 82 for P, 43 for C, and 23 for I-C females; $F(2,46) = 8.29$, $p<0.001$). Paired females had both more litters (total = 14 for P, 6 for C, and 3 for I-C females; $\chi^2 = 16.6$, $df = 2$, $p<0.001$) and more pups per litter (mean = 5.5 for P, 5.0 for C, and 3.3 for I-C females; $F(2,19) = 4.43$, $p<0.03$), than the two groups of crowded females. Furthermore, paired females had their litters significantly earlier than did females from the two crowded categories, $F(2,25) = 15.32$, $p<0.001$. Counting the date of the first litter as Day 1, average latencies for parturition were 23 days for P females, 52 days for C females, and 54 days for I-C females. (Animals not bearing litters were excluded from analysis.) Weights of offspring at 5 days of age were not significantly different; however, even though the paired females' litters were larger, their pups averaged a greater weight gain by the time of weaning than did those of C and I-C females (means = 22.14 g gained between 5 and 30 days of age for offspring of P females, and 18.66 g for offspring of both groups of crowded females; $t = 2.24$, $df = 22$, $p<0.05$).

DISCUSSION

On most of the behavioral and reproductive measures, the paired animals were clearly different from the two crowded groups. In both open field and social interaction

TABLE 1

BEHAVIORAL MEASURES OF THE MALES

Group	Open Field Marking in 10 min		Social Interaction Marking in 30 min		Sniffs and Contacts in 30 min		Aggressive Behavior in 30 min	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
P	23.38	14.11	62.75	31.22	137.75	31.92	16.62	14.66
I-C	3.53	3.69	9.62	13.54	118.38	30.59	28.46	15.13
C	4.60	4.33	8.87	11.88	111.87	21.73	13.93	12.52

TABLE 2

ANATOMICAL MEASURES OF THE MALES

Group	Body Weight (g)		Absolute Testes Weight (g)		Relative Testes Weight*		Absolute Ventral Gland Weight (g)		Relative Ventral Gland Weight†	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
P	70.76	6.43	1.13	0.07	0.0158	0.0010	0.14	0.04	0.0020	0.0004
I-C	72.15	7.52	1.02	0.11	0.0141	0.0006	0.12	0.04	0.0018	0.0005
C	79.44	5.00	1.06	0.26	0.0142	0.0008	0.14	0.04	0.0017	0.0005

*Testes weight (mg)/Body weight (g) †Ventral gland weight (mg)/Body weight (g)

marking and in nonaggressive social interaction contacts, the paired males were clearly superior to crowded males. Paired females far surpassed crowded females at all stages of reproduction, from initial implantation of ova to weaning of the offspring. They conceived earlier, had more pups, fewer pup deaths, and heavier pups at weaning.

It is interesting, however, that the only anatomical differences in the present experiment were increased body weights and decreased testes weights of the crowded males. There were no anatomical differences among groups of females in the measures recorded. Lactation insufficiency may have been involved in post-parturition deaths and lower weight gain in the crowded groups, but could hardly have accounted for failures of implantation and/or intrauterine deaths. Since crowded males' testes were smaller than paired animals', decreased copulatory activity or sperm quantity or quality may be implicated [14].

There was no indication that the observed reproductive failure was mediated by adrenocortical hormones. Adrenal weights, both relative and absolute, were unaffected by density in both males and females. Since fine changes in adrenal morphology or chemistry might not be reflected in gross weight changes, serum cortisol measures were obtained for males, and glands of all animals were examined histologically. There was no significant difference in cortisol levels of males, and no differences in size or number of lipid droplets or in the areas (either relative or absolute) of lipid-staining tissue in the adrenal cortex. The adrenal medulla also showed no hypertrophy, which may be taken as evidence against mediation by catacholamines. Several investigators have failed to find adrenal hypertrophy in response to crowding in several species of mice [1, 3, 17, 18]. Furthermore, Terman [18] did find reduction in size of reproductive organs of both sexes of deermice in spite of the lack of adrenal hypertrophy, and Christian [3] presented similar results for female deermice, though not for males. Christian [3] suggests direct central nervous system mediation of crowding effects in these animals, possibly involving amygdaloid nuclei [6].

The isolate-crowded condition was originally instituted in an attempt to normalize the crowded laboratory environment. Gerbils in the wild do live in herds [12], and in their burrows population densities may resemble that found in our crowded conditions. However, wild gerbils are able to leave the crowded burrows and explore, gather food, etc., in relative seclusion. It was hypothesized that marking and social interaction measures and reproductive success would be intermediate between the high scores on all measures compiled by paired animals and the low scores of crowded animals. However, a rather different pattern emerged. In marking and sniff and contact scores, I-C males were indistinguishable from C males (i.e., had low scores), while in aggressive encounters they scored higher than either other group. Furthermore, this aggressiveness was also observed in their home cages at the termination of each period of isolation beginning at about the time of sexual maturity. Two males in the I-C condition died, with numerous bleeding wounds. Several other male and female animals had less severe wounds.

The social unrest in the I-C cages seemed to be a factor in the reproductive failure of these animals. Even the C females fared better than the I-C females during at least the early stages of reproduction, when they were still in the experimental conditions. They had more implanted ova and more pups at birth than the I-C females. These differences became less obvious as weaning approached, due to greater death rate among the C offspring. However, during this time the females were isolated from other adults, and any reproductive deficiencies at this stage were carried over from the prenatal period and did not result from any contiguous social strife. The reason for the greater post-parturition death rate among C offspring, compared with I-C offspring, is not known. It should be noted that average latencies to parturition for the two crowded groups were nearly identical, so that their apparent delayed maturation can be attributed solely to crowding and not to fighting, since little evidence of fighting was observed in the C cages.

Thus, rather than creating a more favorable environment, the I-C condition led to more social strife and, according to several measures, less reproductive success than did the C condition, in which there was continuous crowding. Apparently, the continuously crowded animals adapted to their situation and as noted in a previous article [9], appear to have become less responsive to social stimuli. Long periods of isolation are known to increase aggressiveness and/or "emotionality" in rats and mice [7,10]. To our knowledge, this is the first indication that repeated short periods of isolation may lead to similar results. The effect seems not to be bound up with territorial defense, since I-C males' ventral gland marking was depressed in the open field and social interaction tests and since their isolated territory was not part of the home cage. The effect does, however, seem to depend on sexual maturity, since no fighting was observed until the animals were approximately 90 to 100 days of age.

In summary, crowding depressed all social and reproductive factors measured but did not evoke an adrenocortical stress response. Periods of intermittent isolation in a physically enriched environment did not ameliorate the effects of crowding, but rather exacerbated them, leading to more fighting both in the home cage and in the social interaction tests and leading to somewhat worse reproductive success than that of continuously crowded animals.

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