Colony differences and behavior in Long-Evans rats

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A series of three experiments was performed on male Long-Evans rats from two colonies, Blue Spruce Farms (BSF) and Mississippi State University (MSU). The purpose of the present study was to account for the differences in rate of mouse killing by male Long-Evans rats from different colonies reported by Thompson and Thorne (1975). A pilot study replicated the findings of Thompson and Thorne. Experiment I found that the offspring of animals whose parents were obtained from BSF and bred at MSU killed at a significantly higher rate than animals whose parents were part of the MSU breeding stock. Thus, genetic and/or preweaning variables were implicated in accounting for the results reported by Thompson and Thorne. Experiment II found no differences between BSF offspring (mostly killers) and MSU offspring (mostly nonkillers) on measures of handling characteristics, activity, and learning of a simple discrimination task. Experiment III was intended as a replication of Experiment I, but no differences in the rate of killing were found between BSF and MSU rats. Due to methodological flaws, the present study was unable to account for the differences seen by Thompson and Thorne (1975). Possible explanations for the failure of Experiment III to replicate Experiment I were discussed.

Not all rats kill when first presented with a domestic mouse. In a selected survey of the mouse-killing literature performed by the senior author, the rate of killing of animals in control groups from 27 studies which used Long-Evans rats was from 1% to 63%, with a mean of 25% and a median of 19%. The large amount of variability is not surprising in light of the number of variables shown to affect muricidal behavior. Some of the variables that have been studied are food deprivation (Malick, 1975; Paul, Miley, & Baenninger, 1971), exposure to mice in early life (Dennenburg, 1973; Myer, 1969), strain differences (Richman, Gulkin, & Knoblock, 1972; Thorne, Aaron, & Latham, 1973) and the aggressiveness of the mother (Flandera & Nováková, 1974).

Recently, colony differences in behavior have been reported. Wise (1974) found that strain-related and supplier-related differences in commercially purchased laboratory rats accounted for major differences in ethanol intake. Similarly, Thompson and Thorne (1975) found that the colony from which rats of the same strain were obtained affected the rate of mouse killing. Thompson and Thorne reported that Long-Evans male rats obtained from Blue Spruce Farms (BSF) in Altamont, New York, showed a high initial rate (80%) of mouse killing, while Long-Evans males which had been maintained for several generations by the Psychology Department at Mississippi State University (MSU) showed a much lower rate (18%).

Since the housing, surgical, and testing procedures were identical for MSU and BSF rats following the latter's arrival at Mississippi State University, the differences between the two colonies must be attributed to

a set of conditions existing prior to the arrival of the BSF animals. Thompson and Thorne (1975) suggested two obvious differences—environmental and genetic. A number of environmental differences, such as the amount of previous handling and the presence of domestic mice in the rearing quarters, are known to exist. In light of the number of variables known to affect the muricidal response, it would not be surprising to find that a seemingly minor environmental difference accounted for the major difference in killing seen by Thompson and Thorne.

Similarly, a genetic basis for the observed behavioral difference is not unlikely if we consider the number of generations of inbreeding that have occurred at BSF and at MSU. That strains may be selected and inbred to result in animals showing high or low performance on a given task is well documented (McClearn, 1962). Karli, Vergnes, and Didiergeorges (1969) reported that, after using mouse-killing rats for breeding for 2 years, the rate of mouse-killing in animals with olfactory bulb lesions increased. Also, of the previously mentioned reviewed studies using Long-Evans males, the only experimenter whose animals showed a killing rate of 33% or above had not introduced any new stock into his colony since 1963 (Myer, 1971).

Since the implications of colony differences within strains are as serious, if not more so, than strain differences themselves, the purpose of the present study was to attribute the between-colony differences observed by Thompson and Thorne (1975) to either genetic and/or early environmental effects or to postweaning environmental differences.

EXPERIMENT I

Before beginning this study, we considered it neces-

sary to replicate Thompson and Thorne's (1975) finding that the BSF animals killed at a high initial rate, since there was some indication that the high rate seen by Thompson and Thorne was an artifact due to deprivation during shipping. Thus, special instructions were given to BSF, and 11 males and 5 females were received in shipping crates containing a large amount of food. All animals were placed in single cages and tested for mouse killing approximately 72 h after their arrival at MSU. The results replicated the previous findings of Thompson and Thorne since 73% (8 of 11) of the males killed. In addition, 40%, or two out of five, of the females killed.

The present study was designed to compare the muricidal behavior of the male offspring of BSF and MSU parents reared under identical conditions in the laboratory at Mississippi State University.

Method
Subjects. The subjects were eight 60-to-90-day-old male Long-Evans rats whose parents were obtained from BSF and bred at MSU, and eight 60-to-90-day-old male Long-Evans rats whose parents were part of the MSU breeding stock.

The breeding stock obtained from BSF consisted of 11 males and 5 females. All animals were placed singly in hanging cages measuring 17.78 x 25.40 x 17.78 cm and given a single muricidal test. After testing, all of the females and three of the males were placed in group cages for breeding. At this time, five female MSU rats were placed in group breeding cages with three MSU males. When the females were determined to be pregnant, they were placed in single cages lined with a fine wire mesh until the pups were born. Shortly after birth, all litters were culled to approximately 10 pups. The dams and the pups remained in the maternal cages until weaning, at which time the pups were sexed and placed in group cages containing three or four animals until 60-90 days of age. At that time they were placed in single cages and given a muricidal test after a short period of adaptation.

Procedure. The muricidal test consisted of placing an adult albino mouse into the cage of the subject. The animals were observed continuously for the first 20 min. If muricide occurred within this period, the latency to kill was recorded. If the subject did not kill during the observation period, a check was made after 24 h and any live mice or the remains of dead ones were removed.

Results and Discussion

Statistical analysis by means of a Fisher test revealed that BSF animals killed at a significantly higher rate than the MSU animals (p < .01). The percentages were 87.5% (seven of eight) and 12.5% (one of eight) for the BSF and MSU rats, respectively. Too few of the MSU animals killed to compare latencies.

From these findings, it would seem possible to attribute the differences found by Thompson and Thorne (1975) to a genetic or preweaning variable. Subsequent results in an attempted replication (Experiment III) led us to suspect that this was an untenable explanation.

Since a significant difference in the rate of killing was seen between the BSF and MSU offspring, Experiment II was designed to compare the animals on behavioral measures other than muricide.

EXPERIMENT II

Several studies have compared killer and nonkiller rats on a number of behavioral measures other than muricide. For example, Vergnes, Boehrer, and Karli (1974) reported that mouse-killing rats showed a higher level of emotionality than nonkillers, as measured by the number of boluses in an open-field test. Similarly, Nováková, Flandera, and Sandritter (1974) found that mouse killers were less able to habituate exploratory behavior, and required a greater amount of time to acquire an auditory conditioned reflex.

Not all studies have found differences, however. Knutson and Hynan (1973) found no differences in regard to shock-induced fighting, while Thorne, Patterson, and Topping (1975) found no differences on a number of reactivity measures, open-field activity, and a visual discrimination task. Vergnes et al. (1974) also found no differences on a number of measures other than the number of boluses.

Since almost all BSF rats in Experiment I killed, while almost none of the MSU rats did so, comparing animals from the two colonies became essentially a comparison of killer and nonkiller rats. In addition, the comparison tested the hypothesis that the variable or variables leading to mouse-killing differences might affect other behaviors as well. Thus, the animals from Experiment I were compared on activity and reactivity measures and on a simple discrimination task motivated by escape from footshock.

Method
Subjects. The subjects were eight MSU rats and seven BSF rats from Experiment I. One BSF rat died prior to the beginning of the present study.

Apparatus. The apparatus used to assess locomotor activity was an open-field box measuring 76.2 x 76.2 x 25.4 cm. The box was painted flat black and the floor was divided by white lines into 25 equal squares. The top was covered with hardware cloth.

The discrimination apparatus was a Thompson-Bryant two-choice box from which the choice chamber had been removed, leaving a startbox and goalbox. The startbox had a grid floor through which footshock could be administered to motivate the animal. A Plexiglas door separated the start area from a 7.6-cm grid in front of two openings to the goalbox. The doors to the goalbox were blocked by 10.2 x 16.5-cm stimulus cards. Each stimulus card consisted of heavy posterboard on which was pasted either a piece of black or a piece of white construction paper. In order to prevent the animal from using olfactory cues to make the discrimination, the cards were inserted into clear plastic sleeves which were washed with water after the testing of each animal. Painted gray cards were used during the preliminary training.

Procedure. All animals were given 4-day tests of handling characteristics and open-field activity. Emotionality was rated on a five-component scale reported by Thorne et al. (1975).

Immediately prior to testing, each animal's cage was removed from the cage rack and carried to a nearby table. The first three components were scored with the animal in its cage, while resistance to handling was measured with the animal on a gloved hand approximately 1 ft above and away from the home cage. Testing sessions lasted approximately 1 min, and vocalization was recorded throughout the session.

Following the rating of handling characteristics, each animal was placed in the center of the open-field apparatus, and the number of squares crossed by the rat's hind feet in a 1-min period was recorded. The number of rearings made by each animal was also recorded. Finally, the number of boluses was counted.

Training began on the discrimination task 7 days after completion of the handling and activity task. Preliminary training consisted of five trials in which an animal was forced to leave the startbox and push aside a gray card to enter the goalbox. The following day, practice on the discrimination trial (white-black) began. On each trial, the subject was placed into the startbox for 5 sec. After 5 sec, the Plexiglas door was raised and the animal was given approximately 10 sec in which to make a choice. If the rat chose the black card, a mild footshock was administered, while choice of the white card allowed the animal to enter the goalbox. The position of the cards, but not the plastic sleeves, was varied according to a modified Gellerman sequence, with a correction procedure employed. All subjects were given 10 trials per day, with an intertrial interval of about 1 min. Training continued until the subject reached a criterion of nine correct responses in 1 day. The number of errors an animal made prior to the day criterion was attained constituted the measure of learning ability.

Results and Discussion

Reliability checks for the rating and counting procedures were made through the use of an independent observer. Correlation coefficients of $r = +.99$ and $r = +.98$ were found for the number of squares crossed and number of rearings, respectively. The large number of ties and zero ratings recorded during the measurement of handling characteristics prevented the computation of a correlation coefficient for this data. The assessment of reactivity to handling has been found to be a quite reliable procedure in several previous studies in this laboratory, however.

The BSF and MSU groups did not differ significantly on any of the behavioral measures. The 4-day totals for reactivity (BSF mean = 9.5, MSU mean = 13.3), number of squares crossed (BSF mean = 69.6, MSU mean = 63.5), number of rearings (BSF mean = 19.3, MSU mean = 24), and number of boluses (BSF mean = 9.9, MSU mean = 12) were analyzed by means of a Mann-Whitney U test. Performance on the discrimination task by the two groups was nearly identical (BSF mean = 9, range: 7-16; MSU mean = 9.5, range: 8-17), a finding also reported by Thorne et al. (1975). The lack of a difference between killers and nonkillers disagrees with the report by Nováková et al. (1974), who found killers to be more reactive to handling and less capable of learning than nonkillers.

A possible resolution of the discrepancies between the reports of Nováková et al. (1974) and Thorne et al. (1975) lies in the fact that only a relatively small number of rats kill "naturally," i.e., without being deprived or drugged or exposed to a very powerful stimulus. In order to get a large enough group of killers to make a reasonable comparison with nonkillers, Thorne et al. used animals that had been tested with wild field mice. In an unpublished series of studies, Thorne found that virtually all rats exposed to wild mice kill, whereas less than 20% kill domestic mice.

Thus, if we assume there is a difference between "induced" killers and "natural" killers, and that Nováková et al. used primarily "natural" killers while Thorne et al. used "induced" killers, then the lack of comparability in the data becomes explicable. This argument, of course, is highly speculative and awaits further experimentation. In order to be applicable to the results of the present study, we would have to assume that the killing in the BSF groups was "induced" rather than "natural." There is evidence for this assumption in Experiment III, which was an attempted replication of Experiment I.

EXPERIMENT III

Because of the relatively small sample size in Experiment I, the present study was performed. The only difference between this experiment and Experiment I was in the preweaning housing, i.e., the present subjects were reared in large wooden boxes, while the previous animals were reared in small wire cages. In retrospect, this seemingly minor difference appears to have been crucial for the difference in results obtained.

Method

Subjects. The subjects were 17 60-to-90-day-old male Long-Evans rats whose parents were obtained from BSF and bred at MSU, and 20 60-to-90-day-old male Long-Evans rats whose parents were part of the MSU breeding stock. All subjects were taken from litters raised in wooden boxes with interior dimensions of 30.48 x 45.72 x 30.48 cm. At weaning, the pups were sexed and placed in group cages containing three or four animals until 60-90 days after birth. At this time, they were placed in single cages and given a muricidal test after a brief period of adaptation. Ad lib food and water conditions were maintained at all times.

Procedure. The procedure was identical to that in Experiment I.

Results and Discussion

The results of Experiment III resulted in a failure to replicate Experiment I. The percentages of animals killing were 29% (5 of 17) and 25% (5 of 20) for the BSF and MSU groups, respectively. Statistical analysis by means of a Fisher test revealed no significant differences ($p > .05$). Latencies were evaluated by means of a Mann-Whitney U and also showed no significant differences between the two groups ($p > .05$). The percentage of mouse killers in the BSF group was consistent with the percentage of mouse killers previously found at MSU.

There is indirect evidence that the BSF animals in Experiment I were "induced" killers. On a number of occasions prior to weaning of the pups, the cages containing the BSF pups were found to be out of food. Some of the BSF pups subsequently developed constrictions around their tails, which eventually fell off. While these animals were discarded, the possibility remains that a number of subjects were deprived during early stages of their development. That deprivation results in an increase in mouse killing has been reported by Malick

(1975), Paul, Miley, and Baenninger (1971) and Whalen and Fehr (1964).

The results of the present experiment indicate that the high rate of killing found by Thompson and Thorne (1975) may have been due to the influence of the different environmental histories of the BSF and MSU animals.

GENERAL DISCUSSION

The contradictory results of Experiments I and III leave unresolved the question of whether environmental or genetic variables accounted for the difference in BSF and MSU rats observed by Thompson and Thorne (1975). The results from Experiment I, which otherwise would seem to implicate genetic or preweaning variables, cannot be accepted uncritically because of the possibility of early deprivation and because of the results of Experiment III.

An alternative explanation for the results of Experiment I is that the mouse-killing behavior of the mother in part determines the mouse-killing response of her offspring. Flandera and Nováková (1974) reported that descendants of nonaggressive (nonkiller) rats reared by aggressive (killer) foster mothers from birth killed at a rate of 65%. By contrast, the offspring of nonaggressive mothers raised by their own mothers killed at rates of 0% and 10%, depending upon their age at the time of the test. Despite the fact that Flandera and Nováková failed to control for the effects of the cross-fostering procedure, adequately controlled studies by Dennenburg (see Dennenburg, 1973) with mice raised by rats have shown that the aggressiveness of the mother is an important variable in determining the aggressive behavior of the offspring.

Unfortunately, no records were kept in the present study identifying which of the female BSF breeding stock were mouse killers. However, since 40% of the female breeding stock were mouse killers, there is a definite possibility that the mothers of the BSF group in Experiment I were mouse killers, while the mothers of those in Experiment III were not.

The results of Experiment II were more conclusive. No behavioral differences were found between killers and nonkillers. These findings replicate those of Thorne et al. (1975). While the behavioral measures used are relevant to emotionality and learning ability, they are less clearly related to predation. If the muricidal response is truly an example of predatory aggression, it is not surprising that no differences were found on these measures.

The results from Experiment III were fairly conclusive and indicate that the differences between BSF and MSU rats observed by Thompson and Thorne (1975) stem from environmental differences. A major possibil-

ity in this regard is the presence or absence of mice in the rooms in which rats are raised. This variable is under investigation at the present time.

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