Communal nursing in mice: Strain-specific effects of multiple mothers on growth and behavior[1]

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Female mice of two inbred strains (A/J and C57BL/6J) raised litters with a fixed ratio of offspring to single or multiple mothers under same- and mixed-strain conditions. Communal nursing enhanced growth in offspring of both strains; for the A/J strain, higher levels of activity were obtained particularly for those offspring raised with mixed-strain mothers and littermates.

A recent report by Sayler & Salmon (1969) demonstrated that inbred albino mice of the BALB/c strain raised in a communal nest with multiple mothers under a constant ratio of mothers to young showed a faster rate of growth as evidenced by increased body weight at 19 days of age. The present experiment was undertaken to determine if these observations of increased weight gain could be extended to two other inbred strains of mice (albino A/J) and pigmented (C57BL/6J). Assessment of the offspring on several measures of activity was also undertaken to determine if the communal-nursing situation enhanced behavioral development. Additionally, litters of each strain were raised in a communal-nursing situation to evaluate the effects of mixed-strain rearing on growth and behavioral development.

METHOD

Nulliparous female mice of two inbred strains (albino A/J and black C57BL/6J), born and raised in this laboratory from stock obtained from The Jackson Laboratory, Bar Harbor, Maine, were mated to male siblings at 55 ± 5 days of age. Mice were housed under standard laboratory conditions in translucent plastic polyethylene cages (12.5 x 27.5 x 13.75 cm) with pine shavings as bedding material and mouse chow (Purina) and water available ad lib. Lights were on from 6 a.m. to 8 p.m. with temperature maintained at 22 ± 1.5°C. Exhaust fans provided approximately 10 air changes per hour and a constant background noise. Upon evidence of copulation (vaginal plug examination), males were removed, and the female was left undisturbed until parturition. At birth, each litter was examined and culled to five. This number represents the average litter size for these strains in our laboratory. Litters born on the same day were assigned with their natural mothers randomly to one of several treatment conditions. In the A/J strain, five litters were reared by same-strain, single mothers in the ratio of 1 mother : 5 offspring, and 12 litters were reared in pairs by same-strain multiple mothers in the ratio of 2 mothers : 10 offspring. In the C57BL/6J strain, five litters were reared by same-strain single mothers (1:5), and six litters were reared in pairs by same-strain multiple mothers (2:10). Six litters each of the A/J and C57BL/6J strains were paired together in the ratio of 2 mothers : 10 offspring. The number of litters described represents the total number of litters that completed the experiment after attrition. Eight litters had to be eliminated from the total sample, sometimes because of the death of one offspring, which altered the communal-nursing ratios.

Offspring from each litter were evaluated on a variety of developmental measures including observations of morphological characteristics, spontaneous and elicited activity on several tests of locomotor and swimming behavior. These tests were conducted on Days 1, 7, 14, and 21 (weaning), and a subsequent sample tested at 30 days of age. Only measures of weight, activity in the open field and on the inclined plane at 21 days of age, and activity in the water-submersion test at 30 days of age are to be reported. The open-field test consisted of a wooden box (45 x 45 x 21 cm) painted a flat gray with squares (5 cm) marked off. Mice were placed in the center of the field and received one 2-min trial. The number of squares traversed during this time period was recorded. A high score reflects a greater level of activity. The inclined plane was a mesh surface (45 x 45 cm) set at a 25- or 40-deg angle. Mice were placed in the center and permitted to proceed either to the top or to the bottom of the plane. As the majority of animals proceed to the top, the test is frequently used as a measure of negative geotaxic activity. Since this was not true of all mice, the measure recorded was the total time to reach the top or bottom for both the 25- and 40-deg angle setting.

Between weaning at 21 days and further testing at 30 days, mice were housed by sex, strain, and treatment under the same conditions described previously. The water-submersion test was conducted at 30 days with the apparatus, procedure, and parametric data from these strains reported previously (Werboff, Haggett, & Anderson, 1967). Mice were tested in water maintained at 46°C and with a weight in the ratio of 2 g : 30 g of body weight attached to their tails. Time from initial immersion to submersion for 5 consecutive seconds is recorded as the submersion time. A low score reflects higher levels of activity, since the animal that expends his energy rapidly submerges more quickly.

RESULTS AND DISCUSSION

Offspring of both strains reared in a communal-nursing environment with multiple mothers were heavier at 21 days of age than those reared by single mothers, in spite of the constant ratio of mothers to offspring (1:5 or 2:10). These data are presented graphically in Fig. 1A, in which offspring of each strain from the three experimental treatment conditions may be compared. Although statistical analysis of these data by one-way analysis of variance yielded inconclusive results, the direction of the data is consistent with that reported previously for the BALB/c strain (Sayler & Salmon, 1969). Observations of the animals demonstrated clearly that, within the communal-nest situation, a condition of multiple mothering existed, even in the mixed-strain treatment.

Behavioral assessment conducted at 21 days of age with the open-field test and inclined plane, and at 30 days with a test of water submersion, clearly demonstrated that on these measures of activity, there were strain-specific effects of the communal nursing treatments. The results of the open-field test are presented in Fig. 1B. These data were subjected to an analysis of variance and only the differences between treatment groups for the A/J strain were statistically significant ($F = 4.31$, $df = 2/66$, $p < 0.05$). Comparisons between the individual groups by the Newman-Keuls method show that these differences can be attributed to the increased activity of the A/Js reared with C57BL/6J mother and littermates as compared to A/Js reared with A/Js either singly or communally ($p < 0.05$ for both).

The data for the inclined-plane test are presented in Fig. 1C. A lower time score reflects greater levels of activity. For both the A/J and C57BL/6J strains, analysis of variance yielded significant results with

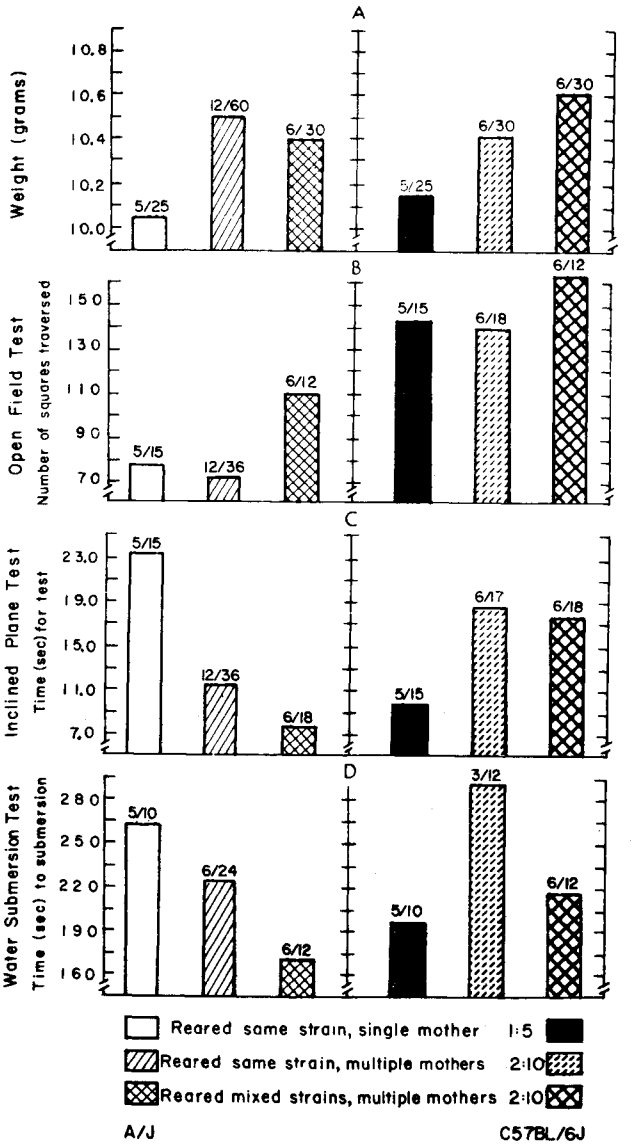

Fig. 1. Scores on various measures of growth and behavior of inbred mice (A/J or C57BL/6J) reared with same-strain mother in ratio of 1:5 or 2:10 (mothers : offspring) or mixed-strain mothers in ratio of 2:10. Numbers above columns refer to sample size tested: number of litters/number of offspring. (A) Mean weight in grams at 21 days of age. (B) Mean number of squares traversed in 2 min on open-field test at 21 days of age. (C) Mean time in seconds to traverse 22.5 cm of inclined plane set at 25 and 40 deg with test at 21 days. (D) Mean time in seconds to submersion in water set at 46°C with mice having weighted tails in ratio of 2 g to 30 g of body weight and test at 30 days of age.

communal nursing groups having higher activity in the A/J comparison ($F = 8.98$, $df = 2/66$, $p < 0.01$) and lower activity in the C57BL/6J comparison ($F = 3.95$, $df = 2/47$, $p < 0.05$). Evaluation of individual groups within each strain by the Newman-Keuls method showed that for the A/J strain, the group reared with their single mothers was significantly less active than both multiply reared groups ($p < 0.01$ for both), and there was no statistically reliable difference between the two communally reared groups of A/J. For the C57BL/6J strain, the singly reared group was significantly ($p < 0.05$) more active than both multiply reared groups.

Analysis of variance of the data in Fig. 1D from the water submersion test showed that the communal nursing condition resulted in significantly more rapid time to submersion for the A/J strain only ($F = 5.16$, $df = 2/43$, $p < 0.01$). Comparisons of individual treatment groups showed that A/J groups reared either singly or communally by the same strain mothers had longer time to submersion than the A/Js reared with C57BL/6J littermates and mixed-multiple mothers ($p < 0.01$ and $p < 0.05$, respectively, for the single- and multiple-mother conditions). Although the communally nursed C57BL/6J litters had longer time-to-submersion scores than groups reared by single mothers, this difference was not statistically significant.

There was a strain-specific effect of the communal-nursing or multiple-mothering conditions that consistently resulted in an increased level of activity for the A/J strain on the open-field, inclined-plane, and water-submersion tests. The fact that the mixed-rearing condition (A/J with C57BL/6J) potentiated the effects of the communal-nursing situation was not unexpected. A number of reports (Bovet-Nitti, Oliverio, & Bovet, 1968; Brumby, 1960; Reading, 1966; Ressler, 1962, 1963; Tenczar & Bader, 1966) are available to indicate that the effect of cross-fostering between strains of mice is to alter growth patterns and behavioral characteristics, with the offspring adopting some characteristics of the foster mother's strain. On all behavioral measures reported here, the C57BL/6J strain was significantly more active than the A/J strain when comparison was made between litters reared with their single mothers. Thus, the effect of increased stimulation from the active C57BL/6J littermates and mother resulted in raising the activity level of the A/J offspring. Similarly, the greater number of animals sharing the same limited living space in the communal A/J situation would also increase the amount of available stimulation and may account for the increased level of activity. The fact that the C57BL/6J strain was not affected in this same manner suggested that the base level of activity, which is considered to be genetically determined, may set the limit below which a particular strain may not respond to a given treatment. The effects of communal nursing and multiple mothering on growth and development, and behavior appear to be strain-specific and must be viewed in the context of the unique, genetically determined characteristics of each strain.

REFERENCES

BOVET-NITTI, F., OLIVERIO, A., & BOVET, D. Effects of cross-fostering on avoidance learning and freezing behavior of DBA/2J and C3H/He inbred mice. Life Sciences, 1968, 7, 791-797.

BRUMBY, P. J. The influence of the maternal environment on growth in mice. Heredity, 1960, 14, 1-18.

READING, A. J. Effect of maternal environment on the behavior of inbred mice. Journal of

Comparative & Physiological Psychology, 1966, 62, 437-440.
RESSLER, R. H. Parental handling in two strains of mice reared by foster parents. Science, 1962, 137, 129-130.
RESSLER, R. H. Genotyped-correlated parental influence in two strains of mice. Journal of Comparative & Physiological Psychology, 1963, 56, 882-886.
SAYLER, A., & SALMON, M. Communal nursing in mice: Influence of multiple mothers on the growth of the young. Science, 1969, 164, 1309-1310.
TENCZAR, P., & BADER, R. S. Maternal effect in dental traits of the house mouse. Science, 1966, 152, 1398-1400.
WERBOFF, J., HAGGETT, B. N., & ANDERSON, A. Swimming performance of mice: Time to submersion as a function of water temperature. Physiology & Behavior, 1967, 2, 39-43.

NOTES

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Sucrose preference in rats following amygdaloid lesions

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Preference for seven increasingly concentrated sucrose solutions was examined in amygdala-lesioned and control rats. Although the amygdaloid Ss tended to show less preference for .01 and .03 M sucrose, their performance was well within normal limits for the remaining concentrations. It is suggested that amygdaloid hyporeactivity to positive taste stimuli may be brief in duration.

A number of experiments have implicated the amygdaloid complex in motivational processes. Grossman (1963, 1964) found that electrical or chemical stimulation and ablation within the amygdaloid complex of rats reliably altered food and water intake. Schwartzbaum (1960, 1961) has also shown that operant response rates of monkeys are insensitive to changes in magnitude of reward and prolonged food deprivation following extensive amygdaloid lesions. Recently Kemble & Schwartzbaum (1969) found that short-term licking rates for 2%, 8%, and 32% sucrose solutions were reduced, while daily quinine solution consumption was temporarily *increased*, in rats following amygdaloid lesions. These results suggest the possibility that the amygdaloid complex may function, in part, to modulate the incentive properties of taste stimuli. The present experiment further explores this possibility, using a wider range of sucrose concentrations and the more traditional two-bottle preference test.

SUBJECTS

The Ss were 17 male albino Sprague-Dawley rats, 140-160 days old at the beginning of the experiment. These Ss previously served in an experiment to determine the effects of amygdaloid lesions on general activity levels.

All operations were carried out under clean conditions with sodium pentobarbital anesthesia (50 mg/kg). Amygdaloid lesions (N = 10) were produced by passing 2.0-mA current from a Grass radio frequency lesion maker through the uninsulated tip of a 24-ga hypodermic needle for 20 sec. Four Ss received control operations, and the remaining (N = 3) Ss served as normal control Ss. There were no reliable differences between the two control groups, and their data were pooled for further analyses.

All tests were conducted in S's (7 x 14 x 7 in.) home cage, which had been adapted to accept the drinking spouts of two 100-ml drinking cylinders which were mounted on the front of the cage approximately 4 in. apart. Seven sucrose solutions were used as test substances, with concentration increasing in equal log-molar steps (.001, .003, .01, .03, .1, .3, 1.0 M). Ss were tested for 2 days at each sucrose concentration, with the position of the sucrose cylinder reversed each day. The remaining graduated cylinder always contained tap water.

At the conclusion of the experiment, 40-micron coronal sections were prepared and stained with cresyl violet through the area of the amygdaloid lesions.

RESULTS AND DISCUSSION

The amygdaloid lesions in this experiment consistently damaged the cortical, basal, and central amygdaloid nuclei, while the medial (N = 8) and lateral (N = 7) amygdaloid nuclei frequently escaped damage. Occasional damage to the optic tracts (N = 2) and caudate-putamen (N = 3) was also noted but produced no detectable changes in performance.

Initial comparisons failed to suggest any reliable differences between the amygdaloid and control groups in total fluid consumption or body weight. Moreover, both groups showed a reliable increase in fluid consumption with

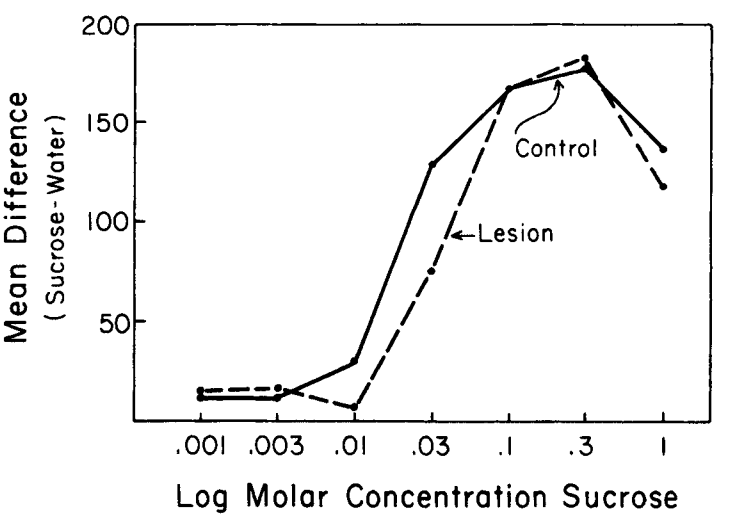

Figure 1.