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THE EFFECTS OF LITTER SIZE ON BEHAVIORAL DEVELOPMENT AND ADULT EMOTIONALITY IN THE RAT

BY

ELIZABETH N. PULLY

A THESIS SUBMITTED TO THE GRADUATE FACULTY OF THE UNIVERSITY OF RICHMOND IN CANDIDACY FOR THE DEGREE OF MASTER OF ARTS IN PSYCHOLOGY

MAY 1977

APPROVAL

THE EFFECTS OF LITTER SIZE ON BEHAVIORAL DEVELOPMENT

AND ADULT EMOTIONALITY IN THE RAT

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ACKNOWLEDGEMENTS

Many fine people helped carry me through the mire of diseased rats, funky rat excreta, and the reams of papers covered with numbers and other hieroglyphics associated with this thesis experiment and paper preparation. First, I thank my brothers, Randy and Thomas, for accompanying me on late night (early morning) jaunts to the laboratory and counting rat bowel movements. Second, I would like to thank Rita for her assistance with cage-cleaning and other odious tasks on otherwise pleasant Sundays. My appreciation also goes to those who served on my committee: to Dr. Walker, for his help with making sense out of numbers and to Dr. Tucker, for reading my paper and for encouragement. I especially want to credit Dr. Kozub for his patience, encouragement, questions, answers, and omissions. And I thank Jane Williams for typing (from a trying rough draft).

Very special thanks are extended to my parents, who kept the faith throughout a prolonged endeavor. My mother, for visiting loathesome rodents with me, for typing, being tactful, receives my gratitude. And I want to express appreciation to my father for helping me build an open field, drawing some terrific graphs, Xeroxing copies, cleaning cages, capturing crazed, escaped rats, and encouraging rather strongly. Lastly, I extend reluctant mixedfeeling thanks to Norwood and the rest of the rats who survived long enough for me to finish the study.

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ABSTRACT

The effects of the early experience variable of litter size is examined at two developmental time periods (infancy and adulthood) in the rat. The study departs from the majority of the early experience experimentation in its use of a naturally occurring independent variable in place of the typical handling and shocking procedures.

Large (n = 9), medium (n = 5), small litters (n = 3) were developed by pooling rat pups at birth and then distributing them to randomly selected, lactating females. Observations of behaviors (including maternal grooming) during the preweaning stage of development disclosed substantial differences in response activity in the three litter size groups. Weaning weights also varied significantly across the experimental groups. Generally, the results of infant behavior observations and weights indicated that the medium litter size was the most efficacious in terms of development and weaning weights. The findings obtained from the observations of infant behaviors were attributed to differences in environmental stimulation afforded by the three rearing conditions.

Analysis of adult open field behaviors (i.e., ambulation and defecation) failed to reveal any variation between the experimental groups. Adult weights also did not differ. Results were interpreted associatively. Strain differences, age of adult testing, and the testing situation were also discussed as

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possible factors influencing the results. And, conceivably, the conclusion that litter size effects were transitory and disappeared in adulthood can be made.

Introduction

Research on the effects of early experience on the rat indicates that stimulation encountered in the preweaning period of development produces more viable, less emotional, and better performing adults (e.g., Denenberg & Karas, 1961; Levine & Broadhurst, 1963; Levine, 1956). The experimenters usually administered varying amounts and degrees of shock and handling as the forms of antecedent stimulation. Strain differences (Levine & Broadhurst, 1963) and temperature change (Schaefer, et:al., 1962) have been implicated in the search for crucial factors through which infantile stimulation exerts its influence on subsequent emotionality and behavior. However, relatively little attention has focused on naturally occurring variables (e.g., litter size, maternal care) within the initial experience of the infant rat. Since several studies have reported or suggested that such variables are significant factors in the determination of later-life performance (e.g., Seitz, 1954; King, 1969), it becomes obvious that further investigation is necessary in order to establish firm empirical validation of early experience effects.

Support for the hypothesis that handling decreases adult open field emotionality is both behavioral and physiological. Early experience experiments have typically employed shock and

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handling as the methods of producing infantile stimulation. Denenberg and Smith (1963) revealed that handling prior to weaning (at 21 days of age) reduces open field defecation scores and increases ambulation. Similar studies (i.e., Scott et al., 1951 and Denenberg & Morton, 1962) concur with the conclusion that handling reduces open field emotionality. Examining changes in plasma corticosterone levels following exposure to the open field situation, Levin et al. (1967) found that rats handled in infancy were reliably less emotionally responsive (i.e., had lower steroid levels) than the control rats.

When presented with an active avoidance task during adulthood, rats handled from Day 1 to Day 20 were significantly superior (in the acquisition of an avoidance response) to rats who received either no handling at all or handling from Days 50 to 70 (Levine, 1956). Further demonstration of the facilitative effects of handling during infancy upon adult avoidance learning has been provided by Levine et al. (1956) and Denenberg (1962).

In addition to the studies which have shown the effect of early stimulation (via handling) upon adult performance variables, the relationship of early experience to later viability has also been explored. Denenberg & Karas (1961) found an increase in viability which they attributed to handling experience during the first 20 days of life. Hence, rats handled throughout infancy survived significantly longer than untreated controls when placed on a terminal food and water deprivation schedule during adulthood. The finding was replicated, later, by Denenberg (1962).

Not only has handling been shown to produce less emotional, more adaptive behavior, but shock administered during a comparable period in development has been shown to produce similar effects. Denenberg and Smith (1963) handled or shocked rats during the second ten days of life and found that open field ambulation scores were higher and defecation scores were lower for both treatment groups than those for the unmanipulated controls. Although the treatment groups did not differ significantly, the shocked rats were generally less emotional. Their conclusions provide support for an experiment by Levine et al (1956) which also revealed that rats shocked daily during infancy are similar in emotionality to the handled, unshocked subjects. Further delving into the variable of electrical shock administered during infancy, Denenberg and Kline (1964) demonstrated that early shock exerts a facilitative effect on the acquisition of active avoidance responses in adulthood. Earlier, Denenberg and Smith (1963), however, found that early shock experience impairs subsequent adult avoidance performance. In other words, the shocked rats took significantly longer to respond than either the handled or control rats in an active avoidance task. Henderson (1967), using BALB/c mice in a matched pairs design, failed to find significant effects of early shock experience on either open field or avoidance behaviors.

Following the demonstration of the importance of infantile experience on subsequent performance, experimentation became directed toward the isolation of critical periods and the

discovery of the crucial variables in the production of the handling phenomenon. Schaefer (1968) described an experiment aimed at delineating maximally effective developmental periods for reduction of emotionality in adulthood. Three groups of subjects were handled during the first, second, or third weeks of life. A fourth group received treatment throughout the first three weeks of life. The control group was left undisturbed. Open field indices were used in ascertaining subsequent adult emotionality. Reliable reduction in defecation and increase in ambulation were exhibited by the group which received handling only during the first week of life. Members of the group which received treatment during the third week of life crouched significantly more than the nonhandled group. The group which was handled throughout the three weeks did not differ from the control group. Levine and Lewis (1959) had provided earlier confirmation for a critical period placed during the first week of infancy for the production of the handling phenomenon. Also, Denenberg (1962) demonstrated that handling in the first five days of life is maximally important in lengthening survival time when the rat is placed on a terminal deprivation schedule in adulthood.

Other factors explored in relation to the effects of early experience include strain differences and temperature change. Levine and Broadhurst (1963) found that handling produces more significant changes in emotionality (as measured by defecation and ambulation) in emotionally reactive strains of rats. Schaefer et al. (1962) revealed that temperature change is the crucial

variable in production of the handling phenomenon. Provided that the rat's body temperature drops at least 2° C, reduction in adulthood emotionality can be achieved--without employing the actual handling procedure. Further, handling without the concomitant temperature change is ineffective (Schaefer, 1968). However, if the body temperature drops $8 - 10^{\circ}$ C during infancy, the rats will take longer to acquire an avoidance response in adulthood (Schaefer et al., 1962).

Most researchers, then, agree that early stimulation effects a kind of "emotional immunization" (Levine & Mullins, 1968). Thus, exposing an organism to stress early in development serves to reduce emotional responses to subsequent stressful situations. However, hypotheses on the exact nature of the functional relationship between early experience and later behavior are rather muddled and contradictory. In 1962, Denenberg postulated that an inverted U-shaped function exists between the two variables. He reached this conclusion after testing groups (which had undergone five different durations of handling in infancy) in an active avoidance set-up. Rats which were subjected to five days of handling made more avoidance responses than those who received only three days of treatment. Better still were the rats which had ten days of handling. The worst performers of all were the rats in the 20-day group. The results indicated that there can be too much, as well as too little, stimulation during infancy. Concurring with the hypothesis of the inverted U-shaped function is Schaefer's (1968) finding that handling experience throughout the

first three weeks of infancy impaired adult open field performance while handling during the first week of infancy improved performance in the open field situation. Later, Denenberg (1964) maintained that the greater the amount of stimulus input during the preweaning period of development, the lower the adult level of emotional reactivity. In one study, the early experience of three minutes of shock per day during the second ten days of life subsequently reduced emotionality in the rats to such an extent that avoidance learning was considerably impaired (Denenberg & Smith, 1963). This generalization draws further support from Denenberg & Karas (1961). Employing handling and shocking as early experience variables, Goldman (1965) disclosed a curvilinear relationship between infant experience, sex, and adult open field ambulation (the measure she used as defining emotionality). The results revealed that handling during infancy decreased adult emotionality, whereas shocking did not. Hence, Goldman denied the monotonicity hypothesis. Still another study indicated that the relationship may not be adequately expressed merely as inverted U-shaped or monotonic. Bell and Denenberg (1963), though describing the overall relationship between early experience and later avoidance performance as curvilinear, found a triple interaction between the age at which the stimulation is administered, the intensity of the infantile stimulation, and the intensity of the stimulation when the subsequent learning task is presented (in adulthood). Henderson (1967), however, failed to discover any significant interactions in his early experience experiment.

The prevalent interpretation of the effects of early stimulation on adult behavior nonetheless maintains that early stimulation endows the organism with the ability to respond less emotionally and hence, more effectively in a novel (stressful) situation. The view is most often empirically grounded on behavior in the open field (since studies using avoidance behavior are not always supportive.) There exist, however, serious doubts pertaining to the implicit assumption that the operationalizing of the concept of emotionality through open field behavior can be adequately justified (Hinde, 1966).

The concept of emotionality, as commonly used in the early experience literature, is a loose one. Behaviorally, the concept is defined in terms of an inverse relationship between defecation and ambulation scores obtained in the open field situation. Pare (1964) examined open field ambulation and defecation measures in order to ascertain whether or not the assumed inverse relationship actually existed. His data failed to yield a significant correlation between the two behavioral indices under examination. He concluded that ambulation and defecation are independent behaviors and questioned the validity of using ambulation as an index of emotionality.

Hayes (1960) drawing from open field activity scores and emergence latencies, maintained that activity is inhibited by fear since rats with the longer latencies to emerge into open field were also the least active when confined to the open field situation. Whimbey and Denenberg (1967), however, pointed out the

inconsistency of open field activity scores, especially those obtained on the first day of testing. Not only has the data relevant to open field activity been contradictory, but also the interpretations offered of that data. As Welker (1959) indicated, the initial increase in ambulation exhibited in open field testing may be an escape response instead of the commonly assumed exploratory or curiosity response (which would indicate that the response is an emotional one.)

In a factor analytic study, Whimbey and Denenberg (1967) examined the relationship between defecation and activity scores obtained in the open field situation. Activity scores taken from Days 2, 3, 4, and 14 weighed heavily on the "exploration" factor. Scores from Day 1 weighed only slightly on that factor. Five defecation measures, as well as the activity scores from Day 1, loaded positively on the "emotional reactivity" factor. Hence, a high activity score obtained on the first day of testing in the open field situation may mean either high emotionality or high exploratory behavior, or both. Whimbey and Denenberg verified that an organism's exploratory drive level and emotional reactivity are not inversely related functions along the same continuum as is commonly assumed. Instead, the two factors are independent. Further, they contend that the defecation measure is the only open field index of emotionality to be consistent.

In addition to the conceptual difficulties encountered in evaluating experiments on the effects of early experience on adult behavior, there exist also several methodological problems which

preclude the drawing of unequivocal conclusions. Two sources of uncontrolled variance, pertaining to either subject or test conditions, confound experimental results and render them ambiguous. Test conditions, such as room surroundings, apparatus and temperature are usually kept constant. However, time of testing and observer bias, two potentially confounding variables, are often neglected. Henderson (1963) found time of testing to be a significant factor affecting the scores of the open field activity test and proposed that testing at a given time of day would allay the confounding. Observer bias can be eliminated by either employing automatic scoring devices or standard doubleblind techniques. Subject conditions such as age, sex, and rearing are commonly controlled while genetic and maternal care factors are not. Henderson (1963) demonstrated that the genetic factor significantly influences open field ambulation scores. Split-litter techniques or modified cross-fostering can effectively eliminate this source of variance.

Results of early experience studies which have included appropriate controls for the subject conditions outlined by Henderson (1963) characteristically differ from the results of experiments which have omitted such controls. Henderson (1967) failed to find significant early shock effects on either the open field defecation or ambulation measures when controlling for litter effects in the BALB/c mouse. Including controls for litter effects in his experimental design, King (1969) did not discover any significant early experience effects or any trends toward significance

on either body weight or maturational variables.

In a more recent study, King (1970) included a statistical control for the litter variable in an attempt to determine the effect of early experience on later-life emotionality in the rat. He found no significant main effects of either infantile handling or litter on subsequent performance in the open field or emergence situations. Further, he ascertained no trends toward significance. Thus, King's (1970) study contradicts those of Henderson (1963, 1967) in which significant main effects of litter were found on open field defecation and ambulation indices.

While some experimenters have been intent upon instituting statistical controls for litter effects, others (e.g., Seitz, 1954; Ader & Grota, 1970; Broadhurst & Levine, 1963) have looked at litter variables as influential factors in the later development and behavior of the organism. Thus, litter size was included in studies as an independent variable and the possibility of an early litter effect on later open field behavior recognized.

Investigating the effects of experience during infancy upon body weight and behavior in the rat, Seitz (1954) employed litters of 12 and 6 pups. Observations of maternal behavior made from Day 5 to Day 15 revealed that mothers with litters of six made significantly more maternal responses (e.g. nursing, retrieving pups, nest building) toward their young than mothers assigned to litters of 12. As early as three weeks after birth and as late as two months after birth, the mean body weight of rat pups reared in small litters was significantly greater than the mean body weight

of the large litter pups. Members of small litters also consumed significantly more food following a deprivation period and required less time to obtain food in a novel situation than members of large litters when tested at five months of age. Open field testing, conducted when the rats were seven months of age, revealed that rats reared in litters of six explored significantly more and defecated significantly less than rats reared in litters of 12. Thirteen-month old rats from small litters also emerged from the home cage significantly more times than rats from large litters. Seitz concluded that infantile experiences are a more profound influence on behavior in adulthood than similar (postweaning) or adult experiences. His findings in the open field situation based on the early experience variables of litter size and maternal behavior, concur with the findings of other studies (e.g., Denenberg & Smith, 1963) which employed the preweaning variables of shock and handling.

In their 1970 study of maternal behavior in the rat, Ader and Grota contradict somewhat Seitz's observations on maternal behavior. They found that, regardless of the size of the litter, the mother rat preserves a clear diurnal cycle. Thus, she spends the greatest amount of time with her pups midway through the 12hour light period and the least during the dark period. The duration of the time spent with the pups is the same regardless of the size of the litter. Since the time spent with the pups is the same, it seems logical to suppose that the same quality and quantity of maternal responses occurs during that time for both

large and small litters, even though the amount of individual attention bestowed upon members of large litters would necessarily be less.

In relation to Seitz's finding that the body weights of pups from small litters were greater than those of large litter pups, Kennedy (1967) found that rats from litters of 12 reached virtually the same weight as rats from litters of five by three weeks of age. He suggested that the supply of milk becomes adapted to the demand. Thus, a mother having a litter of 12 increases her own food intake in order to accommodate her young. Although this discovery is inconsistent with that of Seitz, Kennedy did find weight differences when using litter sizes more outside the "normal" range. At 21 days of age, rats reared in groups of three weighed 40 grams while those reared in groups of 15 weighed less than 20 grams. Further, pups from litters of three grew faster to a higher target weight. Rats from the large groups underwent a period of compensatory growth following weaning and were able to restore all but the most extreme body weight losses accrued during their undernourished infancy. In other words, delays in development were made up after weaning. The differences in body weight between large and small group females disappeared by six months of age while differences among the males' body weights remained.

King (1969) found litter effects on both body weight and some maturational variables (including eye opening, body and tail length.) The effects of litter on body weight, he observed, attenuates as the rat grows older. King suggested that litter

size may transmit the litter effects. Rats reared in large litters were shown to open their eyes later, have shorter body and tail lengths, and weigh less at weaning. Lore and Avis (1969) also discovered that rats from litters of three weighed more at weaning than rats from litters of nine.

Using litter sizes of ten to twelve and three, Lore and Moyer (1973) detected reliable weight differences in their rat subjects at adulthood, as well as at weaning, thus contradicting both Kennedy (1967) and King (1969). Rats from litters of three were heavier when weaned and at 71 to 84 days of age than the rats from the larger litters.

Subsequent research investigating the possible effect of litter size on adult behavior has not always been in concurrence with Seitz's (1954) study. Broadhurst and Levine (1963) reported a lack of reliable differences in open field defecation and ambulation scores, as well as in avoidance responses between rats from litters of two to three and rats from litters of five to nine. They conceded, however, that the small litter's lower defecation and higher ambulation may be taken as evidence of a reduction in emotionality. In rebuttal, Denenberg (1963) pointed to his earlier confirmation (using litters of four and eight) of Seitz's findings and proposed that a reasonable explanation for the apparent contradiction lies in strain differences of the rats used in the two studies. Seitz (1954) employed subjects of the Wistar strain while Broadhurst and Levine (1963) used Maudsley Reactive and Nonreactive stock.

Using litters of both Wistar and Long-Evans rats, Lore and Moyer (1973), in partial agreement with Seitz (1954) and Denenberg (1963), found that subjects from the small (n = 3) litters were more active in the open field situation than rats from the large (n = 10-12) litters. They failed, however, to unveil any significant difference in the number of fecal boli produced by members of small and large litters in the open field.

Lending support to Broadhurst and Levine's (1963) inferences, Grota and Ader (1969) manipulated litter size in order to study the effects of early life experiences on adult emotionality, adrenocortical reactivity, and susceptibility to ulcers. Using litters of four pups and 12 pups, they found no differences in emotional reactivity as measured by the reaction to handling (open field) test or susceptibility to gastric erosions. However, plasma corticosterone levels were significantly higher for rats raised in litters of 12 which suggests that large litter rats may be more "emotional."

Priestnall (1973) employed mice (Mus Musculus) in an experiment to test the effects of litter size on open field behavior. Although mice from small litters explored more in the open field, the two groups were virtually the same in terms of defecation. Earlier, La Barba et al. (1971) had distributed BALB/c mice into litters of nine and three. In the open field situation the small litter subjects were reliably more active and the large litter subjects defecated significantly more. Hence, the experimenters concluded that the large litter mice were more emotionally reactive

than the small litter mice.

Since the results of studies on infantile experience which have included litter effects or litter size as experimental variables have fallen short of clarifying the relationships of early experience and later behavior, and instead, compound the confusion, it seems necessary to undertake further research in the area. The present study is directed toward determining what relationships, if any, exist between litter size, preweaning behavior, and later behavior in the open field.

Intuitively, litter size would seem to affect both the quality and quantity of infantile stimulus input, both from the mother and from the litter mates. Indeed, a few experimenters have alluded to the possibility. Priestnall (1973) guessed that small litter pups receive more attention from their mother than large litter pups. Likewise, La Barba et al. (1971) speculated that the greater emotional reactivity of mice reared in large. litters might be due to a decrease in maternal stimulation. Only a few experimenters (e.g., Seitz, 1954; Ader & Grota, 1970) have attempted to go beyond intuitive assumption toward empirical validation of differences in maternal behaviors. Seitz's (1954) observations, while representing an effort to assess maternal behaviors toward litters of two sizes, are nonetheless confounded by his procedures of weighing the pups every three weeks and conducting frequent behavioral tests during infancy. In other words, the experimenter's "handling" of the litters undoubtedly interfered with naturalistic observations of the mother rats' responses.

Another shortcoming uncovered in Seitz's study is the glaring lack of observations prior to the fifth day of the pup's life. Many later studies (e.g., Schaefer, 1968) have credited the first week of the infant rat's life with maximal importance in the production of the handling phenomenon. Schaefer observed the pattern of maternal absences during the preweaning period. He found that until Day 3 or 4, the mother's pilgrimages from her cage are short and uniformly distributed. After Day 3 or 4, the absences lengthen in duration and increase in frequency. Thus, Schaefer's study yielded data to support the importance of the first few days of rat infancy. An experiment, such as Seitz's, which has neglected to include any observations during this developmental period, can hardly be taken as the last word on early experience effects.

Ader and Grota (1970), in their observations of the rhythmic processes of maternal behavior, avoided some of Seitz's pitfalls by employing dual chamber maternity cages with automatic recording devices for obtaining frequencies and durations of the times the mother rat spent with her pups from birth to weaning. They found that the mother spent more time with her young during the 12-hour light period and less during the dark period. The cyclicity they found persisted even though the duration of the periods the lactating female spends with her young progressively decreased from birth until Day 18 or 19. Thus, additional data were found which pointed to the importance of the first few days of the rat pup's life---the days that Seitz eliminated from his observations.

While a paltry number of experimenters (e.g., Seitz, 1954; Ader & Grota, 1970) produced studies relating litter size to maternal behavior, there is a noticeable dearth of research efforts concerning litter size and the ontogeny of infant rat behaviors. With the exception of a study by Bolles and Woods (1964), there are few experiments which bear directly upon behavior and development in the rat. Since the general finding gleaned from early experience research suggests that the preweaning stage has considerable impact on later adult behavior, the paucity of information on this period represents a surprising oversight.

Observing litters composed of about nine pups each, Bolles and Woods (1964) charted the development of behavior and quantified the amount of time given to functional responses (i.e., sleep, consummatory, locomation, grooming, exploration, social) in the infant rat. Sleep, though fitfull and constantly interrupted by burrowing and climbing for preferred positions, was the predominant activity of the infant rat subjects. However, there was evidence of a decline in total sleep time as a function of age. By Day 19, the pups slept more peacefully (that is, they devoted less sleeping time to climbing and burrowing.)

At first the pups spent much of their waking time nursing. Until Day 10 when the rats began to slack off, lengthy nursing periods, which endured for as long as 30 minutes, were noticed. The pups exhibited their first interest in hard food on Day 15 and in the water fount on Day 17. Thus, consummatory responses of

eating and drinking began to take the place of nursing in their behavioral repertoire.

Generally, the infant rats displayed more locomotion responses than adults. Shortly following birth, the rats commenced dragging themselves around the cage by their forelegs. They began crawling about on four legs with their bellies dragging on Day 3. On Day 10 the rat pups substituted walking for crawling and on Day 13 they ran. Also, on Day 13 the pups started fighting and playing with their littermates. Jumping became proficient by Day 15 and climbing by Day 18.

Bolles and Woods included observations on the three components of grooming activity: face washing, scratching, and fur licking. The predominant grooming response by the maturing rats, face washing, appeared and became functional between Days 3 and 5. Scratching evolved later between Days 10 and 11. The fastest. increase was found in fur licking, which first appeared on Day 13 and which is the predominant grooming activity in the adult rat. Social grooming became common around Day 15.

In the category of exploratory behavior (i.e., expansion of environmental stimulation when the rat becomes isolated), sniffing was unequivocally evidenced by Day 11. On Day 13 the rats first manipulated and carried boluses, nesting material, and food pellets. Generally, infant rats handled and mouthed objects more than their adult counterparts.

Although Bolles and Woods confirmed the existence of diurnal cyclicity in the mother rats' activity, they failed to discern any

evidence of cyclicity in the infants' behavior. The finding held throughout the preweaning period.

Although Bolles and Woods (1964) compensated for the deficiency of observations on the infant rat, there still remain questions concerning the relationship of litter size and infant rat behaviors. Several studies inferred that the two variables are related. Both Kennedy (1967) and King (1969) suggested that rats reared in small litters mature faster physically. Additionally, Seitz (1954) found small litter rats weighed more than those from large litters. However, none offered any mention of behavioral maturation. It seems logical to surmise that differences in behavioral development do in fact exist since there are indications of differences in physical development. But without empirical evidence, the supposition remains a guess. The present study will look at the responses cited by Bolles & Woods (1964) in order to ascertain whether or not the rate or character of behavioral maturation of small litter rats differs from that of large litter rats.

Hence, the present study will not focus singlemindedly upon the exhibition of litter size effects on adult behavior. Infant behaviors will also be scrutinized. An effort will be made not only to know the effects of early experience on adult behavior, but also the effects of early experience on infant behavior.

Method

Subjects

Fourteen litters containing a total of 62 Sherman albino rats provided subjects for the present study. At the onset of the observations of infant behaviors, the $\underline{S}s$ were approximately 24 (±8) hours old. Adult open field testing commenced when the Ss reached 98 days of age.

Apparatus

Adult open field testing was conducted in a plywood box with a 36-inch square base and 18-inch high walls. The inside surfaces of the box were painted flat black except for white lines which divided the floor into 25 squares with 7-inch sides. The field was illuminated by a 25w red light bulb suspended (from one corner of the box) approximately 26 inches from the floor.

Procedure

Twenty-two female rats, presumably at the end of the second week of pregnancy, were obtained from a laboratory supply house. Housed in standard laboratory cages, the females were provided with food and water <u>ad libitum</u>. Nesting material, consisting of torn-up strips of paper towel, was placed in the cages at the end of the third week of gestation. The animals were maintained under a light/dark schedule of 12 hours on/12 hours off throughout the course of the study.

Eight 40w white light bulbs remained on during the 12-hour period of light. During the 12-hour period of darkness, eight 25w red light bulbs furnished illumination for the investigator. Temperature was maintained at $70^{\circ}F \pm 5$.

Of the 22 "pregnant" females, 17 delivered a total of 127 rat pups. Litter sizes ranged from three to 15. Litters (born within 24 hours of each other) were taken from the natural mothers and pooled in a standard laboratory cage containing tornup paper towel strips. From the resultant four poolings of rat pups, 14 litters were formed. Four of the litters contained nine pups each, four litters contained five pups each, and six contained a total of three pups each. The litters were then placed with 14 randomly selected lactating females. The remaining rat pups were either sacrificed or placed with the unchosen lactating females and disregarded for the remainder of the study.

The four litters of nine pups comprised the large litter group (Group 1), the four litters of five each comprised the medium litter group (Group 2), and the six litters of three each made up the small litter group (Group 3). Of the four litters in Group 1, three were randomly selected for the infant behavior observations. The remaining litter was retained under identical conditions in case a litter had to be replaced. Three litters in Group 2 were selected for the observations with the fourth litter used as a back-up group. Five Group 3 litters were used for observations and the sixth kept in reserve. Back-up litters in both Group 2 and Group 3 had to be used during the observation of infant behaviors due to infant mortality in the other litters.

Following assignment of rat pups to the three litter groups and selection of the litters within each group to be employed in the observation of infant behaviors, three (randomly selected) pups in each Group 1 and Group 2 litter and all the pups in Group 3 were marked with a permanent black marking pen. Markings were made

on different parts of pups' backs in order to differentiate the <u>Ss</u> within each litter. Markings were remade every three days throughout the observational phase of the study.

Observations of infant responses in each litter size group began on Day 1 of the pups' lives and continued throughout infancy until weaning at Day 21. The observations of the selected infant behaviors took place once daily during the 12-hour period of darkness. The hour during which behaviors were recorded was randomly selected for each day of the pups' infancy. Within each of the three groups (small, medium, and large litter), one litter was randomly selected for each observation period. The three marked pups in the selected litters were the <u>Ss</u> for their groups, and <u>E</u> recorded their responses. Further, the order in which the small, medium, and large litter <u>Ss</u> were viewed was randomized throughout the taking of behavioral data.

The individual observation periods (of each litter group) were 20 minutes in duration and divided into 30-second time intervals. By scanning the three marked <u>Ss</u> in each litter, the <u>E</u> ascertained and recorded the ongoing behaviors within the litter for each 30-second interval. Hence, time samplings of infant behaviors were gathered for 21 days for each of the three litter size groups. Standard recording sheets were employed by the observer in order to facilitiate an accurate behavioral record.

The infant behaviors which were observed and recorded are those which comprise the category of functional activities previously developed by Bolles and Woods (1964).

The first group of behaviors that were observed were those related to sleep and rest. The activities in this class occurred alone or with littermates. Lying in the nursing position (without actually nursing) or partly obscured behaviors were considered sleep and rest responses. Consummatory activities such as nursing, eating, and drinking were also recorded.

The three components of grooming (washing face, scratching, and licking fur) were recorded. Maternal grooming was also observed and treated as a separate category.

Locomotor responses were recorded and included crawling, walking, running or jumping, and climbing. Exploratory responses such as sniffing, manipulating objects, and rearing were also observed as a distinct category.

Although incorporating several behaviors observed in other contexts, social responses were noted. These included burrowing, fighting and playing, and social grooming.

Following weaning, all of the rats in each of the three groups were weighed and then housed individually in standard wire laboratory cages under the same conditions of light/dark, temperature, and feeding previously described for the pregnant females.

At 98 days of age, 12 rats from each of the three groups were randomly picked to participate in adult open field testing. Testing was conducted during a stable two-hour period on four consecutive days. Each <u>S</u> was placed in a corner of the field by an independent assistant and ambulation was recorded by <u>E</u> (who was unaware of the <u>S</u>'s litter size group). The total ambulation

score for an individual \underline{S} was composed of the number of times the head and forepaws crossed a line into a new square. Trials were three minutes long. Following the removal of the \underline{S} from the field, the number of fecal boluses was counted. The total was taken as the defecation score. After the boluses were counted, the field was wiped clean. The order of testing the \underline{S} s was randomized for each period of testing by the assistant.

After the completion of the adult open field testing, the <u>Ss</u> were again weighed. They were 102 days of age at the completion of the study.

Results

Infant Behaviors

Tables 1 through 3 display the frequencies of occurrence of functional activities in each of the experimental groups during infancy. In order to expedite presentation and analysis of the 63 behavioral samplings, observations taken on three consecutive days were grouped together. Hence, seven developmental time periods (i.e., Days 1-3, 4-6, 7-9, 10-12, 13-15, 16-18, 19-21) were employed in presenting and analyzing the data. Each category of functional activity (e.g., grooming, locomotion) was considered separately though responses within the categories (e.g., face washing, fur licking) were combined for the statistical analyses. Following the individual examinations of the categories of infant responses, the three litter size groups were viewed in terms of overall rate of development.

The results of observations of maternal grooming, actually a

•	Days of Age							
Behavior	1-3	4-6	7-9	10-12	13-15	16-18	19-21	Total
Sleep/rest								
Alone	11	0	1	0	0	0	0	12
With litter	94	69	183	163	107	215	55	886
Nursing position	41	8	8	7	0	0	0	64
Partly obscured	151	101	26	9	23	10	5	325
Consummatory								
Nursing	117	278	157	94	168	157	0	971
Eating	0	0	0	0	5	1	180	186
Drinking	0	0	0	0	0	1	6	7
Grooming								
Wash face	0	4	6	21	20	7	18	76
Scratch fur	0	0	6	25	20	13	23	87
Lick fur	0	0	0	6	8	9	33	56
Locomotion			i.					
Crawl	17	18	89	115	31	17	18	305
Walk	0	0	3	23	38	22	22	108
Run/jump	0	0	0	0	10	6	12	28
Climb	0	0	0	3	26	15	51	95
Exploratory	ļ							
Sniff	0	0	0	2	23	20	49	94
Manipulate	Ō	Ō	Ō	2	8	12	17	39
Rear	Ō	0	0	0	0	4	12	16
Social			н 					
Burrow	83	38	35	24	16	4	8	208
Play/fight	0	0	0	0	2	6	18	26
Groom	0	0	0	16	23	15	10	64
Maternal Grooming	26	24	26	30	12	6	. 3	127

TABLE 1 Frequency of Occurrence of Functional Activities in Group 1 (Litter Size = 9)

	Days of Age							
Behavior	1-3	4-6	7-9	10-12	13-15	16-18	19-21	Total
Sleep/rest			,					
Alone	14	13	3	4	1	0	3	38
With litter	129	178	190	85	157	143	169	1051
Nursing position	20	30	24	4	1	0	0	79
Partly obscured	128	86	28	14	9	2	1	268
Consummatory								
Nursing	1.57	46	79	79	84	189	0	634
Eating	0	0	0	0	0	23	100	123
Drinking	0	0	0	0	1	0	6	7
Grooming								
Wash face	0	1	9	23	33	4	4	74
Scratch fur	0	1	5	13	36	10	17	82
Lick fur	0	0	0	6	41	8	13	68
Locomotion								
Crawl	35	122	104	98	32	14	19	424
Walk	0	0	0	36	24	11	33	104
Run/jump	0	0	0	2	11	12	12	- 37
Climb	0	0	2	8	1	35	51	97
Exploratory								
Sniff	0	0	2	38	23	21	59	143
Manipulate	0	0	. 0	9	26	4	6	45
Rear	0	0	0	0	1	4	11	16
Social								
Burrow	27	23	10	12	13	8	3	96
Play/fight	0	0	0	0	1	4	12 9	17
Groom	0	0	10	49	32	20	9	120
Maternal Grooming	30	40	74	60	13	28	12	257

Frequency of Occurrence of Functional Activities in Group 2 (Litter Size = 5)

TABLE 2

ГΑ	BI	E	3

					Days of	Age		
Behavior	1-3	4-6	7-9	10-12	13-15	16-18	19-21	Total
Sleep/rest								
Alone	14	47	27	7	4	2	0	101
With litter	155	120	188	117	322	45	8	955
Nursing position	24	28	16	13	5	2	. 0	88
Partly obscured	113	66	50	21	15	9	35	309
Consummatory								
Nursing	66	63	124	164	51	225	75	768
Eating	0	0	0	0	0	17	22	39
Drinking	0	0	0	0	0	0	10	10
Grooming								
Wash face	0	1	1	7	6	14	13	42
Scratch fur	0	1	6	3	13	15	22	60
Lick fur	0	0	1	6	17	31	30	85
Locomotion						•		
Crawl	77	128	84	78	24	13	- 4	408
Walk	0	0	0	7	10	18	31	66
Run/jump	0	0	0	0	0	2	18	20
Climb	0	0	0	0	1	11	49	61
Exploratory								
Sniff	0	0	0	41	30	53	86	210
Manipulate	0	0	0	3	8	12	35	58
Rear	0	0	0	1	2	6	16	25
Social								
Burrow	15	4	2	2	9	9	.7	48
Play/fight	0	0	0	0	0	2	20	22
Groom	0	0	0	2	9	25	39	75
Maternal Grooming	76	82	41	68	14	29	20	330

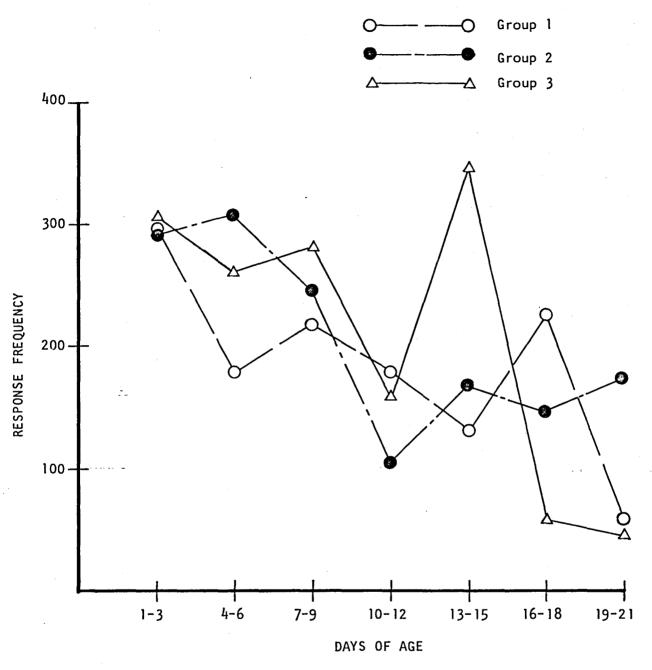
Frequency of Occurrence of Functional Activities in Group 3 (Litter Size = 3)

form of infant stimulation rather than response, are presented at the end of the present section.

Figure 1 contains the frequencies of sleep/rest responses emitted by the three experimental groups during the periods of infant behavioral observations. Each point in the graph represents a three-day block of observed incidences of sleeping alone or with littermates and lying in the nursing position or partly obscured. Expected frequencies were calculated for each one of the seven blocks of three days' observations for the three litter size groups. A comparison of the observed and expected frequencies (Table 4) yielded a Chi square value of 378.95 (12 degrees of freedom), significant at the .01 level. Thus, the differences in sleep/rest activity obtained among the three groups over the period of infancy are reliable.

Though all groups of rat pups slept or rested over one-third of the time they were observed, comparisons of the actual and theoretical (total) response frequencies yielded a Chi square ---value of 11.98, 2 df (p<.01) which indicates the presence of reliable differences between groups. Further analyses revealed that the <u>Ss</u> from the medium-sized litters engaged in the functional activity more often than the <u>Ss</u> from the large litters ($\chi^2 = 8.16$, 1 df, p<.01) during infancy. Small litter <u>Ss</u> also slept more frequently than the large litter <u>Ss</u> ($\chi^2 = 10.06$, 1 df, p<.01). Comparing frequencies of sleep/rest responding in Group 2 and Group 3 Ss resulted in a nonsignificant Chi square value of .10 (1 df).

Viewing infancy in three parts (i.e., Days 1-6, Days 7-15, and Days 16-21) also disclosed differences in sleep/rest activity



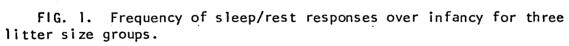


TABLE	4
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Comparison of Sleep/Rest Activity in Three Litter Size Groups^a

Group		DAYS OF AGE								
Group	1-3	4-6	7-9	10-12	13-15	16-18	19-21	Total		
1	(275.52) 297	(229.91) 178	(229.29) 218	(136.84) 179	(198.47) 130	(131.91) 225	(85.06) 60	1287		
2	(307.42) 291	(256.53) 307	(255.84) 245	(152.68) 107	(221.45) 168	(147.18) 145	(94.91) 173	1436		
3	(311.06) 306	(259.56) 261	(258.87) 281	(154.49) 158	(224.07) 346	(148.92) 58	(96.03) 43	1453		
Total	894	746	744	444	644	428	276	4176		

^aExpected frequencies are given in parenthesis.

between the three litter size groups. Hence, reliable differences existed between the three groups during Days 1-6 ($\chi^2 = 14.98$, 2 df, p<.01). Group 2 <u>Ss</u> slept significantly more than Group 1 <u>Ss</u> ($\chi^2 = 14.10$, 1 df, p<.01). Moreover, Group 3 <u>Ss</u> also engaged in sleep/rest activity more often than Group 1 <u>Ss</u> ($\chi^2 = 8.12$, 1 df, p<.01). Group 2 and Group 3 pups did not differ from each other, however, in sleep/rest response frequency ($\chi^2 = .82$, 1 df).

During mid-infancy (Days 7-15), differences in the amount of time spent sleeping and resting persisted between the three experimental groups (χ^2 = 44.59, 2 df, p<.01). Group 3 <u>Ss</u> slept reliably more often than either Group 1 pups (χ^2 = 30.44, 1 df, p<.01) or Group 2 pups (χ^2 = 32.86, 1 df, p<.01). Divergences between Group 1 and Group 2 <u>Ss</u> were no greater than would be expected by chance (χ^2 = .04, 1 df).

Differences in the sleep/rest activity of the three experimental groups remained during the last six days of infancy, Days 16-21 (χ^2 = 116.52, 2 df, p<.01). Still, the large and medium litter <u>S</u>s failed to differ in the functional activity (χ^2 = 1.8, 1 df). However, both Group 1 and Group 2 infants slept or rested more often than Group 3 infants. The Chi square value obtained from comparing Group 1 and Group 3 response frequencies was 8.77, 1 df, which surpasses the value needed for significance at the .01 level. Comparison of response frequencies from Group 2 and Group 3 pups resulted in a Chi square value of 112.38, 1 df, significant at the .01 level. As presented in Figure 1, time engaged in sleep and rest activity decreased over infancy. In the initial three days after birth, <u>Ss</u> from the three experimental groups slept over half of the time that they were observed. During the final days of infancy, sleep/rest responding dwindled to 11.1% in Group 1 and 8% in Group 3. Group 2 pups displayed a slight augmentation in the activity prior to weaning although the final frequency was considerably lower than the initial frequency.

Viewing the individual responses subsumed under the general category of sleep/rest activity (in Tables 1, 2, and 3), there are few remarkable differences between the three experimental groups. Group 3 <u>S</u>s, who were reared in the smallest litters, spent more time sleeping alone than <u>S</u>s from the larger litters, an expected finding.

The data obtained from observing consummatory responses (i.e., nursing, eating, drinking) are presented graphically in Figure 2. Excepting response frequencies collected on Days 1-3, 10-12, and 16-18, Group 1 pups consistently engaged in more consummatory behavior than pups from either of the two smaller litter size groups. In fact, over the entire period of infancy, Group 1 <u>S</u>s nursed, ate, or drank 30.8% of the time, whereas Group 2 and Group 3 <u>S</u>s nursed, ate, or drank only 20.3% and 21.6% of the time, respectively.

Expected frequencies for the consummatory responses of the three groups were calculated and appear in Table 5. The consequent divergencies from the observed frequencies produced a Chi square value of 287.77, 12 df (p<.01). Therefore, the obtained differences in the emission of consummatory behaviors by the rat pups

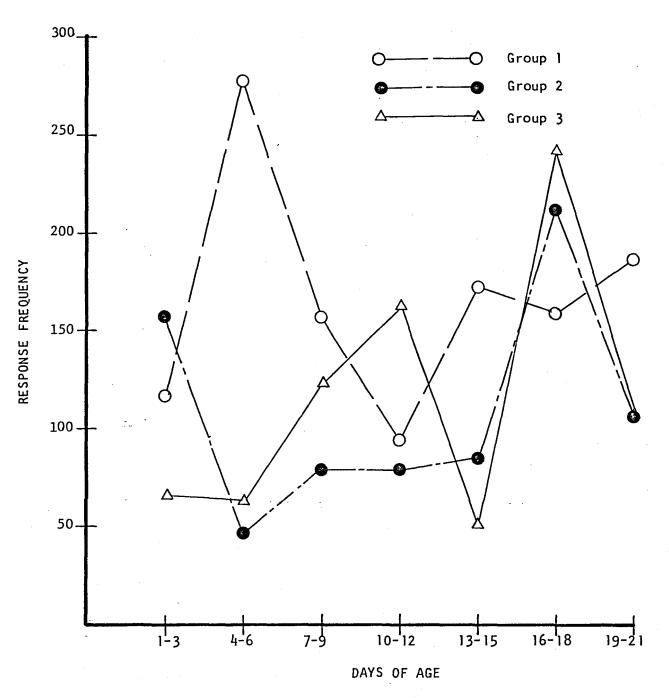


FIG. 2. Frequency of consummatory activity during infancy in three litter size groups.

Group		DAYS OF AGE								
Group	1-3	4-6	7-9	10-12	13-15	16-18	19-21	Total		
1	(144.17)	(164.10)	(152.66)	(142.90)	(131.03)	(259.94)	(169.19)			
-	117	278	157	94	173	159	186	1164		
2	(94.63)	(107.71)	(100.20)	(93.80)	(86.00)	(170.61)	(111.05)			
	157	46	79	79	85	212	106	764		
3	(101.19)	(115.18)	(107.15)	(100.30)	(91.97)	(182.45)	(118.76)			
ر	66	63	124	164	51	242	107	817		
Total	340	387	360	337	309	613	399	2745		

TABLE 5

Comparison of Consummatory Activity in Three Litter Size Groups

reared in small, medium and large litters were statistically reliable.

The three litter size groups varied significantly not only in total frequency of consummatory behaviors, but also in the amount of consummatory activity both before (i.e. Days 1-6) and after (i.e., Days 13-21) the appearance of eating and drinking responses. Comparing the total number of consummatory responses emitted by the <u>S</u>s reared in the three litter conditions produced a Chi square value of 103.18, 2 df, which is significant at the .01 level. Additional Chi square tests revealed that the large litter <u>S</u>s spent more time nursing, eating, or drinking than either Group 2 <u>S</u>s (χ^2 = 82.98, 1 df, p<.01) or Group 3 <u>S</u>s (χ^2 = 60.78, 1 df, p<.01). No differences in total number of consummatory responses were found between Groups 2 and 3 (χ^2 = 1.78, 1 df).

In the first six days of infancy, variations in the three groups' consummatory responding produced a Chi square value of 155.56, 2 df (p<.01), indicative of reliable differences. Group 1 <u>Ss</u> again out-consummed both Group 2 <u>Ss</u> (χ^2 = 61.64, 1 df, p<.01) and Group 3 <u>Ss</u> (χ^2 = 135.04, 1 df, p<.01). Moreover, the pups reared in medium litters nursed, ate, or drank more than Group 3 pups (χ^2 = 16.50, 1 df, p<.01).

During Days 13-21 consummatory response frequencies once again indicated the existence of reliable differences between groups (χ^2 = 20.55, 2 df, p<.01). Group 1 <u>S</u>s nursed, ate, or drank reliably more than either Group 2 <u>S</u>s (χ^2 = 14.36, 1 df, p<.01) or Group 3 <u>S</u>s (χ^2 = 15.16, 1 df, p<.01). Group 2 and

Group 3 pups did not vary in the amount of consummatory activity $(\chi^2 = .02, 1 \text{ df})$.

Inspecting further the consummatory activity of the infant rats, other dissimilarities are revealed. While Group 1 and Group 2 rats ceased nursing after Day 18, rats in Group 3 litters persisted in the behavior through Day 20. Moreover, the onsets of eating and drinking were delayed in Group 3, occurring on Days 18 and 19, respectively. Group 1 <u>S</u>s, on the other hand, commenced consumming solid food on Day 14 and Group 2 <u>S</u>s on Day 16. Drinking from the water fount was first observed on Day 17 in Group 1 litters and on Day 14 in Group 2 litters. While Group 1 and Group 2 <u>S</u>s ate more frequently than Group 3 <u>S</u>s (a logical finding), the frequency of drinking varied little from group to group (see Tables 1, 2, and 3).

Frequencies obtained from the observations of grooming, the third category of functional activity under study, are graphed in Figure 3. Over the period of infancy, grooming behavior frequency increased steadily in Group 3. Meanwhile, Group 1 and Group 2 pups exhibited grooming more frequently though more erratically. In computing expected frequencies for grooming behaviors, Days 1-3 were eliminated due to the absence of observations of the responses. Data from Days 4-6 and Days 7-9 were grouped together due to insufficient observations of grooming on those days. Resultant comparisons (which are displayed in Table 6) of expected and observed frequencies yielded a Chi square value of 103.87, 8 df, (p<.01). Hence, grooming response frequencies over the preveaning

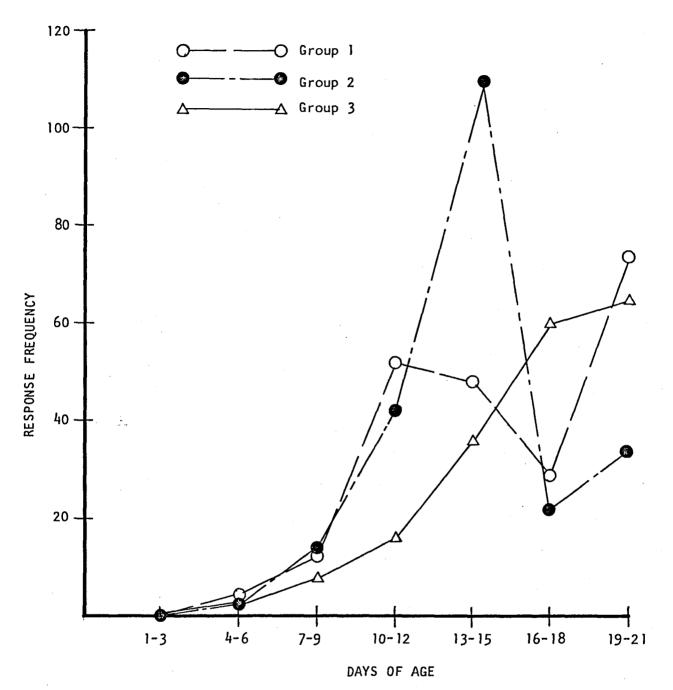


FIG. 3. Frequency of grooming responses over infancy for three litter size groups.

······································		DAYS OF AGE								
Group	4-9	10-12	13-15	16-18	19-21	Total				
1	(14.16) 16	(38.24) 52	(67.44) 48	(38.59) 29	(60.14) 74	219				
2	(14.93) 16	(39.11) 42	(68.98) 110	(39.47) 22	(61.51) 34	224				
3	(12.47) 10	(32.65) 16	(57.58) 36	(32.95) 60	(51.35) 65	187				
Total	42	110	194	111	173	630				

TABLE	6
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Comparison of Grooming Activity in Three Litter Size Groups

stage of development are significantly different for the three litter size groups.

A Chi square test performed on the total number of grooming responses made by rat pups in the three litter conditions during infancy failed to produce statistically significant differences $(\chi^2 = 3.91, 2 \text{ df})$. During Days 4-12, however, the three groups varied reliably from one another as disclosed by a Chi square value of 19.00, 1 df (p<.01). The large litter <u>Ss</u> groomed more frequently than the small litter <u>Ss</u> $(\chi^2 = 18.76, 1 \text{ df}, p<.01)$ as did the medium litter <u>Ss</u> $(\chi^2 = 12.20, 1 \text{ df}, p<.01)$. However, the grooming frequencies attained by Group 1 and Group 2 <u>Ss</u> were essentially the same $(\chi^2 = .80, 1 \text{ df})$.

Comparisons of the three groups' grooming frequencies from Day 13-15 resulted in a statistically significant Chi square value (48.78, 2 df, p<.01). Rat pups from the medium-sized litters groomed more often than pups from both the large litters (χ^2 = 24.32, 1 df, p<.01) and the small litters (χ^2 = 37.50, 1 df, p<.01). The large and small litter <u>S</u>s did not differ reliably (χ^2 = 1.72, 1 df).

Divergencies in the three groups' grooming activity during the final six days of infancy also produced a reliable Chi square value (i.e., 26.25, 2 df, p<.01). Both the small and large litter <u>Ss</u> groomed more frequently than the medium litter <u>Ss</u>. The Chi square test on grooming frequencies between Group 2 and Group 1 produced a value of 13.90, 1 df (p<.01). Comparisons between Group 2 and Group 3 resulted in a Chi square value of 26.30, 1 df,

which exceeds the critical value necessary for significance at the .01 level. Group 1 and Group 3 pups, however, exhibited essentially the same number of grooming responses during the last days of the preweaning period ($\chi^2 = 2.12$, 1 df).

Group 3 <u>S</u>s not only groomed less (on the whole) than Group 1 and Group 2 infants, but also commenced grooming later in infancy. Face washing was initially noted in Group 1 and Group 2 litters on Day 6, while the behavior did not appear in the smallest litters until Day 9. Fur scratching also appeared on Day 9 in Group 3, but had previously begun on Day 8 in Group 1 and on Day 6 in Group 2. On Day 12, rat pups reared in Groups 1 and 3 were first noticed to lick their fur. Group 2 <u>S</u>s licked their fur earlier on Day 10.

According to the data presented in Tables 1 and 2, fur scratching was the predominant grooming activity in Groups 1 and 2 during the preweaning stage of development. In fact, fur scratching accounted for 39.7% of the grooming in Group 1 and 36.6% in Group 2. Group 3 <u>S</u>s, on the other hand, licked their fur almost half (i.e., 45.4%) of the time that they groomed.

Data collected from viewing locomotor behaviors, including crawling, walking, running/jumping, and climbing, are graphed in Figure 4. All three groups appeared to exhibit an initial increase in locomotion, succeeded by a decrease which was, in turn, followed by an increase in the behavior over the final three days of observations. Following computation of expected frequencies of locomotor activity for the three groups over the entire preweaning stage of development (which are shown in Table 7), the Chi square

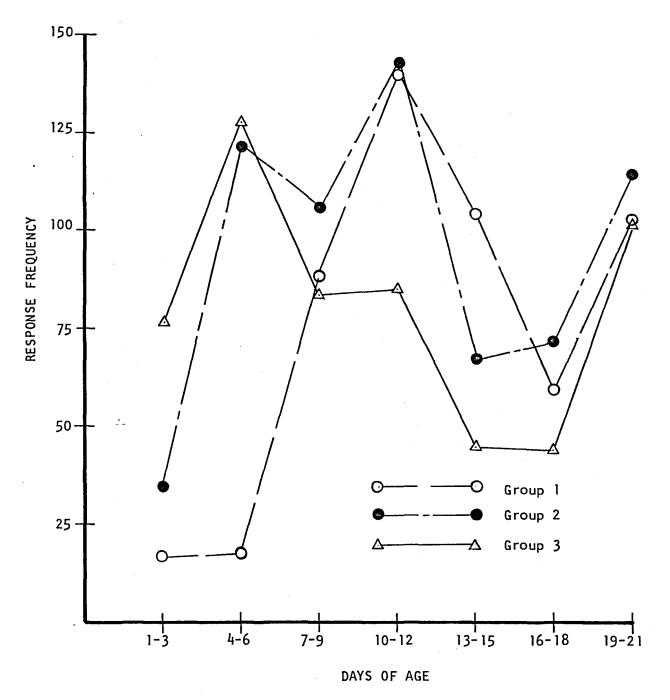


FIG. 4. Frequency of locomotor responses over infancy for three litter size groups.

0	DAYS OF AGE								
Group	1-3	4-6	7-9	10-12	13-15	16-18	19-21	Total	
1	(39.07)	(81.16)	(84.49)	(112.05)	(66.02)	(53.30)	(96.91)		
1	17	18	89	141	105	60	103	533	
2	(48.52)	(100.80)	(104.94)	(139.17)	(82.00)	(66.20)	(120.36)		
	35	122	106	144	68	72	115	662	
	(41.41)	(66.03)	(89.57)	(118.78)	(69.98)	(56.50)	(102.73)		
3	77	128	84	85	45	44	102	565	
Total	129	268	279	370	218	176	320	1760	

TABLE 7

Comparison of Locomotor Activity in Three Litter Size Groups

test was performed. A χ^2 value of 215.53 (12 df) was obtained, which is significant at the .01 level. Therefore, differences found among the three groups' exhibited locomotor activity are reliable ones.

The performance of the Chi square test on the total number of locomotor responses emitted by the three experimental groups produced a χ^2 value of 15.38, 2 df, which is significant at the .01 level. Subsequent comparisons determined the presence of reliable differences between Groups 1 and 2 (χ^2 = 13.92, 1 df, p<.01) and between Groups 2 and 3 (χ^2 = 7.66, 1 df, p<.01). In both cases, pups reared in the medium-sized litters crawled, walked, ran/jumped, or climbed more than pups reared in either the large or the small-sized litters. The small and large litter groups did not differ significantly (χ^2 = .94, 1 df).

Frequencies of locomotor responses during the initial six days of infancy likewise differed reliably according to the obtained χ^2 value of 116.10, 2 df, p<.01. Further, Chi square tests revealed that Group 2 <u>Ss</u> made significantly more locomotor responses than Group 1 <u>Ss</u> (χ^2 = 77.52, 1 df, p<.01). Moreover, Group 3 <u>Ss</u> made more locomotor responses than Group 1 <u>Ss</u> (χ^2 = 120.42, 1 df, p<.01). In addition, the application of the Chi square test to frequencies of locomotor activity in Groups 2 and 3 produced a χ^2 value of 6.36 (1 df). Hence, group 2 pups appeared to exhibit more locomotor responses during the early days of infancy than either the large or the small litter Ss.

In the middle part of infancy, Days 7-15, differences between the groups endured (χ^2 = 29.69, 2 df, p<.01). Both the medium and large litter groups exceeded the small litter groups in locomotor activity. Comparing Groups 1 and 3 yielded a χ^2 value of 26.66, 1 df, (p<.01) and a comparison between Group 2 and 3 produced a χ^2 value of 20.34, 1 df (p<.01). An obtained χ^2 of .44, 1 df, however, indicates that Group 1 <u>Ss</u> diverged little from Group 2 <u>Ss</u> in locomotion frequencies. Differences in locomotion found in the earlier phases of the preweaning period disappeared during Days 16-21 (χ^2 = 5.13, 2 df).

Within two days following birth, infants from each of the three experimental groups began dragging themselves about by their forelegs (i.e., crawling). Group 1 rats commenced walking on Day 9, Group 2 rats on Day 10, and Group 3 rats on Day 12. Running/jumping first occurred on Day 13 in Group 1 litters, on Day 11 in Group 2, but not until Day 18 in Group 3. Similarly, pups climbing the walls were not observed until Day 14 in Group 3 litters, but had previously been noticed on Day 12 in Group 1 and Day 9 in Group 2.

According to the data presented in Tables 1, 2, and 3, climbing seemed to be the fastest increasing and most frequent form of locomotion in the later days of infancy for all three experimental groups. Following initial increases, crawling gradually was replaced by walking and climbing. Running/jumping never attained the frequency of the other forms of locomotion although Group 2 <u>S</u> indulged in the behavior slightly more than

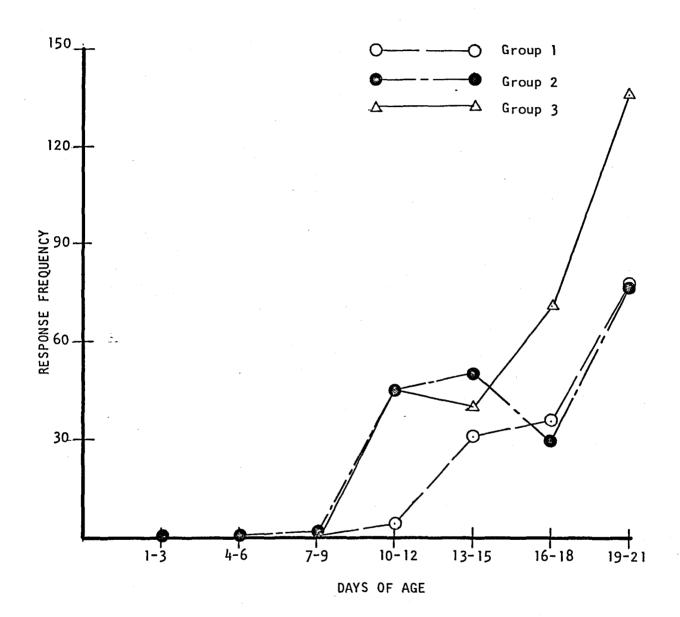
Ss in the small and large litters.

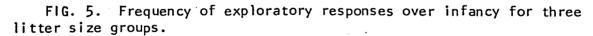
Figure 5 displays the frequency of exploratory activity over the 21 days of infancy for each of the three litter size groups. While exploration increased over infancy in all three groups, the most drastic increment occurred in Group 3. Group 3 pups also devoted considerably more time to exploration than <u>Ss</u> in the other groups.

Since exploratory responses were not observed in any of the litters until Day 9, Days 1-6 were omitted and Days 7-12 combined in the computation of the expected frequencies. A Chi square value of 45.18 (6 df) was derived from comparisons of the observed and expected frequencies (which are presented in Table 8). The obtained χ^2 lies at the .01 significance level. Thus, the frequencies (of exploratory responses) over infancy for the three litter size groups are reliably different.

The application of the Chi square test to the total number of exploratory responses by the three litter size groups across infancy produced a χ^2 value of 49.05, 2 df, which is significant at the .01 level. Succeeding tests revealed that Group 2 infants explored more than Group 1 infants (χ^2 = 8.56, 1 df, p<.01) and that Group 3 infants explored more than Group 2 infants (χ^2 = 15.94, 1 df, p.<01). Furthermore, the frequency of Group 3 exploratory activity exceeded that of Group 1 (χ^2 = 46.92, 1 df, p<.01).

In the first week of exploration (Days 7-15), significant differences were found between the experimental groups (χ^2 = 31.01, 2 df, p<01). Both Group 2 and Group 3 Ss explored more often than





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Comparison of Exploratory Activity in Three Litter Size Groups

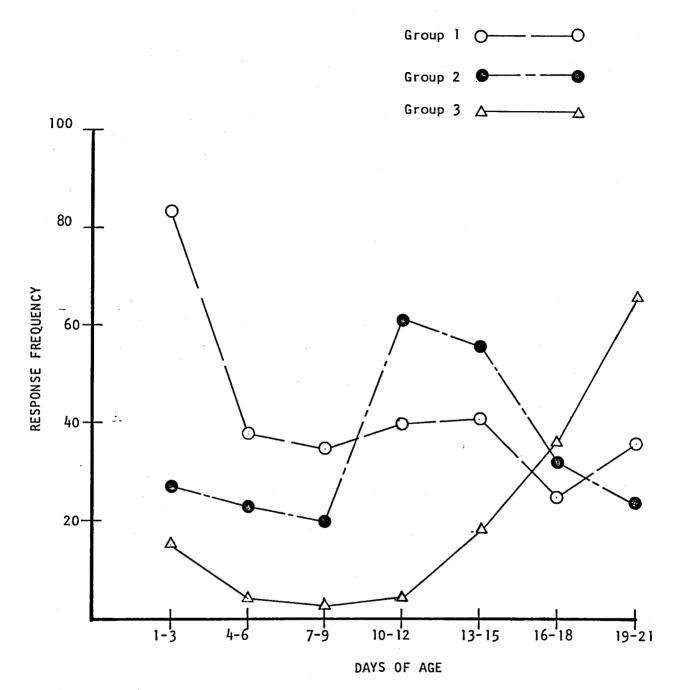
		. DAYS OF AGE								
Group	7-12	13-15	16-18	19-21	Total					
1	(22.60)	(27.91) 31	(31.37) 36	(67.12) 78	149					
2	(30.95) 49	(38.21) 50	(42.95) 29	(91.89) 76	204					
3	(44.45) 45	(54.88) 40	(61.68) 71	(131.97) 137	293					
tal	98	121	136	291	646					

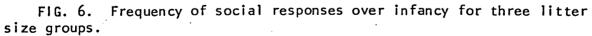
the large litter <u>Ss</u>. The Chi square test on Group 1 and Group 2 frequencies yielded a χ^2 of 30.56, 1 df, while the test on Group 3 and Group 1 frequencies produced a χ^2 of 20.84, 1 df. Both values exceeded the critical value for significance at the .01 level. Differences in exploratory frequencies between Group 2 and Group 3 were unreliable (χ^2 = 1.06, 1 df).

Divergencies in exploration remained in the second week of the observations (χ^2 = 45.73, 2 df, p<.01). Group 3 pups explored more often than Group 1 pups (χ^2 = 27.44, 1 df, p<.01) and Group 2 pups (χ^2 = 33.90, 1 df, p<.01). Group 1 and Group 2 exploratory activity was essentially the same (χ^2 = .35, 1 df).

The first exploratory response observed was sniffing, which occurred on Day 9 in the medium-size litters, Group 2. On Day 10 the present investigator noted sniffing in Group 3 and on Day 11, in Group 1. The first evidence of the pups' manipulating boluses, food pellets, or nesting material took place on Day 10 in all three groups. Group 3 <u>Ss</u> reared on Day 12, while <u>Ss</u> in Groups 1 and 2 did not exhibit the response until Days 17 and 15, respectively. Tables 1, 2, and 3 reveal that sniffing was the predominant exploratory response in all three groups. Manipulating was the next most frequent response in the category, followed by rearing.

Observed incidences of social behaviors are presented in Figure 6. While the frequency of social activity (i.e., burrowing, playing, fighting, and social grooming) appeared to decline over infancy in litters of nine, <u>S</u>s in litters of three exhibited a steady increase in the behaviors. Social responding in Group 2 litters





decreased slightly over the first nine days of observations, followed by a sharp increase in mid-infancy. From Day 13 until weaning, the Group 2 pups gradually lessened their social activity. On the whole, the large litter rats made more social responses throughout the preweaning stage of development than rats reared in the smaller litters.

Obtained and theoretical frequencies of social responding are shown in Table 9. Comparisons of expected and observed frequencies produced a Chi square value of 183.17, 12 df (p<.01), thus confirming the reliability of differences in social responding in the three experimental groups over infancy.

A Chi square test on the total numbers of social responses over infancy in the three litter conditions yielded a χ^2 of 52.53, 2 df, which is significant at the .01 confidence level. Subsequent tests disclosed that both the large and medium litter groups exceeded the small litter group in number of social responses. The χ^2 resulting from the comparison of Group 1 and Group 3 frequencies was 52.84, 1 df, and 24.74, 1 df, from the comparison of Group 2 and Group 3 frequencies. Both values are significant at the .01 level. The medium and large litter groups were not reliably different ($\chi^2 = 5.60$, 1 df).

Reliable differences in social activity were also present in the initial days of infancy (χ^2 = 86.36, 2 df, p<.01). Group 1 <u>Ss</u> diverged significantly not only from Group 3 <u>Ss</u> (χ^2 = 74.32, 1 df, p<.01) but also from Group 2 <u>Ss</u> (χ^2 = 29.48, 1 df, p<.01). Additionally, the medium litter pups initiated a greater number of

;	DAYS OF AGE								
Group	. 1-3	4-6	7-9	10-12	13-15	16-18	19-21	Total	
1	(54.30) 83	(28.24) 38	(24.76) 35	(45.61) 40	(49.96) 41	(40.40) 25	(54.73) 36	298	
2	(44.28) 27	(23.02) 23	(20.19) 20	(37.19) 61	(40.74) 56	(32.94) 32	(44.63) 24	243	
3.	(26.42) 15	(13.74) 4	(12.05) 2	(22.19) 4	(24.31) 18	(19.66) 36	(26.63) 66	145	
Total	125	65	57	105	115	93	126	686	

TABLE 9

Comparison of Social Activity in Three Litter Size Groups

social responses than the small litter pups (χ^2 = 13.92, 1 df, p<.01).

During Days 7-15, differences in social activity remained $(\chi^2 = 78.25, 2 \text{ df}, \text{ p<.01})$. Group 3 <u>Ss</u> again made fewer social responses than either Group 1 <u>Ss</u> $(\chi^2 = 60.46, 1 \text{ df}, \text{ p<.01})$ or Group 2 <u>Ss</u> $(\chi^2 = 79.32, 1 \text{ df}, \text{ p<.01})$. The medium and large litter groups did not vary reliably $(\chi^2 = 1.74, 1 \text{ df})$.

Although differences in social responding were present during the final six days of the preweaning period ($\chi^2 = 17.45$, 2 df, p<.01), they were quite the opposite of earlier existing differences. In other words, the social activity of the small litter rats exceeded that of both the large litter rats ($\chi^2 = 10.32$, 1 df, p<.01) and the medium litter rats ($\chi^2 = 13.40$, 1 df, p<.01). Still, Group 1 and Group 2 did not differ from one another ($\chi^2 = .22$, 1 df).

Burrowing, though observed from Day 1 in all three groups, was far more frequent in Group 1 than in the other two groups, as apparent from the data presented in Tables 1, 2, and 3. In fact, about 69.8% of the social activity observed in Group 1 was categorized as burrowing.

Fighting/playing responses, which were not seen until Day 14 in Group 1, Day 13 in Group 2, and Day 18 in Group 3, comprised a larger portion of social activity in Group 3 than in the other two groups. Specifically, 15.2% of Group 3 <u>S</u>s' social responding was fighting/playing. On the other hand, only 7.3% of Group 2 <u>S</u>s' social activity and 8.7% of Group 3 <u>S</u>s' involved fighting/playing.

Social grooming, the third response subsumed under the category of social activity, first occurred on Day 9 in Group 2 and on Day 10 in Groups 1 and 3. Over half of the social activity in Group 2 and Group 3 litters was social grooming, whereas only 21.5% of the social responses observed in Group 1 could be labeled social grooming.

In an attempt to determine the influence of litter size on the overall process of development, ages of onset of the 14 behaviors which were not present immediately after birth were compiled and ranks affixed for each of the experimental groups. Higher ranks were thereby assigned to the earlier appearing behaviors. A Kruskal-Wallis One Way Analysis of Variance, corrected for ties, was then applied to the data. The computed H value of 24.29, 2 df, was significant at the .01 level. Therefore, celerity of development differs with size of litter. Moreover, since the sum of ranks obtained in Group 2 was 350 (compared with a sum of 298 in Group 1 and 255 in Group 3), it would appear that Group 2 contained the more precocious rat pups.

Results of the observations of maternal grooming are shown in Figure 7. Generally, it can be concluded that the mothers with the smaller litters groomed their young more often than the mothers with the larger litters. Indeed <u>Ss</u> in litters of three received the greatest amount of maternal grooming and <u>Ss</u> in litters of nine received the least. Overall, the incidence of maternal grooming activity declined as the infant rats aged. However, pups in litters of five experienced an augmentation of maternal grooming

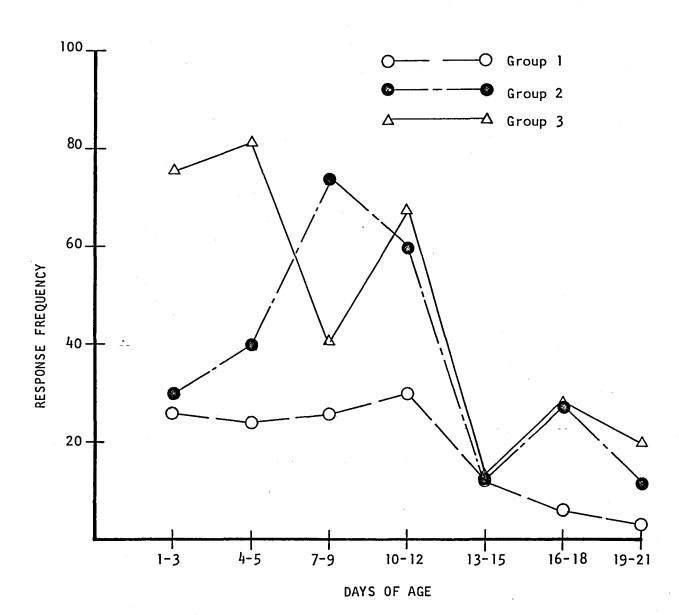


FIG. 7. Frequency of maternal grooming responses over infancy in three litter size groups.

activity from Days 4-9, which was followed by a persistent decrease. Both actual and expected frequencies of maternal grooming are shown in Table 10. After the observed frequencies of maternal grooming were compared with the theoretical frequencies, a Chi square value of 47.90, 12 df (p<.01) was found. Therefore, differences observed in maternal grooming over infancy in the three litter size groups were not due to chance.

Likewise, the Chi square test performed on the total number of maternal grooming responses in the three litter conditions was reliable (χ^2 = 88.85, 2 df, p<.01). While the medium litter group received a greater amount of maternal grooming than the large litter group (χ^2 = 44.02, 1 df, p<.01), the small litter group exceeded not only the large litter group (χ^2 = 90.18, 1 df, p<.01) but also the medium litter group (χ^2 = 9.08, 1 df, p<.01) in maternal grooming activity.

In the first six days of the preweaning period of development, significant variations in the amount of maternal grooming bestowed upon members of the different sizes of litter commenced $(\chi^2 = 71.26, 1 \text{ df}, p < .01)$. Groups 1 and 2, however, received about the same amount of maternal stimulation $(\chi^2 = 3.34, 1 \text{ df})$ while Group 3 experienced more maternal stimulation than either Group 1 $(\chi^2 = 56.04, 1 \text{ df}, p < .01)$ or Group 2 $(\chi^2 = 33.96, 1 \text{ df}, p < .01)$.

From Days 7 - 15, differences in maternal stimulation persisted (χ^2 = 29.12, 2 df, p<.01) although the nature of the differences changed. Hence, Group 2 <u>Ss</u> received about the same amount of maternal grooming as Group 3 <u>Ss</u> (χ^2 = 2.14, 1 df) while

Group	DAYS OF AGE								
	1-3	4-6	7-9	10-12	13-15	16-18	19-21	Total	
_	(23.48)	(25.97)	(25.08)	(28.10)	(6.94)	(11.21)	(6.23)		
1	26	24	26	30	12	6	3	127	
2	(47.51)	(52.55)	(50.75)	(56.87)	(14.04)	(22.68)	(12.60)		
	30	40	74	60	13	28	12	257	
	(61.01)	(67.48)	(65.17)	(73.03)	(18.03)	(29.12)	(16.18)		
3	76	82	41	68	14	29	20	330	
Total	132	146	141	158	39	63	35	714	

TABLE	10
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Comparison of Maternal Grooming in Three Litter Size Groups

experiencing more than Group 1 rats (χ^2 = 29.02, 1 df, p<.01). Group 3 continued to have a greater number of maternal grooming observations than Group 1 (χ^2 = 15.84, 1 df, p<.01).

In the final phase of infancy, differences between groups still existed (χ^2 = 26.95, 2 df, p<.01) and remained basically the same (in nature) as was noted in the prior week of observations. Thus, Group 2 and Group 3 litters did not differ (χ^2 = .92, 1 df) and more maternal grooming responses were observed in Group 2 litters than in Group 1 litters (χ^2 = 19.62, 1 df, p<.01) or in Group 3 litters (χ^2 = 27.58, 1 df, p<.01).

Weaning Weights

Immediately after the completion of the infant behavioral observations (on Day 21), all of the remaining Group 3 <u>Ss</u> (n = 12) and 12 randomly selected <u>Ss</u> from Groups 1 and 2 were weighed. The collected weight data were then subjected to a single factor analysis of variance in order to ascertain whether or not (and how) rats reared in the three conditions differed in terms of body weight. The results of the ANOV are presented in Table 11. Based upon the obtained F value of 20.56 (2.33 df), it can be assumed that variations in body weights manifested at weaning by the rats are the product of different rearing conditions and not chance.

Since a significant F ratio was obtained, an <u>a posteriori</u> test was subsequently performed on the data to pinpoint the location of the differences. The Newman-Keuls test was therefore applied to the data. The results procured from rank ordering mean

TABLE 11

Source	SS	df	MS	F
Treatment (groups) Experimental error	2951.64 2368.82	33	71.78	20.56**
Total	5320.46	35		

Analysis of Variance: Weaning Weights

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**p<.01

weaning weights (employing an alpha level of .01 with r and 33 degrees of freedom) indicated that the average weaning weight (i.e., 68.22 grams) of Group 2 <u>S</u>s was reliably greater than that of Group 1 <u>S</u>s (i.e., 46.08 grams) or Group 3 <u>S</u>s (i.e., 58.39 grams). Further, rats reared in litters of three weighed reliably more than rats reared in litters of nine. Hence, the medium size litters appeared to be the most efficacious rearing conditions for the production of heavy 21-day old rats.

Adult Open Field Behaviors

A 3 x 4 factorial design with repeated measures on the second factor was employed in analyzing each of the adult open field behaviors under study--ambulation and defecation. The purpose of each analysis was not only to educe the possible consequences of infant rearing conditions on adult behavior but also to determine the effect of day of testing on open field responding.

The mean ambulation scores for the three experimental groups during open field testing are presented in Table 12. Differences were evaluated by means of a two factor analysis of variance with repeated measures on the second factor, day of testing. Results of the test appear in Table 13. Size of litter during infancy (Factor A) exerted only a fortuitous effect upon ambulation scores of adult <u>S</u>s. Moreover, interaction between litter size group and day of testing was not statistically reliable. Day of testing (Factor B), however, produced significant

TABLE 12

Mean Ambulation Scores During

		Day of Testing			
Group	1	2	3	4	
1	91.25	57.75	54.83	65.42	
2	101.75	51.25	50.92	52.25	
3	89.67	64.50	57.17	64.25	

Adult Open Field Testing

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Source	SS	df	MS	F
Between <u>S</u> s	68933.50	35		
Litter size (A)	588.29	2	294.15	.142
<u>S</u> s within groups	68345.21	33	2071.07	
Within subjects	68992.50	108		
Day of testing (B)	36951.83	3	12317.28	25.93**
AB	3014.55	6	502.43	1.06
BxSs within groups	47026.12	99	475.01	

TABLE 13

Analysis of Variance: Adult Open Field Ambulation

**p<.01

variations in the ambulation scores according to the obtained F ratio of 25.93, df = 3/99 (p<.01).

Since the F value for Factor B exceeded the critical value necessary for significance, the Newman-Keuls method for testing differences was applied to the mean ambulation scores over the four days of open field testing. The results of the comparisons revealed that the Day 1 mean ambulation score (i.e., 94.22) differed significantly from the average ambulation scores obtained on Day 2 (i.e., 57.83), Day 3 (i.e., 54.31), and Day 4 (i.e., 60.64). No other differences were found.

Mean defecation scores obtained by the three litter size groups are shown in Table 14. Reliability of the differences in defecation scores was tested by the analysis of variance technique. Results of the test are presented in Table 15. Once again, the litter size variable (Factor A) exhibited no effect (beyond chance) on the adult behavior under observation, defecation. Furthermore, day of testing (Factor B) and the interaction between the two factors produced nonsignificant F ratios. Hence, it can be assumed, quite safely, that litter size during infancy is of no consequence on open field defecation in adulthood.

Adult Weights

After the completion of the open field testing, the <u>S</u>s were again weighed in order to determine whether or not differences in body weight found at 21 days of age persisted into adulthood. At 102 days of age, the mean weight for Group 1 rats was 416.28 grams.

		Day of Testing					
Group	1	2	3	4			
1	.92	2.25	1.58	1.50			
2	2.25	3.25	2.17	2.08			
3	.50	1.50	1.75	1.67			

TABLE 14

Mean Defecation Scores During Adult Open Field Testing

Source	SS	df	MS	F
Between <u>S</u> s	351.33	35		
Litter size (A)	31.73	2	15.87	1.64
<u>S</u> s within groups	319.60	33	9.69	
Within <u>S</u> s	546.00	108		
Day of testing (B)	22.36	3	7.45	1.44
AB	11.16	6	1.86	. 36
Bx <u>S</u> s within groups	512.48	99	5.18	

TABLE 15

Analysis of Variance: Adult Open Field Defecation

Group 2 rats achieved an average body weight of 395.85 grams. 403.18 grams was the mean weight of <u>Ss</u> in Group 3. Interestingly, Group 2 rats weighed less than the rats from the other groups even though their weaning weights were significantly greater.

Following application of a single factor analysis of variance, it became obvious that the reliable variation found at weaning between the experimental groups had vanished in adulthood. Results of the analysis of variance are presented in Table 16.

Discussion

The results of the infant behavioral observations confirm the general hypothesis that litter size affects both the quantity and the quality of stimulus input during the preweaning stage of development. At different times during infancy, dissimilarities between the three experimental groups existed within each of the seven observed categories of functional activities. In addition, the present findings address several of the more specific hypotheses and assumptions noted by previous investigators.

Rat pups reared in the three litter size groups differed not only in terms of response activity but also in the nature and amount of maternal and littermate stimulation received. Based upon the empirical observations, various relationships among the functional behaviors can be deduced.

Past discussions of the effects of litter size on maturation, body weight, and adult performance variables have frequently relied upon rationalizations based on nutritional variables

TABLE 16

Source	SS	df	MS	F
Treatment (groups) Experimental error	2569.40 224637.63	2 33	1284.70 5807.20	.22
Total	227207.03			

Analysis of Variance: Adult Weights

(e.g., Kennedy, 1957; King, 1969; Priestnall, 1973). That is, the speedier maturation, greater body weight, and decreased emotionality found in rats reared in small litters were accounted for by the greater supply of mother's milk they had available. By the same token, large litter rats, described as developmentally retarded, light (in weight), and overly emotional, were assumed to have undergone a milk shortage during infancy. The present study indicates, however, that variations between rats reared in small and large litters were probably due to differences in milk quality rather than milk quantity.

Group 1 <u>Ss</u> nursed more frequently during the first 21 days of life than <u>Ss</u> reared in the smaller litters. If, as Kennedy (1957) maintained, milk supply becomes adapted to the demand, it would appear that the Group 1 pups had (at the minimum) the same amount of milk available as Group 2 and Group 3 pups. The higher frequency of consummatory responding in Group 1 litters would, therefore, have to be attributed to the decreased nutritional value of Group 1 mothers' milk.

Two additional observations support the assumption of decreased quality of maternal milk in the large litter. First, the weaning weight data indicated that rats reared in litters of nine weighed less than rats from litters of either five or three. The greater frequency of nursing activity in Group 1, then, fell short of fulfilling nutritional demands due to the poor quality of milk consummed. Second, rat pups reared in litters of three persisted in nursing activity longer than pups in either Group 1 or

Group 2. Furthermore, the small litter Ss were the last to commence eating and drinking. Apparently, milk available to Group 3 rats quite sufficiently accommodated increasing developmental demands. Group 1 and Group 2 pups, on the other hand, acquired the new responses at an earlier age in order to consume solid food and thereby supplement and fulfill growth needs unmet by inferior quality milk. Perhaps the availability of highly nutritious milk in the smallest litters decreased or attenuated stimuli which normally signaled eating and drinking responses. The greater nutritional value of maternal milk in the smallest litters was not wholly beneficial. The infants reared in the litters of five, Group 2, weighed more than infants from the other groups, including the pups raised in the smallest litters. Evidently, the nutritional value of Group 2 milk, while able to meet early growth demands, was not so great as to lead to the postponement of eating and drinking responses (as had occurred in Group 3), thus preventing developmental deficits (both physical and behavioral) later in infancy.

Increased maternal stimulation in small litters has also been employed in explanations of litter size effects. Several experimenters hypothesized the existence of differences in maternal stimulation (e.g., Priestnall, 1973; La Barba et al., 1971), and one (i.e., Seitz, 1954) empirically validated the differences. Generally, the assumptions are grounded in the logic of Denenberg's (1964) monotonicity hypothesis. Hence, rats reared in small litters are subject to more mothering which,

presumably, results in their emotional immunization. The present observations of maternal grooming showed only that pups reared in different litter sizes were exposed to divergent amounts of maternal stimulation. Group 3 <u>Ss</u> received the most maternal grooming, whereas Group 1 <u>Ss</u> had the least amount of maternal grooming. No indication of differences in emotionality were shown upon later testing.

Infant rats, however, are not reared in isolation, but with sibling rats. Recognizing this fact, several researchers have sought to explain the increased emotional reactivity in large litter rats (that they reported) as the function of an overload of littermate stimulation (e.g., Priestnall, 1973; La Barba et al., 1971). The three experimental groups did indeed vary in stimulation afforded by the presence of the littermates as well as by the In fact, the greatest amount of social activity (including mother. burrowing, social grooming, playing/fighting) occurred in Group 1 and the least amount in Group 3. Social grooming was the most frequent in the medium size litters, Group 2, and the least in the large litters, Group 1. The fact that the pups with the most littermates received the most littermate stimulation is entirely reasonable. The observation of Group 1 Ss' apparent aversion to social grooming, however, is puzzling. Perhaps it was precipitated, in part, by early deprivation of maternal grooming and hence, the experience of a kind of social grooming.

Self-grooming activity also varied according to litter size. Both Group 1 and Group 2 <u>Ss</u> groomed more often during the first 21

days of life than Group 3 Ss. Not only did pups reared in the smallest litters groom less than pups reared in larger litters, but they also commenced grooming later in development. The observed delay may have been due, partially, to the fact that material grooming was most common in Group 3. Therefore, stimuli necessary to elicit grooming were probably absent, or at least decreased in intensity. Thus, the three components of grooming (face washing, fur licking, and scratching) appeared later in the Group 3 Ss' lives. Besides variations observed in response frequency and onset, the three experimental groups also differed in terms of their preferred grooming component (i.e., face washing, scratching, fur licking). Hence, scratching was the predominant grooming activity in Groups 1 and 2, whereas Group 3 Ss preferred fur licking. Usually rat pups spend more grooming time face washing while adult rats generally prefer to lick their fur. The extensive prior experience of the mother rats licking their fur may have reinforced the response in Group 3, rendering it the favored grooming activity in infancy.

Stimulus input during infancy was increased further in the young rats by exploratory activity. Group 3 <u>Ss</u> displayed exploratory responses more often than either Group 1 or Group 2 <u>Ss</u>. Likewise, Group 2 <u>Ss</u> explored more frequently than the large litter rats. The greater amount of available space in the laboratory cages and decreased closeness of littermates apparently prodded Group 3 pups (and, to a lesser extent, Group 2 <u>Ss</u>) to augment their afferent stimulation through exploration.

Observed most frequently in Group 2 litters, locomotor behaviors were perhaps indicative of the more advanced development of their skeletal and muscular systems and reflexive responses. Bearing further on the validity of the assumption are the findings of both heavier weaning weights and earlier appearances of most of the functional behaviors under study in the medium-sized litters. Group 3 pups, who did not exhibit more locomotor activity than Group 1 pups, nevertheless weighed more at weaning than the large litter <u>Ss</u>. The finding can be explained, at least partially, by the observation that the rats from the Group 3 litters crawled, walked, ran/jumped, or climbed more than the Group 1 <u>Ss</u> during the first six days of infancy (which, according to the early stimulation research, is a critical period in development.)

Sleep/rest observations revealed that the small and mediumlitter pups slept more (on the whole) during the preweaning stage of development than the large litter pups. The finding can be explained by the greater number of littermates in Group 1 litters with the concomitant high incidence of burrowing (a social behavior associated with early sleep/rest activity). As noted by Bolles and Woods (1964), sleep became more peaceful toward the end of infancy. Thus, the large litter rats compensated for the prior sleep loss with longer periods of uninterrupted repose during the final week of infancy. In fact, the large litter rats slept as much as the medium litter rats and more than the small litter rats from Days 16-21.

The observations of infant and maternal behaviors revealed that rearing in different sizes of litters produces divergent social, psychological, and biological environments for the maturing rat. Environmental variations, in turn, create unique patterns and quantities of tactual, kinesthetic, thermal, visual, and auditory stimulation for each litter size group. Formulations (e.g., Priestnall, 1973; Seitz, 1954), which rely solely upon logical relationships between stimulus quantity and litter size are far too simplistic to adequately fulfill any explanatory purpose. While the quantity of stimulation within the litter is indeed an important variable, type of stimulation and the interrelationships between different forms of stimulation are also quite worthy of inspection. In the present study Group 2 Ss, raised in medium-sized litters, did not receive the most maternal or littermate stimulation. However, pups in Group 2 displayed the fastest development and weighed the most at 21 days of age. Hence, the data collected from observing infant rats during the first 21 days of life indicates that the relationship between litter size and infant behavior is not monotonic but, more likely, curvilinear.

In addition to the litter size variable, several external, non-experimental factors could have, to a certain extent, accounted for some of the obtained results. First, the mother rats were nulliparous prior to the present study. Since maternal behavior in rats is dependent upon past experience as well as stimulation by the young and hormonal secretions, the mothers in this experiment

were somewhat handicapped and may have failed to exhibit appropriate maternal behaviors. Second, the redistribution of the pups may have had an adverse affect on the behavior of the mother rats. Conceivably, the dissimilarity of natural and experimental litters prohibited the complete transfer of maternal behaviors from one situation to the other. Further, the laboratory cages which housed the rats were located in an arid room. Due to the lack of sufficient humidity, many of the infant rats developed a fungal infection of the tail and toes. Remediative use of a humidifier halted the rampant spread of the infection but some damage had already occurred. Besides the actual physical disability, the pups may have experienced some unfavorable behavioral effects as well.

The absence of a relationship between litter size and adult open field behaviors in the present study can be rationalized in a variety of ways (besides the fact that there may be no relationto find.) The first possible explanation has been previously employed, with some success, by Denenberg (1963). He maintained that the incongruent findings of litter size studies were the product of the strain differences of the <u>Ss</u>. Thus, the <u>Ss</u> in the current experiment were Sherman albinos, whereas the more "successful" experiments employed Wistar (Lore & Moyer, 1973; Seitz, 1954), Long-Evens (Lore & Moyer, 1973), or Maudsley Reactive and Nonreactive (Broadhurst & Levine, 1963) strains.

The advanced age of the <u>S</u>s at the onset of open field testing may have likewise accounted for the lack of significant

findings. Both King (1960) and Denemberg and Smith (1963) asserted that early experience effects attenuate as the rats grow older. The disappearance by 102 days of age of the weight differences found at weaning lends some support to the claim. However, other experiments have not always produced results consistent with the age-attenuation hypothesis. Seitz (1954) found reliable differences in both ambulation and defecation when his rats were tested at the ripe old age of 210 days. And, Lore and Avis (1969) did not discover any differences (due to infant experience) when they tested their 46-day old rats in the open field.

Possibly the most credible explanation of the present (contradictory) open field findings lie within the open field test itself. The open field test is not standardized and seems to vary according to the particular investigator employing it. The number and size of the squares have varied from four 18-inch quandrants (Henderson, 1963) to 16 one-foot squares (Hayes, 1960). Whimbey & Denenberg (1967) used 25 nine-inch squares; and the present experimenter employed 25 seven-inch squares. Some investigators (including the current one) have painted the field black with white lines and some made the floor white and the lines black (e.g., Pare, 1964). Another used photoelectric beams instead of paint (Henderson, 1963). Lighting has been variously provided by 150w red reflector floodlamps (e.g., Henderson, 1963) and a 25w red lightbulb (as in the present study). If rat behavior is indeed controlled to a large extent by stimulus novelty and complexity, it seems likely that the different open

field settings could produce differences in behavior. In some fields, then, the rat may ambulate more, due to more complex stimuli. The same animal may also exhibit a decrease in defecation due to the overriding of fear-producing stimuli by the complex, exploration-producing stimuli. Further, the greater the number and the smaller the size of the open field squares, the better the chances of obtaining an inflated ambulation score.

Not only does the physical layout of the open field change from study to study, but the interpretations of the collected data also fluctuate. Hence, the data have been employed as indices of fear, exploration, food seeking, and activity (Bolles, 1967). If open field defecation results can be validly used as a measure of emotional reactivity (a premise of the present experiment), the question then arises of applicability to other situations. Elimination in the open field may be situation specific. Ambulation, on the other hand, has been previously recognized as an inappropriate index of emotion (Whimbey & Denenberg, 1967). In the present experiment, some support was obtained for the position, since Day 1 ambulation scores differed reliably from Day 2, Day 3, and Day 4 ambulation scores. The data point to the possibility that ambulation means different things (i.e., fear or exploration) on different days of testing.

The incongruence of the present findings with previous reports of relationships between early experience and adult emotionality can be explained associatively. The handling procedure (whereby the infant is placed in a tin can with wood

shavings covering the bottom) may provide for response transfer to the open field situation in adulthood because of the presence of similar stimuli. Hence, the infant stimulation exerted a positive transfer effect on adult behavior. Containing both mother and littermates, the nest (which was the source of preweaning experience in the present study) appears quite dissimilar from the black and white plywood field employed in adult testing. So, there would be little, if any, transfer from one situation to the other. Goldman (1965) provided a similar account to explain the effects of preweaning shock on open field and avoidance behaviors in adulthood.

The failure to ascertain differences in adult emotional reactivity due to litter size in infancy could possibly be remediated through the use of different measures of emotionality. Perhaps the use of physiological measures (e.g., plasma corticosterone levels) would be more fruitful and more appropriate as some investigators believe (e.g., Levine & Lewis, 1959). Another perhaps better approach to the study of the effects of litter size entails the abandonment of the indices of emotional reactivity and replacement with more naturally occurring social responses such as reproductive or maternal behavior. Such indicators would not only hold more validity in the study of rat behavior, but also would be more likely to reveal the effects of rearing infants in different sizes of litters on adult behavior (because of the increased likelihood of transfer from one situation to another.)

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