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The location of a lactating mouse in a cage of two compartments, a nest area and a larger wire cage, was continuously recorded for ten days after parturition. Three experimental groups within each of two inbred lines, A/J and C57BL/6J, were tested: first generation, unfostered; second generation, unfostered; and second generation, crossfostered.

In both lines, the time spent away from the litter increased linearly with day (b = 25 minutes per day). C57 mothers spent about 1.1 hours more per day away from the nest than A/J mothers, 3.3 vs 2.2 hours on day 1 and 7.5 vs 5.5 hours on day 10. The average trip length increased linearly with day (b = 0.61 minutes per day); trip lengths for days 1 and 10 were 1.3 and 7.2 minutes, respectively. The average number of trips per day was 103 but the 10 day pattern differed between the lines. In the C57 line it decreased linearly

(b = -6 trips per day) while in A/J mothers an initial increase was followed by a decrease to day 1 levels. Larger estimated differences among the experimental groups and among individuals were found in A/J mothers while a larger proportion of the variation was attributable to daily changes in C57 mothers. Significant line x maternal line interaction terms indicated a fostering effect rather than maternal line effects was present. Crossfostered A/J mothers averaged 1.0 hour less away from the nest, 14 more trips daily and 2.1 minutes less per trip than unfostered mothers. Crossfostered C57 mothers averaged 22 more trips daily. Generation differences were found. Generation 1 individuals of both lines averaged 0.4 hours longer away from the nest daily. Generation 1 A/J mothers averaged 92 more trips daily and had trip lengths 4.1 minutes shorter. Generation 1 C57 mothers averaged 18 more trips daily but no difference in trip length was found. When litter size and weight were included in the regression model, time out per day and trip length increased with the litter parameters in the A/J line. In the C57 line time away from the nest and trip length increased as litter weight increased, but decreased as litter size increased.

In open field testing members of the A/J line had average ambulation and defecation scores of 2.3 and 4.2 under white light and 44.5 and 3.2 under red light. Members of the C57 line had average ambulation scores of 315 under white light and 353 under red light. The defecation score averaged about 0.7 under both regimes.

Line differences in circadian rhythms were noted. A/J mothers averaged 13% of the time away from the nest per day during the 14 hour light interval vs 47% for C57 mothers. Circadian rhythms of both lines were characterized by a prominent activity peak stimulated by darkness onset. In A/J mothers activity began abruptly after darkness onset while C57 mothers activity increases were noted in hours prior to darkness onset. A secondary peak of activity occurred in early morning hours in A/J mothers and at variable times in the C57 mothers. In both lines the second generation unfostered group, which experienced the most constant pre and postweaning environment, showed the least nocturnal tendency. This group in the C57 line displayed the largest proportion of the time away from the litter and trips and had longer trip lengths in light than dark, and had higher levels of general activity in light; the secondary peak of activity occurred after light onset. In the other two C57 groups, behavior measures were higher and the secondary activity peak occurred in darkness. The second generation unfostered A/J group had a third peak of activity during light, unlike the other groups.

# Temporal Maternal Behavior Patterns in Two Inbred Lines of Mice and Reciprocally Crossfostered Individuals

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# TEMPORAL MATERNAL BEHAVIOR PATTERNS IN TWO INBRED LINES OF MICE AND RECIPROCALLY CROSSFOSTERED INDIVIDUALS

## INTRODUCTION

The characteristics and quality of maternal care in rodents have traditionally been examined from two standpoints. Some observations are recorded on a mother while others study maternal care indirectly through characteristics of the offspring. For example, litter size and weight have been used to compare differences in maternal capabilities between females. Measurements of maternal behavior, such as time required to retrieve young to the nest, willingness to defend offspring, and nest quality ratings have been used to detect changing maternal responsiveness to maturing young.

Both methods are based on what Rosenblatt (1970) termed the synchrony of the mother-young interaction. He used the concept of synchrony to describe the phenomena that the mother's behavior is consistent with the needs and behavioral capacities of the young and that it changes as these needs and capacities develop. For example, at the stage when the young develop fur and become more mobile, when their eyes open and when they begin to feed and drink by themselves, maternal retrieving and nestbuilding decline (Rosenblatt, 1969). Synchrony is viewed as the outcome of natural selection in

which the selective pressure was exerted on the interdependent development of behavior in both the mother and offspring (Rosenblatt, 1970).

Within these generalized models of mother-offspring interaction a substructure exists which allows for variation. Such traits as the growth rate of the nursing young have been shown to depend in part upon their genetic capacity for growth and the lactation performance of the mother (Willham, 1972). Furthermore, maternal characteristics of daughters have been shown to be influenced by the maternal characteristics of their dam. In cattle, a negative environmental covariance has been found between a dam's own preweaning growth and her subsequent maternal ability (Hohenboken and Brinks, 1969; Mangus and Brinks, 1969).

The purpose of this experiment was to determine the extent to which the maternal behavior of the dam influences subsequent maternal behavior in her offspring. The maternal behavior of two lines of inbred mice and reciprocally crossfostered individuals were compared in an attempt to partition maternal effects from line effects. Maternal effects, as defined by Legates (1972), are the measured phenotypic expressions arising from influences of the mother on a trait measured in her offspring, apart from the direct influence of the genes she transmits. The line effect represents the contribution of genetic and prenatal factors which are shared among individuals of the line.

Several measures of maternal behavior were considered, all of which reflected temporal patterns of maternal activity. These measures included: the amount of time a female spends away from the litter per day, the number of trips made away from the litter per day and the average trip length. In addition, the circadian pattern in time away from the nest was examined. These behavioral measures were recorded as the litter matured from newborn to 10 days of age. A secondary objective of this experiment was to ascertain the changes in maternal behavior patterns as the litter matured, and relate line differences in circadian rhythms to line differences in open field behavior.

# REVIEW OF THE LITERATURE

While maternal care begins abruptly with the birth of the litter, its expression must be consistent with other forms of behavior. The temporal patterns of maternal care must be integrated with the normal circadian patterns of activity. Factors influencing circadian activity rhythms could alter circadian patterns of maternal behavior, if they exist. Any expression of maternal behavior must also be consistent with the underlying genetic determinants of behavior. One test which has been widely used in the study of the genetics of behavior is the open field test. The two inbred lines of mice used in this study have been shown to differ in their response to this test.

The three fields of research of interest in this study are then maternal behavior, circadian rhythms and open field behavior. They have heretofore been pursued separately, and thus this review is divided into three sections.

## Circadian Rhythms

Twenty-four hour cycles in living organisms were first discovered in plants. Androstenes noted a circadian rhythm in the up and downward movement of leaves in plants of the Papilionaceae (peas) family during the march of Alexander the Great. The astronomer DeMairan in 1729 provided the first experimental demonstration of an

endogenous component in circadian leaf movements by observing that these movements would continue in constant darkness (Bunning, 1967).

The existence of endogenous daily rhythms in animals was demonstrated for the rat by C. P. Richter in 1922 and for the mouse by M. S. Johnson in 1926. Johnson noted that the well marked periodicity in the occurrence of spontaneous activity persisted when the mouse was kept in continuous darkness. Johnson (1939) showed that although rhythmic activity persisted in constant light in Peromyscus, the cycle was modified so that the period was longer than 24 hours.

Since then, an enormous amount of literature has accumulated concerning the relationship between light and circadian rhythms. For example, Bullfinches kept in continuous darkness showed a period of 24 hours, while in continuous light it was 22 hours (Bunning, 1967). In mice the period was 24.5 to 25.4 hours in constant light but only 22.5 to 23.8 hours in constant dark (Hoffman, 1965). Furthermore, changing the intensity of constant light exerted an effect on the period of the rhythm. Although exceptions exist, in general the length of the period of diurnal species has been found to decrease with increasing light intensity, and in nocturnal species it has increased with increasing light intensity. In many cases the frequency (the number of oscillations or cycles per unit of time) varied linearly with the logarithm of light intensity (Aschoff, 1960). Not only was the period altered, but the ratio of activity to rest time and the absolute amount

of activity were found to be greater in bright than in dim light in diurnal species, while the reverse was generally true in nocturnals (Aschoff, 1960, 1963).

Although periods can be altered by maintaining the animal in controlled laboratory conditions, the rhythms have been found to continue with remarkable constancy in the absence of external phase setters. Mice maintained for six generations under constant light continued to display a circadian rhythm in activity (Aschoff, 1960). Both early blinded and congenitally blind rats showed alternating 12 hour phases of activity and inactivity (Richter, 1971). Attempts to modify sleep patterns in the rat by sleep prevention, electric shock conditioning, and early life activity restrictions resulted in no persistent modification of circadian sleep ratios (Webb and Friedman, 1970). Rhythms which are seemingly independent of environmental control have been termed free-running rhythms. Such rhythms have been reported to be temperature independent; that is, cycle length did not change over a wide range of constant temperatures (Brown, 1972).

Under natural conditions circadian rhythms are synchronized to 24 hours by phase setters, or "Zeitgeber." The dominant phase setter is light (Bruce, 1960). The influence of light can be readily demonstrated by inverting the cycle of light and dark. It is an old experimental idea and was used as early as 1757 by J. Hill (Bunning, 1967). An organism so treated adjusted its circadian rhythm to the

inverted cycle. In some organisms one or two days were sufficient for the adjustment, but longer than 8 to 16 days was evidently never necessary. A complete reversal in rats required 8 to 10 days (Bunning, 1967).

The influence of light has also been demonstrated by the ability of organisms to adapt their rhythms to abnormal light-dark cycles. However, this ability to entrain to different cycle lengths was found to be quite limited. Mice could be entrained to day lengths varying from 21 to 27 hours, but with longer or shorter light-dark cycles the animals reverted to an approximately 24 hour rhythm (Bruce, 1960). Furthermore, a short period of light, ranging from a few minutes to a few hours each day at the same time in otherwise continuous darkness, was a sufficient phase setter for many plants and animals. The rhythm shifts in such a way that phases normally associated with day coincide with these light periods (Bunning, 1967). In diurnal species light onset appears to play a more important role in resetting the rhythm than cessation of light, and the initiation of activity often bears a more constant relationship to dawn than cessation of activity plays to sunset (Hinde, 1970).

It has often been assumed that biological clocks are endogenous. Brown (1960, 1972), however, argued that geophysical rhythms are perceived and act as phase setters. Evidence has accumulated which tends to refute this theory. The first was the demonstration of

repeatable individual differences within a species. In flying squirrels, individuals showed different but internally consistent periods of activity; the range in the periods was 22.98 to 24.35 hours, but the variation from day to day for an individual was only several minutes (DeCoursey, 1960). In lizards individual differences ranging from 21.1 to 24.7 hours have been recorded (Bunning, 1967). Thus if a geophysical stimulus were perceived, entrainment of individuals was not synchronized. Secondly, bees trained to visit a sugar source at a certain time under constant conditions continued to visit at intervals of 24 hours after they were flown to another time zone and latitude. Furthermore, bees trained under natural lighting cycles were moved, and the sugar searching activity showed two clear maxima which were related to the two lighting cycles, not location of the laboratory (Renner, 1960). Also, Hamner et al. (1962) have supplied evidence that plants, mammals and insects continue circadian behavior at the South Pole, where rotation of the earth cannot result in geophysical fluctuations which could serve as a phase setter.

This apparently endogenous nature of the clocks has led to the hypothesis of underlying genetic control. In 1932, Bunning crossed plants displaying different periods and found that the offspring had intermediate periods (Bunning, 1967). Recently three mutants of Drosophila have been isolated in which the normal circadian rhythm of pupa emergence was drastically changed. One mutant was

arrhythmic, another had a period of 19 hours and the third had a period of 28 hours. All mutants appear to involve the same functional gene on the X chromosome (Konopka and Benzer, 1971).

Circadian rhythms in mammals are common for physiological variables as well as for basic physical activity. Rhythms have been described for concentration of blood constituents (glucose, eosinophiles, corticosterone), rate of mitosis in several tissues, metabolic changes in nucleic acids and hormone secretion. Rhythms for body temperature, oxygen consumption corrected for locomotor activity, blood coagulation and onset of estrus and ovulation have also been described (Aschoff, 1963). Insects were found to show daily rhythms in susceptibility to insecticides (Cole and Adkinsson, 1965).

Thus, evidence has indicated that maintenance of circadian rhythms has an underlying genetic determination. Properties of these rhythms are remarkably similar throughout the animal and plant kingdoms; rhythms can be entrained to environmental fluctuations but not eliminated by the absence of such phase setters.

## Measures of Rodent Activity

Unlike the basic rhythm, a considerable amount of variation has been found in measures of spontaneous activity, such as wheelrunning. It has been possible to select strains of active and inactive rats (Rundquist, 1933; Brody, 1942). Standard strains of inbred laboratory

mice have been shown to differ in wheelrunning activity and the mode of inheritance has been investigated (Bruell, 1964b; Messeri et al., 1972).

Another group of measures which have been widely used in the study of rodent behavior are those taken in the novel environment test. In this test the animal is placed in a large illuminated area either marked into subdivisions or divided by barriers or an enclosed maze, or some variation of the two. All tests measure the amount of ambulation or movement and the amount of eliminative behavior of the subject. Hall (1934) first used the term "emotionality" to apply to the behavioral and autonomous changes which supposedly accompanied high sympathetic nervous activity. He characterized emotional defecation as that defecation which occurred at a higher rate than in the home cage and which ceased upon repeated exposure to the situation which originally evoked it.

The ambulation score has generally been assumed to be a measure of exploration. The relationship between these two measures and their interpretation has since been the subject of much controversy. In many cases, defecation and ambulation in the open field have shown a significant negative correlation (Archer, 1973). This has been interpreted to mean that high emotionality inhibits exploration and encourages defecation, while low emotionality facilitates exploration and inhibits defecation. However, it has also been suggested that both

low and high fear states are associated with low exploration whereas at intermediate fear states exploration is high, thus producing an inverted U shaped curve (Lester, 1968). The validity, underlying concepts and usefulness of the open field tests have been hotly debated and no concensus of opinion exists. Much of the disparity of results undoubtedly stems from a lack of uniformity in test conditions and the inability to generalize results from rats and mice. For arguments in favor of the usefulness of the emotionality concept see Broadhurst (1969) and Denenberg (1969). For discussions of the limitations of the emotionality concept see Ivinskis (1970) and Archer (1973).

There are a large number of factors which have been reported to modify results obtained from open field studies. First, when only one type of test was used at different times, defecation and ambulation showed a high degree of individual consistency or repeatability (Ivinskis, 1966, 1968; Hegmann and DeFries, 1968). At least part of this repeatability has been found to have a genetic basis. Thompson (1953) showed that 15 standard inbred strains of mice differed markedly in both ambulation and defecation in the open field. Furthermore, the relative ranking of the strains has been found to be remarkably constant at various laboratories and in the various types of tests designed to measure exploratory behavior (Thompson, 1956; McClearn, 1959; Bruell, 1964a; Goodrick, 1971).

Crossbreeding studies determined that exploratory behavior displayed mainly additive inheritance in some strain crosses while non-additive genetic variance was important in others (McClearn, 1961; Bruell, 1964a). Selection was effective in producing mice both high and low in open field activity; realized heritability was estimated to be 0.13. A correlated response in open field defecation was also noted with a realized genetic correlation of -.80 (Defries and Hegmann, 1970; DeFries, Wilson and McClearn, 1970). Broadhurst (1962, 1967) utilized selective breeding based on emotional defecation in rats to establish so-called "reactive" and "nonreactive" strains. Divergence in ambulation scores was also noted, with the correlated response in the same direction as in mice.

It has frequently been found that females are more active in the open field (McClearn and Meredith, 1966; Rosenberg, Denenberg and Zarrow, 1970; Archer, 1973; Russell, 1973). Russel (1973) found a positive correlation between defection score and home cage defecation level in males but not in females. Males also had higher home cage defecation levels than females but correction for this factor failed to eliminate the difference in open field test scores. Rosenberg et al. (1971) found that neonatally castrated rats were more active in the open field than intact male controls, while androgenized female rats scored lower than control females. Thus the difference in scores between sexes probably reflects hormonal influences on responsiveness to the test.

Differences in the level of illumination in the testing area affect ambulation and defecation scores. When standard inbred strains of mice were tested, ambulation scores were higher and defecation scores were lower under red light than white light and under low illumination than high illumination. Pigmented strains were relatively unaffected by changes in illumination. Even under low illumination, however, the albino strains still generally scored lower in ambulation than the pigmented strains (McClearn, 1960; Ross et al., 1966; McReynolds, Weir and DeFries, 1967). Failure to find line differences under low illumination in some tests has been attributed to an experimental artifact produced by automatic scoring techniques, with the mode of locomotion in some low active strains inflating ambulation scores (Goodrick, 1971). Apparently part of the illumination sensitivy is due to the effect of the albino gene. Tests utilizing a spontaneous mutation to albino in a normally pigmented inbred line showed the albino subline to be less active in the open field, but no difference in wheel-running was noted (Fuller, 1967; Henry and Schlesinger, 1967). When albino and pigmented animals of $F_2$ and later generations derived from crossing albino and pigmented strains were compared, some studies have found differences in ambulation and defecation when tested under white light, but the differences disappeared when tested under red light (DeFries, Hegmann and Weir, 1966; DeFries, 1969). Others have failed to note the difference. Goodrick (1973) found that for the first

five minutes of the test pigmented mice were more active than albinos but the differences were reversed in the last five minutes; he failed to find a differential effect of illumination as a function of coat color for the total 15 minute activity score. All results agree that although part of the difference in open field ambulation and defecation may be due to the effect of the albino locus, a large part of the variance is due to segregation at other loci; albino inbred strains are not inactive solely because of differential sensitivity to light (DeFries et al., 1966; DeFries, 1969). DeFries and Hegmann (1970) estimated that segregation at the albino locus accounted for 12% and 26% of the additive genetic variance in open field activity and in defecation, respectively.

Age has been implicated as a factor influencing ambulation in rats. Although all subjects scored similarly in the first exposure to the open field, rats 40 days or younger showed day to day increases in activity while rats 70 days or older failed to show this activity change. This pattern persisted with as many as 60 days of repeated trials. Indeed, the adults showed a decrease for the first three to four trials followed by an increase to the level of the first trial (Bronstein, 1972a, b, 1973).

In addition, a large number of other environmental variables have been reported to influence ambulation and defecation scores. Most are far from universally accepted phenomena with many

conflicting results reported. They are thus presented as possible influencing factors. Generally animals reared in larger groups tend to exhibit less exploratory behavior. Seitz (1954) in rats and LaBarba and White (1971) in mice both reported greater exploratory activity and lower defecation scores in individuals in smaller litters. Warren and Ivinskis (1973) reported greater activity and less defecation in rats housed in isolation vs those group housed. Bell et al. (1971) found that exploration in mice decreased with increasing housing group size, and this effect did not depend on the degree of crowding.

Several workers have tried to relate stimulation in the early life environment to later behavior, and some have reported that animals in the more deprived or restricted environments had higher later life ambulation scores. In particular, rats provided with toys or cage decorations had lower locomotor activity test scores (Nielson, 1970, 1971) and lower open field ambulation scores (Smith, 1972) than those reared without such items. However, rats reared in an environment in which they controlled lighting conditions and the delivery of food and water were more active in the open field than rats which had no control over their occurrence (Joffe, Rawson and Mulick, 1973). Also, severe food deprivation in early life in mice has been linked to higher defecation scores and lower activity in running wheels with the effect being somewhat strain dependent (Manosevitz and McCanne, 1973).

Two early life stimuli which have been studied extensively are electric shocking and handling. Handling is a procedure whereby the young are removed from the nest and isolated from the mother for some nonfatal length of time. At times the two above treatments have been combined with a buzzer or cold treatment. Shocking is considered to be the more noxious stimulation. The most common finding has been that early life handling increases open field ambulation and decreases defecation (Denenberg, 1969; King, 1970; Williams and Russell, 1972). In 1964, Denenberg proposed that emotional reactivity was inversely related to the amount of stimulus input in infancy; i.e., the more stimulation applied to the infant organism, the less emotionally reactive the adult organism. However, Goldman (1969), upon reviewing the literature, proposed that subsequent emotionality was a curvilinear function of the amount of stimulation in infancy with both low and high stimulation producing higher emotionality scores than intermediate levels. While Henderson's (1967) results with inbred strains of mice were in general agreement with this theory, he found that actual treatment response varied with strain.

Much effort has been applied to the study of psychological stressors acting on the mother during pregnancy. Several different procedures have been applied to induce prenatal stress: handling, conditioned avoidance, swimming, open field exposure, noise, and epinephrine, norepinephrine or corticosterone injections.

Theoretically, the prenatal stress is intended to influence offspring behavior via maternal hormonal changes, but some of the techniques used could act directly on the fetus (Archer and Blackman, 1971). The original experiment was performed by Thompson in 1957. His method of inducing stress was avoidance conditioning. Crossfostered controls were maintained. Offspring of mothers receiving the electric shock during pregnancy showed less ambulation and more defecation in the open field. Archer and Blackman (1971) provided an extensive review of the available literature. They concluded that most studies indicated that prenatal stress produced changes in the behavior of the offspring in the direction of decreased ambulation in novel situations. Most have found no difference in the behavior of crossfostered and unfostered animals.

Many other factors appear to interact with prenatal stress to determine later behavior. Some postnatal factors are preweaning experience and early stimulation, group or isolation housing, age at testing and previous test experience. Interestingly, it has been found in separate experiments that prenatal stress had the opposite effect on later behavior of postnatal handling. Young (1964) combined both in the same experiment and reported the same results.

Of particular importance are reports of significant genetic x treatment interactions. Thompson and Olian (1961) studied the effects of prenatal injections of epinephrine on three strains of inbred mice.

In the low activity strain, the offspring of treated mothers were found to be more active than controls. In the high active strain the offspring of treated mothers were less active than controls. Only slight differences between the groups were found in the strain intermediate in activity. In a series of experiments utilizing physical prenatal stress in two inbred strains of mice, it was consistently found that prenatal stress decreased the activity of the high active strain and increased the activity of the low active strain (DeFries, 1964; Weir and DeFries, 1964; DeFries, Weir and Hegmann, 1967).

There are a few reports that offspring behavior was modified by premating stress applied to the mother. Joffe (1965) reported such results in rats using premating avoidance training and concluded the effect was mediated prenatally since all offspring were fostered at birth. He further found a sire interaction with progeny of fathers of high ambulating strains having reduced activity and progeny of low ambulating sires showing increased activity. Denenberg and Whimbey (1963) found that rats born of and raised by females not handled in infancy ambulated more and defecated less than those born of handled mothers. However, when they employed a fostering technique to separate pre- and postnatal influences, a fostering effect was found and results were inconclusive.

The reported influence of environmental variables, such as handling, on later open field ambulation and defecation scores in

rodents suggests that the maternal environment should be an important influence on these scores. Richards (1966) even suggested that the handling procedure produces changes in the stimulus properties of the young which result in altered maternal behavior, and the effects of handling do not necessarily operate directly on the infant. Priestnall (1973) found that mothers whose pups had been handled licked the pups after the treatment more than situations in which the mother had been handled, or controls where neither the mother nor the pups were handled.

Studies to examine maternal influences have utilized both interspecific and intraspecific crossfostering techniques. One extensive series of studies examined mice reared by rat mothers (Denenberg et al., 1964, 1966; Hudgens et al., 1967, 1968). It was established that such mice were less active in the open field. Mice raised with rats after weaning were also less active in the open field. Trends were the same with the two strains of mice tested, one pigmented and one albino. Fostering within a mouse strain had no effect. A further series of experiments (Rosenberg et al., 1970; Paschke, Denenberg and Zarrow, 1971) designed to eliminate the possible effect of rat mother's milk included a rat "aunt" in the same cage as the mouse and her litter. It was found that offspring so treated also had lower ambulation scores when adults. The "aunt" was a rat who had just given birth to a litter but was unable to nurse her young because she

had been thelectomized. The rat "aunts" were found to display maternal behavior toward the mouse pups. Mice reared with control rat "aunts" (those with no maternal tendencies) showed only a slight decrease in open field ambulation. From these findings the authors concluded the rat-mouse interaction and not the rat mother's milk was the critical variable. As a generalization Bols and Wong (1973) found that rat reared gerbils were less active than control gerbils in the open field. Quadagno and Banks (1970) utilized a crossfostering procedure between two species of mice, Mus musculus and Baiomys taylori ater. They found the fostered groups to have activity levels altered in the direction of the foster parent; the activity scores of the fostered groups were intermediate and the unfostered groups showed the extreme values.

Crossfostering within a species has been used to test the hypothesis that different amounts of maternal handling was a generator of interstrain behavior differences. Ressler (1962) examined the amount of parental handling in BALB and C57 strains of mice. He found that BALB parents handled both strains of pups more and BALB pups were handled more irrespective of the postnatal parental strain. He later reported (Ressler, 1963) that both strains engaged in more exploration if raised by BALB rather than C57 parents. Ottinger et al. (1963) reported that rats born of and reared by the high active group had highest ambulation scores; those born of and reared by low

active groups had the lowest ambulation scores; fostered groups were intermediate. They concluded that offspring ambulation had both genetic and maternal components. Other workers have failed to discover differences due to maternal effects. Poley and Royce (1970) found no difference in the amount of maternal stimulation of pups in two inbred strains of mice. Griesel (1964) and Joffe (1965b) both reported that the strain of the foster mother did not affect the adult behavior of rats. DeFries et al. (1967) used ovarian transplantation between inbred strains and their hybrids. They found no difference in ambulation and defecation between inbred mice carried by inbreds and carried by hybrids.

## Maternal Behavior

In an attempt to quantify maternal responsiveness to young, behavioral patterns which are deemed maternal by the experimenter have been established. Unfortunately, it is often difficult to demonstrate that such behavioral indices are valid measures of maternal care. Often scoring systems have been devised which were based on several measurements in an attempt to quantify the strength of the maternal instinct. Such procedures are based on the mistaken assumption that maternal behavior is controlled by a single unitary drive. The fallacies of the unitary drive concept were discussed in detail by Hinde (1970) and Richards (1967). Richards pointed out that

the various maternal activities can occur independently of each other and that the various drives, such as nest building and retrieving, are caused by different endo-organic factors.

Prolonged presence of young can evoke maternal responses in non-lactating females. Maternal behavior found to be exhibited by virgin rats included retrieving pups to a nest, nest building, licking the young and crouching over them in a nursing position (without lactating) (Rosenblatt, 1970). Similar results have also been reported in mice (Noirot, 1964). Maternal behavior exhibited by virgins did not appear to depend on pituitary or ovarian hormones; neither hypophysectomy nor ovariectomy prevented them from exhibiting maternal behavior in response to pup stimulation (Rosenblatt, 1970). However, Leon, Numan and Moltz (1973) argued that ovariectomy does inhibit maternal behavior induction, but a postoperative interval of from 4 to 8 weeks is required to discern the difference. Induction of maternal behavior was reported to have no effect on estrus cycling (Rosenblatt, 1967). Blood transfused from pup induced maternal virgins did not shorten latencies for retrieving pups in recipient virgins (Terkel and Rosenblatt, 1971). However, blood transfused from newly parturient mothers was able to induce maternal behavior in a significant proportion of virgins (Terkel and Rosenblatt, 1973).

The type of nest built by adult female mice has been reported to depend on the reproductive state. Normally, nonpregnant female and

male mice built a small saucer-shaped sleeping nest. A second type of nest was much larger and completely enclosed. This type appeared about the fourth or fifth day of gestation and was called a brood nest. This response has been found only in mice and hamsters (Richards, 1967). Ovariectomized and intact virgin female mice built maternal type nests when presented with day old pups (Gandelman, 1973). Virgin females and day 1 castrated males could be induced to build maternal style nests following treatment with estrogen plus progesterone. Presentation of pups was not necessary for the response. Intact males and females given neonatal injections of testosterone would not respond to estrogen and progesterone treatments (Lisk et al., 1973). Thus two factors are probably involved in maternal nest building: one response is hormonal and the other is mediated by the presence of young. One limitation of the nest building test is that it may not be valid for interspecies mouse comparisons. Wolfe (1970) compared the size of sleeping nests of three species of Peromyscus. He concluded that species that normally construct surface or elevated nests build larger nests under experimental conditions than species which normally nest in underground chambers.

One test used extensively is retrieving, or the returning of pups to the nest. Retrieving appears to be a common response in rodents; not only laboratory rats and mice but the golden hamster, deermice, red squirrels, flying squirrels and ground squirrels have been studied

(Michener, 1971). In some the response is limited to lactating females (Richards, 1967). King (1958) studied retrieving behavior in two subspecies of deermice and found that individual differences between mothers in retrieving behavior were so great that there were no discernible subspecies differences.

Retrieving tests have been most frequently used to assess changes in maternal behavior within an animal rather than to test for differences between groups. Rat mothers were shown to take longer to retrieve pups on the first test than on subsequent tests. The improvement resulted from a decrease in the delay preceding retrieval of the first pup, from more skillful handling of the pups, and from a decrease in the time spent on other activities (Beach and Jaynes, 1956b; Carlier and Noirot, 1965; Michener, 1971). Apparently some of the improvement in the first lactation period is retained until the second lactation period. Carlier and Noirot (1967) reported that rat mothers had better scores during second lactation than during the first, and improvement continued during the second lactation. Beach and Jaynes (1956b) reported no parity differences in improvement between primiparous and multiparous rats when the primiparious mothers were given five retrieving tests the day of parturition.

The most interesting changes are those associated with gestation and lactation. Noirot and Goyens (1971) presented primiparous

mice in day 1, day 4, day 14 and day 19 of gestation a two-day-old pup and observed maternal behavior. Total time displaying maternal behavior dropped below control levels on the first day of gestation; the virgin control level was regained and exceeded between the fourth and fourteenth day and remained fairly constant on the nineteenth day. Late gestation improvement was observed in activities dependent upon olfaction, such as licking and sniffing of pups.

Rosenblatt (1969) has described three phases of maternal behavior in the rat. The initiation phase lasts from the first to the third day postpartum; nursing, retrieving, and nest building are practiced with vigor, and care of the young is initiated almost entirely by the mother. The maintenance period lasts from day 4 to the thirteenth or fourteenth day. During this phase the mother's behavior has stabilized; the female initiates all feeding by approaching the young in the nest, licking them and crouching over them while the pups gradually improve in crawling and suckling. The third phase begins around the fifteenth or sixteenth day and extends until the twenty-first to the twenty-eighth day; during this time maternal behavior declines. The decline in maternal behavior was shown to have a regular order: nest building declined first, followed by retrieving several days later, then nursing, which in some cases continued at a reduced level until the twenty-eighth day.

Thus the phases of the female's maternal behavior cycle are synchronized with the stages of development of the young. The changes in maternal behavior reflect both changes in the maternal responsiveness and changes in the external stimuli from the young. To examine changes in maternal responsiveness, mothers in various stages of the cycle were presented with constant age young. Denenberg, Grota and Zarrow (1963) fostered newborn young to lactating females. They reported that as the number of days the foster mother had been lactating increased, the mortality rate of the foster young also increased and body weight of the survivors at 21 days decreased. Rosenblatt (1969) used constant age pups 5 to 10 days of age to elicit maternal behavior in lactating females and compared responsiveness to the test young to that with their own young. About the same time that nursing, retrieving, and nest building declined in the natural litter situation (with pups in their third to fourth week) they also declined in tests with 5 to 10 day old pups.

To determine stimulus variation with pup age, Noirot (1964) presented groups of naive female mice with infant mice aged 1 to 20 days. Occurrences of retrieving, licking, nest building and lactation position decreased with increasing age of the young. The incidence of nest building and retrieving fell sharply at the thirteenth day, the age when the pups' eyes opened and mobility increased. Rowell (1960) presented foster pups of various ages to hamster mothers in various

lactation phases. Acceptance increased from day 1 to day 7; the highest percentage of acceptance was of pups aged 7 to 10 days; acceptance declined with age of pups thereafter. The female's response also depended upon the age of her own litter.

Maternal behavior in rodents has been shown to depend upon a variety of sensory modes. Tactile, auditory and olfactory stimulation have been found to affect maternal behavior. Females deprived of the opportunity to lick their own bodies may fail to care for their young. It has been suggested that this is due to the lack of development of a continuity between "own body" and young important to the development of the mother-infant bond (Rosenblatt, 1970). However, another study demonstrated that deprivation of self-licking retarded mammary development in pregnancy (Richards, 1967).

Auditory and olfactory cues have been implicated in searching and retrieving behavior. Noirot (1969) exposed naive female mice to pups in a container so only olfactory and auditory stimulation could occur. These females showed more maternal responses to a weak stimulus (a dead pup) than did the untreated controls. Auditory cues from the pups appear to be responsible for the initiation of searching and retrieving behavior. When ultrasonic recorded pup calls were played back to lactating female rats over 50% of them left the nest and engaged in searching behavior (Herrenkohl and Sachs, 1972). Once pups were found olfactory cues seemed to be important to inhibit

cannibalism of the young. After bulbectomy, naive virgin rats cannibalized foster young and later their own young, but maternal behavior was exhibited by bulbectomized virgin rats which had been sensitized by exposure to young and newly parturient rats (Herrenkohl and Sachs, 1972). Gandelman, Zarrow and Denenberg (1971) reported that the incidence of cannibalism in bulbectomized female mice was a function of the age of the pups; one of the cues which attenuated pup killing was the presence of body hair. Thus the effects of olfaction loss were modified by hormonal state, previous experience and other stimuli.

Apparently olfaction is important to the mother-young relationship during the latter stages of lactation. Rat pups approached the odor emitted by a lactating female rat in preference to that of a non-lactating female; no preference was shown if olfactory cues were eliminated (Leon and Moltz, 1971). Because the young showed no preference for their own vs another female at the same lactational stage, they hypothesized the existence of a maternal pheromone. By measuring the attraction of young known to be responsive to the pheromone to females in various lactation stages, they concluded that the pheromone is only released between 14 and 27 days postpartum (Leon and Moltz, 1972). Thus the initiation of pheromone release corresponded to the period of increasing mobility of the pups and of declining scores in maternal behavior by the female; release ceased at normal weaning time. Fullerton, Berryman and Porter (1973)

showed that guinea pig pups up to three weeks of age show no preference for their own mother as compared to another lactating female. The females preferentially approach their own offspring rather than other infants. Thus specificity in the mother-offspring relationship probably depends on female discrimination.

# MATERIALS AND METHODS

This investigation consisted of two experiments. The main experiment was designed to study differences in duration and pattern of maternal behavior between crossfostered and noncrossfostered lactating mice of two inbred lines. The secondary experiment measured open field behavior on similar populations.

## Measurement of Maternal Activity

### Subjects

Two inbred lines of mice, A/J and C57BL/6J, were obtained from the Jackson Laboratory of Bar Harbor, Maine. Twenty females and ten males of each line were purchased in June, 1973. The A/J line is albino and carries color genes for black and brown coat color. The C57 line is black. Both lines are highly inbred. As of December, 1967 the A/J line had been subjected to 139 generations of brother-sister matings and the C57 line had been subjected to 95 generations (Green, 1968).

These two lines of mice have been found to differ in several measures of behavior. The A/J line has scored low in open field activity, high in open field defecation, low in aggressiveness and low in alcohol preference, while the C57 line has scored high in open

field ambulation, low in open field defecation, high in aggressiveness and high in alcohol preference (Green, 1968). In addition, these two lines have been found to be genetically distinct on the basis of the alleles found at polymorphic loci, especially electrophoretic protein variations (Taylor, 1972).

Husbandry

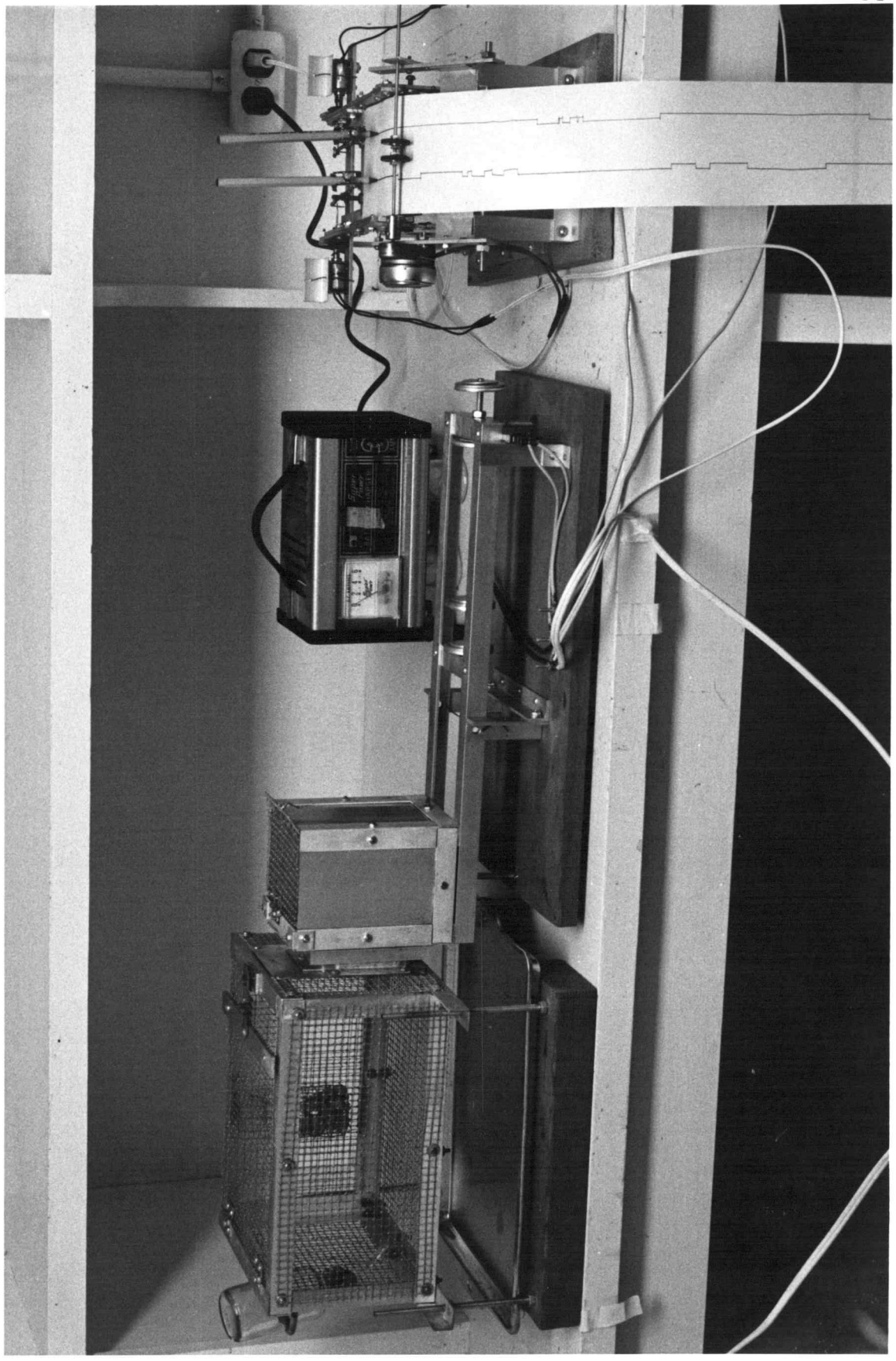
When not in maternal behavior testing the mice were group housed in standard laboratory cages with food and water available ad libitum. Lactating and pregnant females were fed Purina Mouse Chow. Others were fed either Purina Mouse Chow or the standard Oregon State University laboratory ration. The lighting schedule was irregular and was controlled by personnel at the Oregon State University Small Animal Laboratory.

Females were group bred in single sire groupings at approximately five months of age. When a female was observed to be pregnant, she was removed from the breeding cage and either placed in the maternal behavior test cage (to be described) or allowed to litter under normal laboratory routine. Those animals who were not tested were placed in a separate cage and given a can of sawdust for nesting. Litters were weaned at 26-29 days of age. While only females having first litters were used for maternal behavior testing, subsequent litters were used to maintain colonies of the inbred lines.

Equipment

A two-chambered cage was designed which would allow measurement of the amount of time a lactating mouse spent with vs away from the litter. The apparatus is pictured in Figure 1. The larger room was made of wire mesh and measured 28.0 x 20.3 x 15.2 cm. The food dispenser and water bottle were mounted in this room. The smaller room was designed to be the nest area and measured 10.2 x 10.2 x 14.0 cm. The sides and bottom of the nest room were made of tempered aluminum and the top was wire mesh; thus the room was enclosed except for the top and a door between the two rooms. Sawdust and a facial tissue were provided in this room for nesting material. The nest room was attached at the bottom to a bar which had adjustable weights at the opposite end, such that a fulcrum existed between the two ends of the bar. The weights were balanced to the weight of the lactating female. Anytime the female left the nest area and entered the larger room, a microswitch was depressed. It remained depressed until she returned to the littering room. This switch was attached to a continuous event recorder. Paper was fed through the event recorder by a synchronous motor at the rate of 30.4 cm/hour for one recorder and 30.8 cm/hour for the other recorder. The recorder pen-marked the outside of the continuously fed paper when the female was in the litter room and the inside when

Figure 1. Apparatus for the continuous recording of maternal behavior.



the female was away from the litter. Reference times were marked on the event recording daily. Thus, the room where the female was located at any time of the day was known.

Four maternal behavior cages and two dual channel event recorders were used for the experiment. They were located on shelves 0.76 m from the floor in a room isolated from other activity. Usually, human entry into the room was limited to once or twice per day for the purpose of providing food and water and for checking the recorder and weights. This interruption was normally less than 15 minutes. Occasionally, removal of one test subject or the introduction of a test subject required activity in the room of longer duration. Cages were dismantled and washed after each 10 day test. Temperature in the test room was maintained relatively constant, between 21.6-24.5$^{o}$C. Test subjects were maintained under a 14 hour light: 10 hour dark schedule. Lights were on between 0600 and 2000 hours.

Methods

The experimental design was such that females of three groups within each inbred line were tested in the maternal behavior apparatus. One group, termed the first generation unfostered, was comprised of some of the females obtained from the Jackson Laboratory. They were approximately eight weeks of age at the time of arrival, June 6, 1973.

Their litters were born in the maternal behavior apparatus between August 24-December 24, 1973 for the C57 line and between September 4-October 5, 1973 for the A/J line.

Generation 2 individuals were the progeny of generation 1 females who had been either crossfostered to females of the other inbred line at birth or had been reared by their own mother. In the C57 line, crossfostered individuals were weaned between August 8-September 26, 1973 and tested between October 31, 1973-April 12, 1974; second generation unfostered individuals were weaned between October 1-December 6, 1973 and tested between April 24-June 27, 1974. In the A/J line, crossfostered individuals were weaned between August 8-September 26, 1973 and tested between October 30, 1973-March 17, 1974; second generation unfostered individuals were weaned between October 1, 1973-March 28, 1974 and tested between February 12-July 25, 1974. Only females rearing first litters were used for maternal behavior testing, but females which comprised the second generation were the result of both first and subsequent parturitions.

Pregnant females were placed in the maternal behavior cage several days prior to parturition. Mice were checked daily for new litters. On the day following littering, the maternal activity recording was begun and it was continued until the litter was ten days of age. For recording purposes the "day" was defined to be that 24 hour

period beginning and ending at noon. At ten days the mother and the litter were transferred from the maternal activity cage to a regular mouse cage with a can of sawdust for a nest. The litter size and litter weight at ten days were recorded. The litter size and weight at birth were not taken to avoid disturbing the female, and possibly invalidating part of the maternal activity record.

## Data Preparation

The data as recorded consisted of rolls of paper tape which indicated the room location of the female mouse. Over 3.6 km of paper tape recordings was collected. Each tape was divided into segments which represented 15 minutes. Such a segment was about 7.7 cm long. Within each segment the cumulative length of the line indicating that the mouse was away from the nest was measured to the nearest millimeter. This measurement was then converted to the time equivalent. The number of times the female left the nest room was also recorded. These 15 minute values were then summed, and the total time out was divided by the number of trips to produce the three behavior measurements for the desired interval length. These were: total time away from the litter during the time interval, total number of trips away from the litter during the time interval, and average trip length. Measurements for three interval lengths were computed in this study. They were: per day (24 hours), light vs dark (14 hours light, 10 hours dark), and per hour.

For the three behavior measures calculated for a particular interval, time trends and behavior differences of the experimental groups of subjects were examined by analysis of variance techniques. Appropriate models are described in greater detail in the Results section.

## Open Field Study

### Subjects

Subjects were of the same lines as those described for Experiment I; both males and females were tested and some females tested for open field behavior were also tested for maternal behavior. However, the subjects from each line fell into two age brackets. The older group were the same animals termed generation 1, unfostered in the preceding experiment, but in this test they were scored after having raised or sired one or more litters. They were approximately nine months of age at the time of open field testing. The younger group corresponded to the generation 2 unfostered and fostered groups. These individuals were tested in the open field prior to mating, and were from four to five months old at the time of testing.

### Equipment

The open field arena was a 91.4 x 91.4 cm enclosure which was

subdivided by painted lines into 81 10.2 cm squares. The bottom of the enclosure was made of wire mesh surrounded by 30.5 cm white plywood walls. The enclosure was elevated 7.6 cm above the floor; white paper was placed on the floor under the enclosure.

Illumination was provided by two 75w red or frosted white bulbs. These were placed at opposite corners of the arena at a height of approximately 0.6 m from the arena floor. Aluminum shades directed the light onto the arena floor.

Methods

At the start of each test the subject was placed in a corner square. The number of squares entered and the number of fecal boluses deposited in five minutes were taken as the ambulation and defecation scores, respectively. Eighty-five A/J and 89 C57 mice were tested.

Each mouse was subjected to two tests, one under white light and the other under red light. The two tests were conducted at least 24 hours apart. Half of the mice were tested with a white light, red light order; in the other half the order was reversed.

Three series of tests were conducted. These are termed trials in the following analyses. Trial 1 took place from November 14-21, 1973; trial 2 took place from January 16-20, 1974; and trial 3 took place from March 20-22, 1974. The room location of the first trial

differed from that of trials 2 and 3. All of the older animals were tested in the first trial. Fostered individuals were tested in trials 1 and 2. Young, unfostered animals were tested in trials 2 and 3.

Ambulation and defecation scores were analyzed using multiple regression techniques to be described in the Results section.

# RESULTS

From the continuous recording of the room location of the female three measures of behavior could be computed. These were: time away from the litter per time interval, number of trips away from the litter per time interval, and average trip length for the time interval. Because the recording was continuous, several time intervals could be defined and examined.

Thus the analysis consisted of several phases. The first examined the three behavioral measures with the time interval as 24 hours, with each mouse tested having one measurement for each of the first 10 days after littering. A day was defined as starting at noon. In the second phase of the analysis, the 24 hour day was divided into light and dark portions. Thus each mouse tested had 20 measurements. The third analysis used one hour as the time interval and was designed to inspect the circadian rhythms in the activity of the female mice.

A crossfostering design was used to partition the line differences and maternal effects. Each model includes a comparison of mothers that had been fostered in infancy to females of the other line and unfostered mothers. In addition, a comparison of the two chronologically separate groups or generations was included. This will be called the generation effect. Generation was confounded with time,

season, laboratory where raised and a plethora of other unidentified effects. No intentional selection occurred between generation 1 and generation 2. The number of females tested within each line, maternal and generation combination is given in Table 1.

Table 1. Number of lactating females tested within each experimental group.

| Line | Generation 1 | Generation 2 | |
|---|---|---|---|
| | | Unfostered | Fostered |
| A/J | 5 | 7 | 6 |
| C57 | 7 | 8 | 11 |

## Examination of Maternal Behavior Measured at Daily Intervals

### Amount of Time Spent away from the Litter Per Day

Table 2 presents the analysis of variance for the number of hours spent away from the litter per 24 hour period with data from both inbred lines included in the analysis. A list of symbols used in this model and succeeding models is given in Table 3. The data were also analyzed within each inbred line with effects due to individual differences included in the model. The individual effects for mice were nested within each of the three tested groups. Table 4 presents the

Table 2. Analysis of variance of time away from the nest per day.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 389 | | | |
| Error | 348 | 2.568 | | |
| Regression | 41 | 20.711 | 8.06 | .005 |
| Day | 9 | 44.91 | 17.49 | .005 |
| Line | 1 | 73.33 | 28.55 | .005 |
| Maternal Line | 1 | 18.06 | 7.03 | .01 |
| Generation | 1 | 6.30 | 2.45 | .25 |
| Line x Day | 9 | 2.76 | 1.07 | - |
| Maternal x Day | 9 | 0.83 | 0.32 | - |
| Generation x Day | 9 | 0.40 | 0.16 | - |
| Line x Maternal | 1 | 36.33 | 14.15 | .005 |
| Line x Generation | 1 | 0.38 | 0.15 | - |

$R^2 = 89.8\%$

Model:

$$Y = 4.67 - 2.06(D1) - 1.43(D2) - 0.98(D3) - 0.40(D4) - 0.02(D5) + 0.36(D6) + 0.76(D7) + 0.67(D8) + 1.41(D9) + 2.06(D10) + 0.56(C) - 0.56(A) - 0.27(Mc) + 0.27(Ma) + 0.17(G1) - 0.17(G2) + 0.38(CxMc) + 0.38(AxMa) - 0.38(CxMa) - 0.38(AxMc)$$

Table 3. Symbols used in the models.

| Symbol | |
|---|---|
| D1, D2, ... | Day effect for day 1, day 2, ... |
| C | C57 line effect |
| A | A/J line effect |
| Mc | Maternal effect of C57 line |
| Ma | Maternal effect of A/J line |
| G1 | Generation 1 effect |
| G2 | Generation 2 effect |
| U | Effect of unfostered females |
| F | Effect of fostered females |
| 1U | Generation 1, unfostered group |
| 2U | Generation 2, unfostered group |
| 2F | Generation 2, fostered group |

Table 4. Analysis of variance of time away from the nest per day, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 149 | | | |
| Error | 123 | 0.97 | | |
| Regression | 26 | 21.85 | 22.53 | .005 |
| Days | 9 | 20.62 | 21.27 | .005 |
| Generation | 1 | 3.31 | 3.41 | .10 |
| Fostering | 1 | 25.54 | 26.34 | .005 |
| Individuals | 15 | 22.10 | 22.79 | .005 |

$R^2 = 82.6\%$

Model:

$$Y = 4.14 - 1.96(D1) - 1.77(D2) - 0.67(D3) - 0.19(D4) + 0.16(D5) + 0.31(D6) + 0.83(D7) + 0.54(D8) + 1.38(D9) + 1.37(D10) + 0.19(G1) - 0.19(G2) + 0.51(U) - 0.51(F)$$

Table 5. Analysis of variance of time away from the nest per day, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 242 | | | |
| Error | 208 | 0.964 | | |
| Regression | 34 | 21.81 | 22.62 | .005 |
| Days | 9 | 47.72 | 49.50 | .005 |
| Generation | 1 | 4.72 | 4.90 | .05 |
| Fostering | 1 | 0.29 | 0.31 | - |
| Individual | 23 | 8.56 | 8.88 | .005 |

$R^2 = 78.7\%$

Model:

$$Y = 5.27 - 2.00(D1) - 1.66(D2) - 1.41(D3) - 0.67(D4) + 0.04(D5)$$
$$+ 0.38(D6) + 0.60(D7) + 0.82(D8) + 1.70(D9) + 2.20(D10)$$
$$+ 0.18(G1) - 0.18(G2) + 0.04(U) - 0.04(F)$$

analysis of variance table for the A/J line and Table 5 is the comparable analysis for the C57 line.

In all three analyses, the day effect was highly significant. The constant estimates for each line for total time away from the litter are plotted in Figure 2. Mothers of both lines spent an increasing amount of time away from the nest as the litters matured. A significant difference between lines was found; females of the C57 line spent about 1.1 hours longer per day away from their litters than did mothers of the A/J line. The absence of a significant line x day interaction indicates that the rate of increase in time spent away from the nest was approximately the same in both lines. The rate of increase was about 0.42 hours or 25 minutes per day. Neither the maternal line x day nor the generation x day interactions was significant indicating that these environmental differences also failed to alter the rate of increase. The mean square for day effect is over twice as large in the C57 line (Table 5) as in the A/J line (Table 4). Since both analyses had comparable mean square error estimates this indicates greater uniformity in the response of the C57 mothers to the growth of their litters.

Highly significant differences in total time away from the litter were attributable to postnatal maternal factors, with the main effect of A/J rearing being about half an hour increase in time spent away from the litter each day. However, the line x maternal line interaction

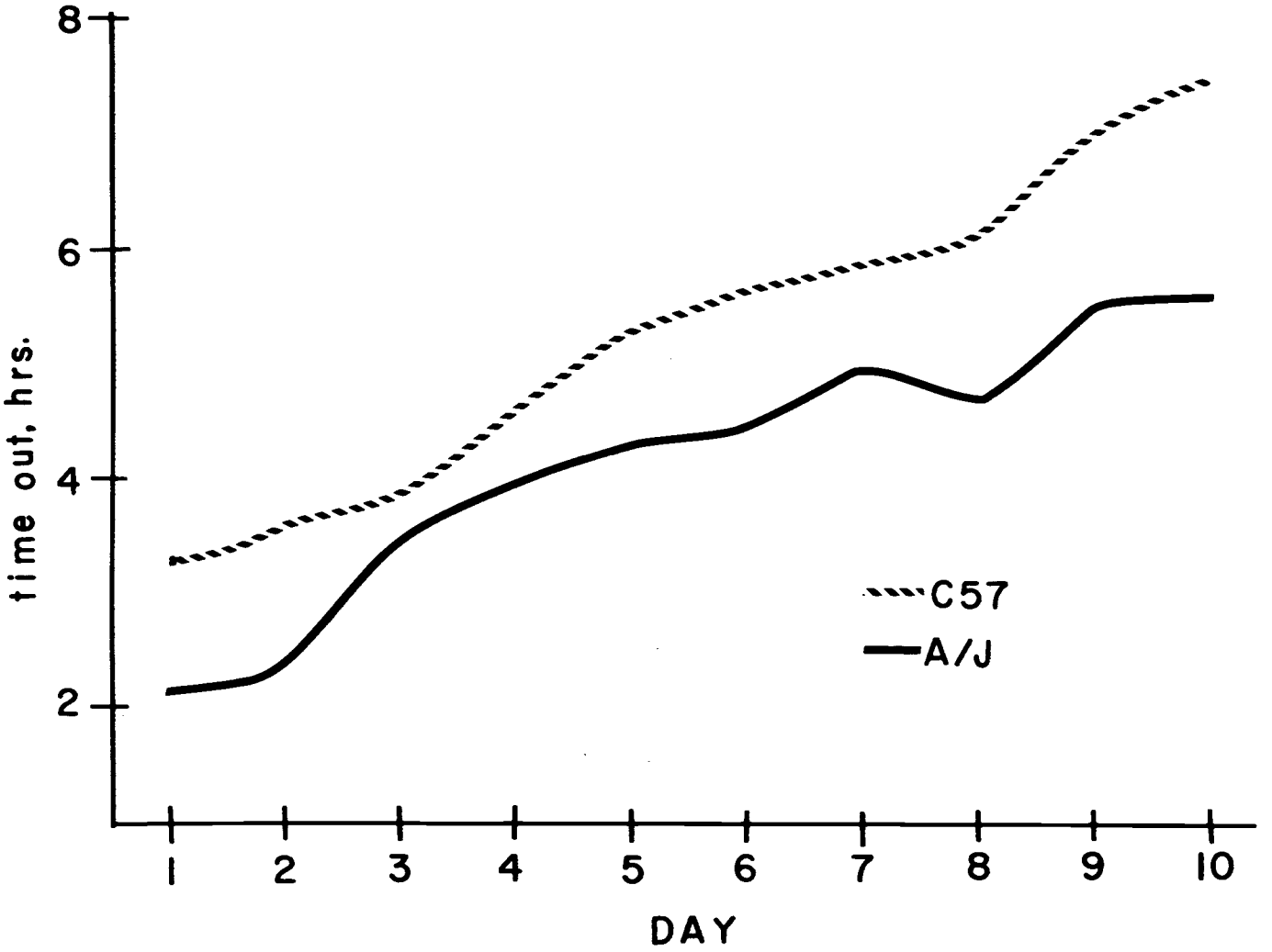



Figure 2. Time spent away from the nest per day for 10 days after parturition.

was highly significant. In both lines, the fostered individuals spent less time away from the litter than the unfostered mothers (Figure 3). In the C57 line the difference was not significant (Table 5). However, in the A/J line, mothers who had been fostered in infancy to C57 mothers spent an average of 1 hour less per day away from their litters (Table 4).

Generation 1 mice averaged about 0.4 hours per day more away from their litters than did generation 2 mice. This difference was not significant in the analysis considering both lines (Table 2) but was significant at the 10% level in the A/J line (Table 4) and at the 5% level in the C57 line (Table 5).

Because of the experimental design, mice of each inbred line belonged to one of three experimental groups: Generation 1, unfostered; Generation 2, unfostered; and Generation 2, fostered. Constant estimate comparisons of these six groups are given in Figure 4. In the A/J line, generation 1 unfostered mothers averaged the most time away from their litters and the generation 2 fostered group averaged the least. In the C57 line, the first generation unfostered group averaged the most time away from the litter but the generation 2 groups were similar.

Effects attributable to individual behavior differences were highly significant in both lines. However, the mean square from the individual effects was much larger in the A/J line (Table 4) than in the C57

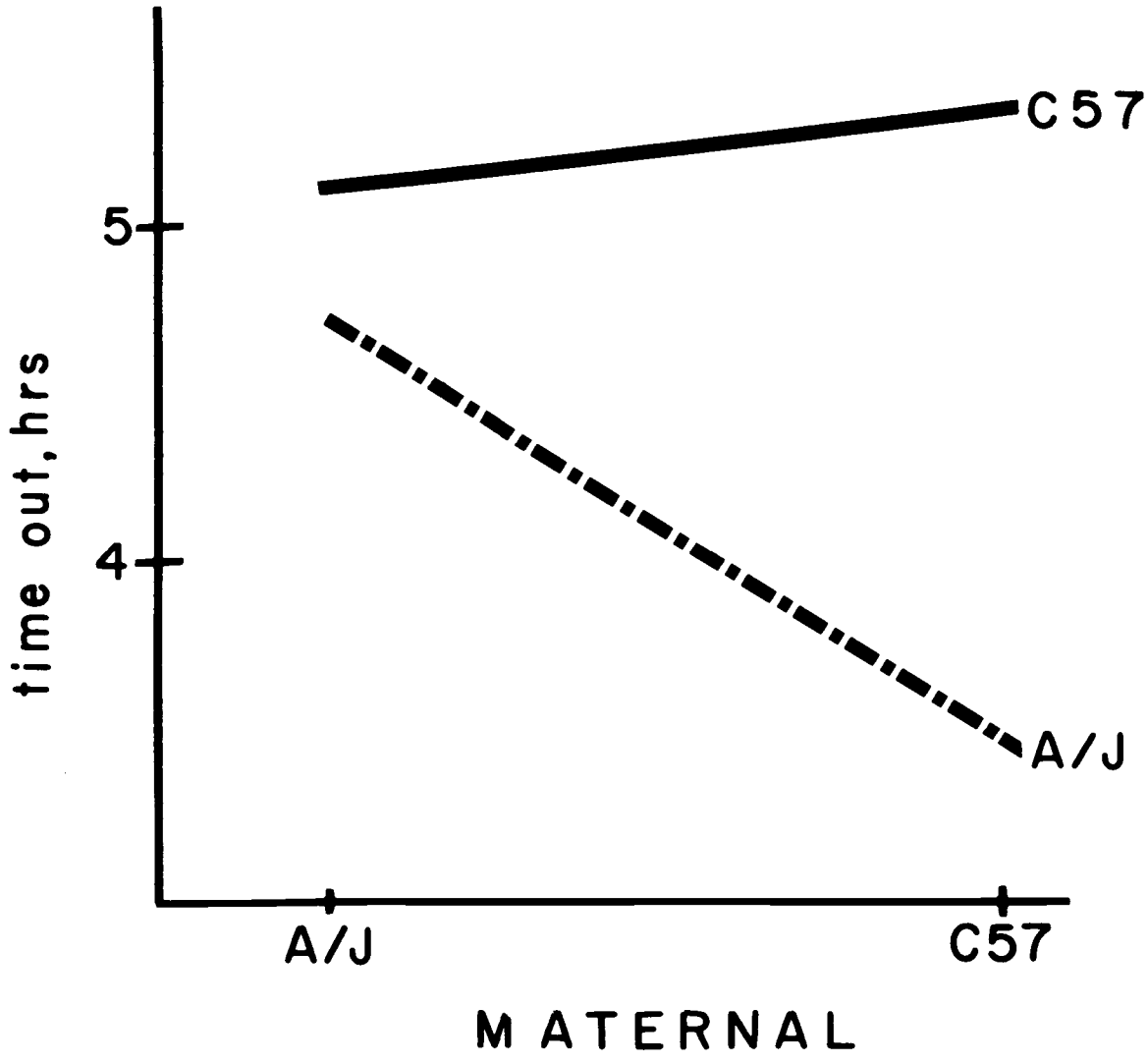



Figure 3. Effect of line and postnatal maternal line on average time away from the nest.

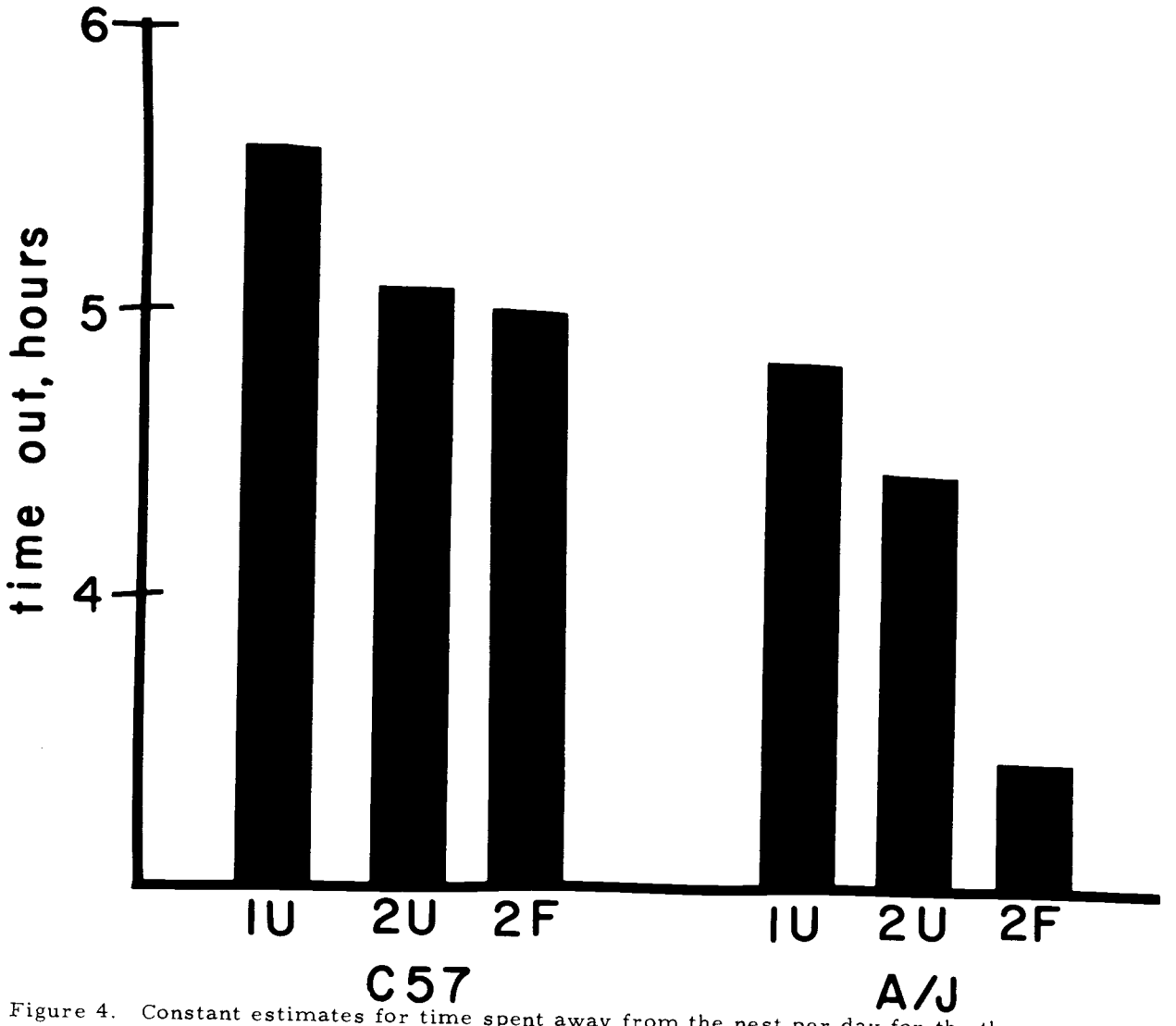



Figure 4. Constant estimates for time spent away from the nest per day for the three experimental groups within each inbred line.

line (Table 5). This indicated a greater extent of repeatable individual patterns existed in the A/J line than the C57 line.

Interestingly, both lines displayed the same amount of variation; mean square regression and the mean square error terms were the same in both analyses. Large differences are found between the lines in the amount of variation attributable to each factor.

Number of Trips away from the Litter Per Day

Each exit from the litter room was considered a trip; duration was not considered in this analysis. Table 6 presents the analysis of variance of the number of trips away from the nest per 24 hour period with data from both inbred lines included in the analysis. As in the previous section the data were also analyzed within each inbred line to examine individual differences. Table 7 presents the analysis of variance table for the A/J line and Table 8 is the comparable analysis for the C57 line.

In all three analyses, a significant change in the number of trips away from the next occurred over the ten days. There was no significant difference between the two lines, but the day x line interaction term was significant. This indicated a difference in the ten day trends between the lines and this is illustratrated in Figure 5. The trend in the C57 line was linear with a negative slope; there was a day

Table 6. Analysis of variance of number of trips away from the nest per day.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 389 | | | |
| Error | 348 | 2110 | | |
| Regression | 41 | 28596 | 13.55 | .005 |
| Day | 9 | 4571 | 2.17 | .025 |
| Line | 1 | 65 | 0.03 | - |
| Maternal Line | 1 | 217 | 0.10 | - |
| Generation | 1 | 181192 | 85.88 | .005 |
| Day x Line | 9 | 4641 | 2.20 | .025 |
| Day x Maternal | 9 | 731 | 0.35 | - |
| Day x Generation | 9 | 2089 | 0.99 | - |
| Line x Maternal | 1 | 28610 | 13.56 | .005 |
| Line x Generation | 1 | 81074 | 38.43 | .005 |

$R^2 = 61.5\%$

Model:

$$Y = 103.5 + 3.8(D1) + 2.1(D2) + 14.0(D3) + 17.3(D4) + 5.9(D5) - 7.9(D6) + 5.3(D7) - 3.5(D8) - 15.5(D9) - 21.5(D10) + 0.5(C) - 0.5(A) - 0.9(Mc) + 0.9(Ma) + 28.2(G1) - 28.2(G2) + 19.2(D1xC) + 21.0(D2xC) + 5.9(D3xC) - 8.4(D4xC) - 4.7(D5xC) - 9.9(D6xC) - 10.6(D7xC) - 7.2(D8xC) - 8.5(D9xC) - 5.8(D10xC) - 10.6(AxMa) + 10.6(CxMa) + 10.6(AxMc) - 18.9(g1xC) + 18.9(G2xC) + 18.9(G1xA) - 18.9(G2xA)$$

Table 7. Analysis of variance of number of trips away from the nest per day, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 149 | | | |
| Error | 123 | 805 | | |
| Regression | 26 | 21805 | 27.10 | .005 |
| Days | 9 | 1664 | 2.07 | .05 |
| Generation | 1 | 201885 | 250.89 | .005 |
| Fostering | 1 | 4808 | 5.97 | .025 |
| Individuals | 15 | 20506 | 25.48 | .005 |

$R^2$ = 85.1%

Model: Y = 101.8 - 5.0(D1) - 8.3(D2) + 7.3(D3) + 13.7(D4) + 11.1(D5)
-7.8(D6) + 15.8(D7) - 6.5(D8) - 5.5(D9) - 14.9(D10)
+45.9(G1) - 45.9(G2) - 7.0(U) + 7.0(F)

Table 8. Analysis of variance of number of trips away from the nest per day, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 242 | | | |
| Error | 208 | 821 | | |
| Regression | 34 | 8083 | 9.85 | .005 |
| Days | 9 | 8330 | 10.15 | .005 |
| Generation | 1 | 11808 | 14.39 | .005 |
| Fostering | 1 | 22774 | 26.25 | .005 |
| Individuals | 23 | 7514 | 9.16 | .005 |

$R^2$ = 61.7%

Model: Y = 104.9 + 28.6(D1) + 22.7(D2) + 20.5(D3) + 6.5(D4) - 4.5(D5)
- 7.6(D6) - 8.0(D7) - 16.3(D8) - 21.6(D9) - 20.3(D10)
+ 9.2(G1) - 9.2(G2) - 11.2(U) + 11.2(F)

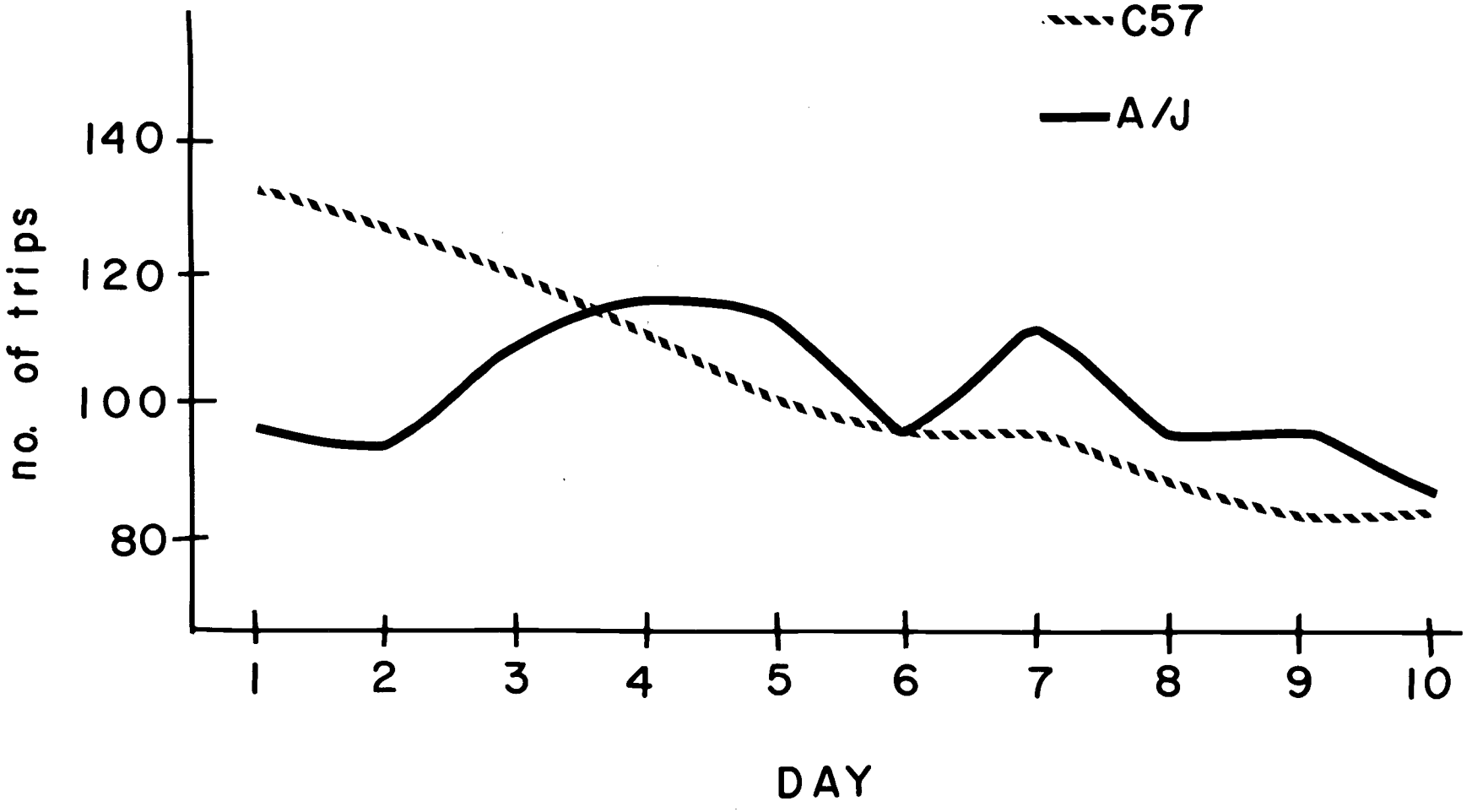



Figure 5. Number of trips away from the nest per day for the 10 days following parturition.

to day decrease in the number of trips away from the nest of about six trips per day. The day effect in the A/J line showed no consistent slope. Estimates for days one and two were approximately the same as those for days eight to ten, with the estimates for the intermediate days generally being larger.

The lack of regularity in the A/J line was also evident in comparisons between Table 7 and Table 8. The mean square error terms in the two analyses were the same but the mean square regression was much larger in the A/J line; thus there was much more total variation in the A/J line. However, the mean square for day effect was much larger for the C57 line than the A/J line, which indicated a greater consistency in mothers of the C57 line in the day to day trends.

No consistent maternal line effects were noted; but the line x maternal line interaction term was highly significant. In both lines, the fostered individuals averaged a larger number of trips per day than their unfostered counterparts (Figure 6). In the C57 line the fostered mothers averaged 22 trips more per day (Table 8), and in the A/J line the fostered females averaged 14 trips more per day (Table 7). These results indicated the differences might be due to the fostering procedure *per se* and did not reflect differences in postnatal maternal influences.

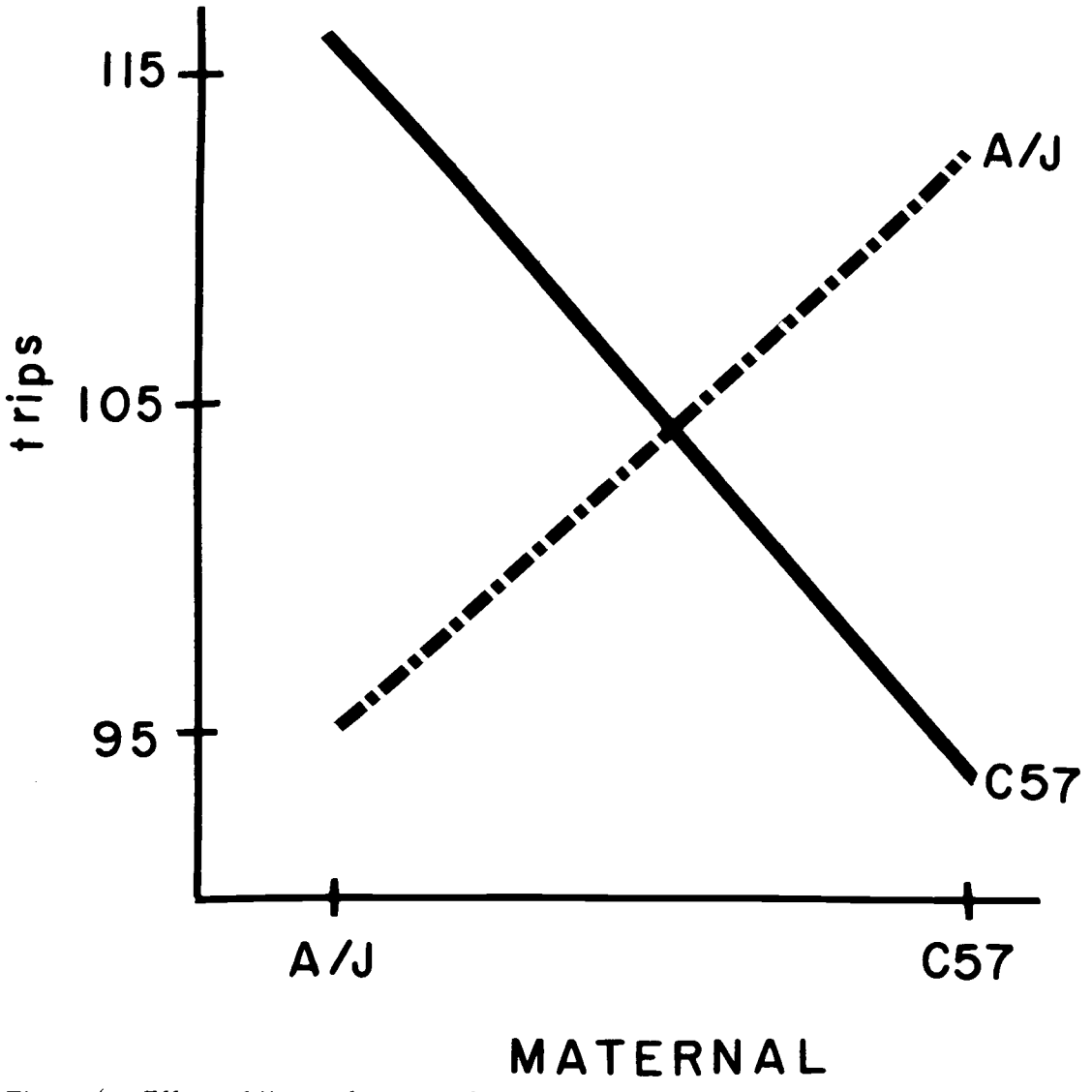



Figure 6. Effect of line and postnatal maternal line on average number of trips per day.

Differences attributable to generation were significant in all three analyses, with the second generation making fewer trips away from the nest per day. However, the magnitude of the difference varied between the two lines. In the A/J line, the second generation estimate was 92 trips per day less (Table 7), while in the C57 line the second generation estimate was 18 trips per day less (Table 8).

The statistical estimates for the six experimental groups are given in Figure 7. Differences between the experimental groups were more extreme in the A/J line. In both lines the generation 2 unfostered group made the fewest trips per day away from the nest. However, in the A/J line the greatest difference was between the generation 1 unfostered group and the two generation 2 groups, while in the C57 line the greatest difference was between the generation 2 unfostered group and the other two groups.

Differences between individuals in average number of trips per day were found in both lines. Again these differences were more extreme in the A/J line (Table 7) than in the C57 line (Table 8).

## Average Trip Length

Each observation for the hours spent away from the litter per day was divided by the number of trips per day; these values were converted to minutes to produce a measure of average trip length. Table 9 presents the analysis of variance table for the average trip

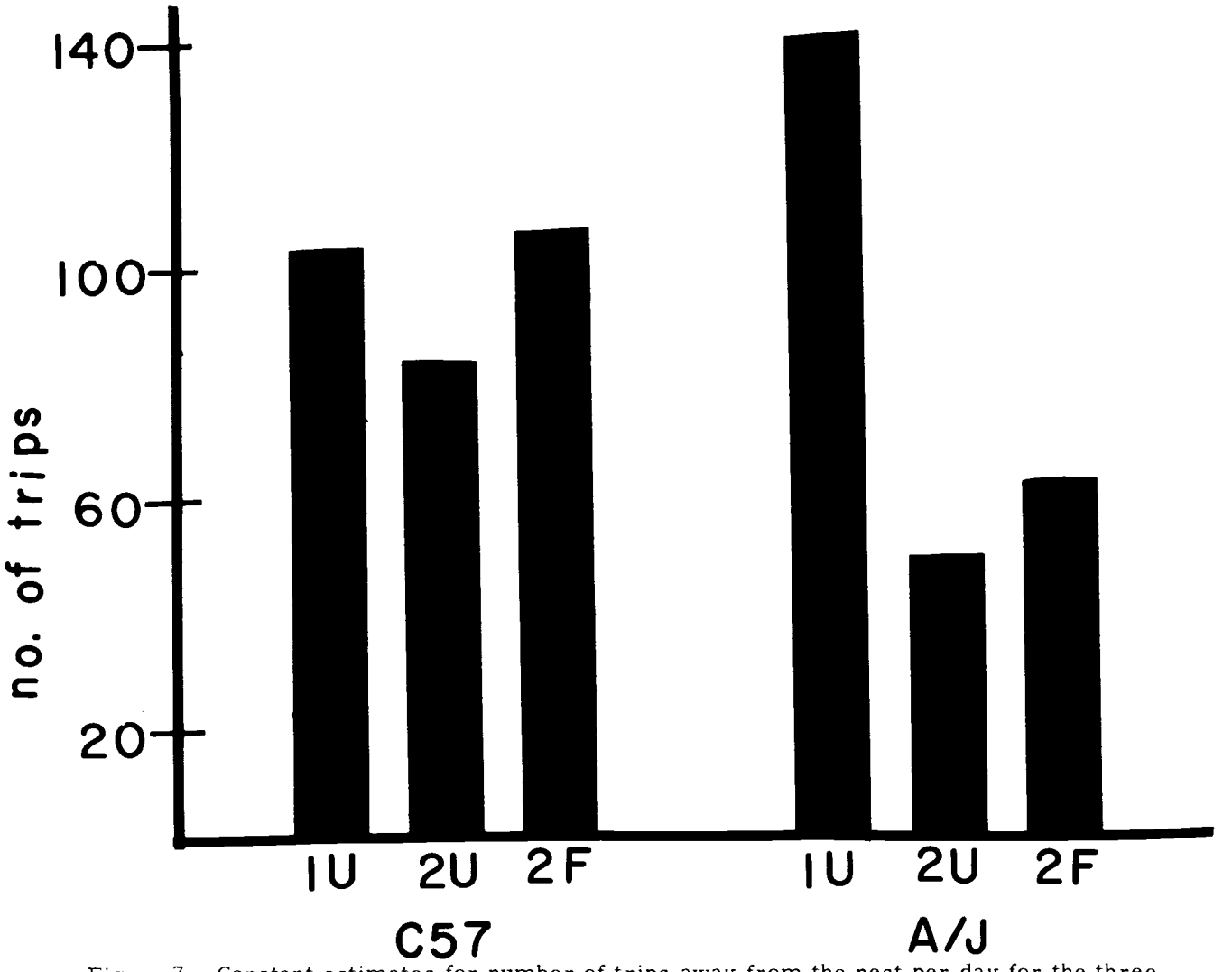



Figure 7. Constant estimates for number of trips away from the nest per day for the three experimental groups within each inbred line.

Table 9. Analysis of variance of average trip length.

| Sourcw | df | MS | F | P |
|---|---|---|---|---|
| Total | 389 | | | |
| Error | 348 | 7.97 | | |
| Regression | 41 | 221.07 | 27.73 | .005 |
| Day | 9 | 107.81 | 13.52 | .005 |
| Line | 1 | 1.73 | 0.22 | - |
| Maternal Line | 1 | 71.40 | 8.95 | .005 |
| Generation | 1 | 295.75 | 37.09 | .005 |
| Day x Line | 9 | 4.86 | 0.61 | - |
| Day x Maternal | 9 | 2.42 | 0.30 | - |
| Day x Generation | 9 | 3.31 | 0.41 | - |
| Line x Maternal | 1 | 232.70 | 29.18 | .005 |
| Line x Generation | 1 | 347.25 | 43.55 | .005 |

$R^2 = 76.6\%$

Model:

$$Y = 3.72 - 2.46(D1) - 1.98(D2) - 1.56(D3) - 1.06(D4) - 0.26(D5) + 0.05(D6) + 0.61(D7) + 0.90(D8) + 2.24(D9) + 3.50(D10) + 0.09(C) - 0.09(A) - 0.53(Mc) + 0.53(Ma) - 1.14(G1) + 1.14(G2) + 0.95(CxMc) + 0.95(AxMa) - 0.95(CxMa) - 0.95(AxMc) + 1.23(GlxC) = 1.23(G2xC) - 1.23(G1xA) + 1.23(G2xA)$$

length per day with data from both inbred lines included in the analysis. Table 10 presents the analysis of variance for the A/J line and Table 11 presents the analysis for the C57 line.

There was no difference between the lines in average trip length. Both lines showed an increase in trip length with day (Figure 8). On day 1 the average trip length was 1.26 minutes and on day 10 the average trip length was 7.22 minutes; the average increase was 0.61 minutes per day. The rate of increase was slightly greater in the A/J line but this was not significant as evidenced by the lack of a day x line interaction. In the C57 line, the rate of increase was relatively constant throughout the 10 days with the average increase being 0.56 minutes per day. The increasing trip length in this line is the result of both increasing time spent away from the litter and the decreasing number of trips with day. In the A/J line the increase in trip length was more rapid in days 9 and 10. The overall average daily increase in trip length was 0.71 minutes, while the increase from day 8 to day 9 was 1.5 minutes per trip and the increase from day 9 to day 10 was 1.6 minutes per trip. These results were a reflection of the linear trend in time out per day and the curvilinear effect in average number of trips.

There was more variation in trip length in the A/J line (Table 10) than in the C57 line (Table 11). Both the mean square regression

Table 10. Analysis of variance of average trip length, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 149 | | | |
| Error | 123 | 6.27 | | |
| Regression | 26 | 99.78 | 15.93 | .005 |
| Days | 9 | 77.64 | 12.39 | .005 |
| Generation | 1 | 409.30 | 65.32 | .005 |
| Fostering | 1 | 115.00 | 14.34 | .005 |
| Individuals | 15 | 92.67 | 14.79 | .005 |
| | | $R^2$ = 77.1% | | |

Model: Y = 3.73 - 3.03(D1) - 2.61(D2) - 1.50(D3) - 0.64(D4)
-0.46(D5) - 0.03(D6) + 0.54(D7) + 1.08(D8) + 2.53(D9)
+4.12(D10) - 2.07(G1) + 2.07(G2) + 1.07(U) - 1.07(F)

Table 11. Analysis of variance of average trip length, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 242 | | | |
| Error | 208 | 1.76 | | |
| Regression | 34 | 30.97 | 17.58 | .005 |
| Days | 9 | 69.5 | 39.45 | .005 |
| Generation | 1 | 0.7 | 0.36 | - |
| Fostering | 1 | 22.3 | 12.66 | .005 |
| Individuals | 23 | 16.1 | 9.12 | .005 |
| | | $R^2$ = 74.2% | | |

Model: Y = 3.82 - 2.21(D1) - 1.90(D2) - 1.80(D3) - 1.03(D4)
+ 0.08(D5) + 0.24(D6) + 0.56(D7) + 1.23(D8) + 2.15(D9)
+2.65(D10) + 0.07(G1) - 0.07(G2) + 0.37(U) - 0.37(F)

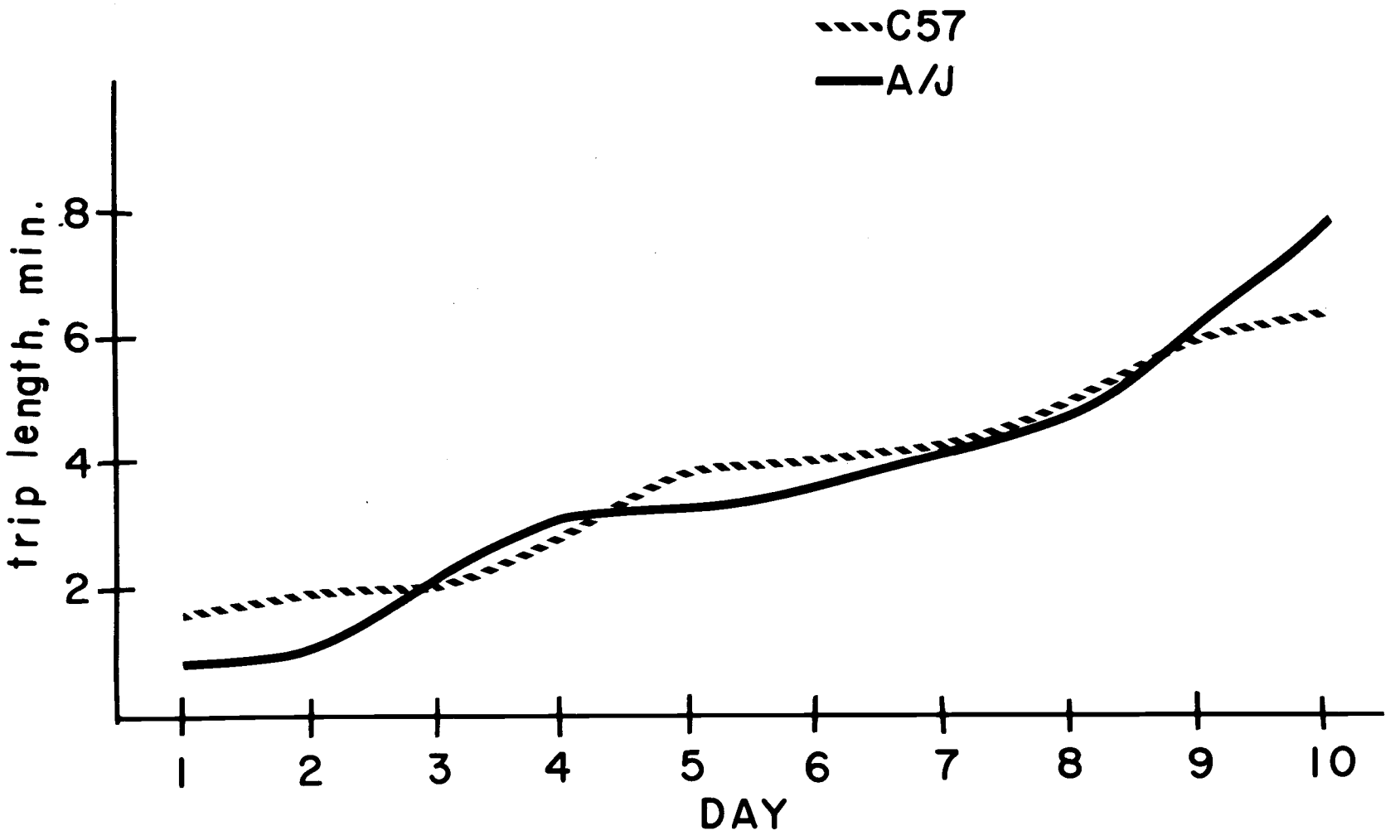



Figure 8. Average trip lengths for the 10 days following parturition.

and the mean square error terms were over three times larger in the A/J line.

Both the maternal effect and line x maternal line interaction terms were highly significant. This interaction is plotted in Figure 9. Both individual line analyses showed a significant maternal effect. In both cases, the fostered females had a shorter average trip length than their unfostered counterparts. In the preceding analyses, fostered individuals had lower measures for time away from the nest and a higher number of trips, thus a shorter trip length was a result of both of these factors. Again the differences are greater in the A/J line than the C57 line. In the C57 line the fostered group had an average trip length 0.7 minutes shorter than the unfostered group (Table 11); in the A/J line the average trip length was 2.15 minutes shorter (Table 10).

Significant differences between generation 1 and generation 2 mice were found in the A/J line (Table 10) but not the C57 line (Table 11). In the A/J line, the generation 1 females had an average trip length 4.1 minutes shorter than did the generation 2 females. This is a reflection of the much larger number of trips per day, since the generation 1 females also averaged more time away from the litter per day.

Statistical estimates for the six experimental groups are given in Figure 10. Differences between the experimental groups were more

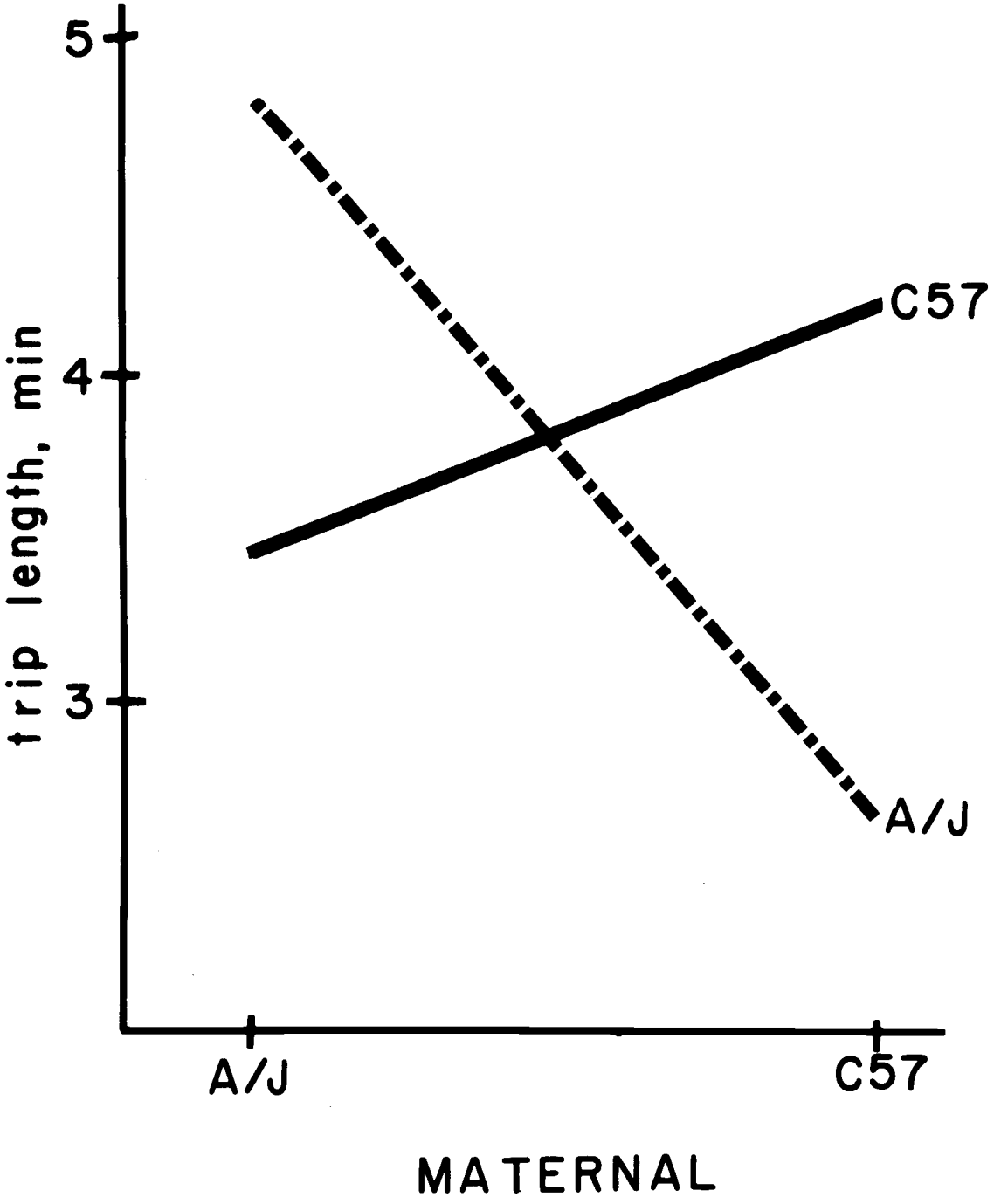



Figure 9. Effect of line and postnatal maternal line on average trip length.

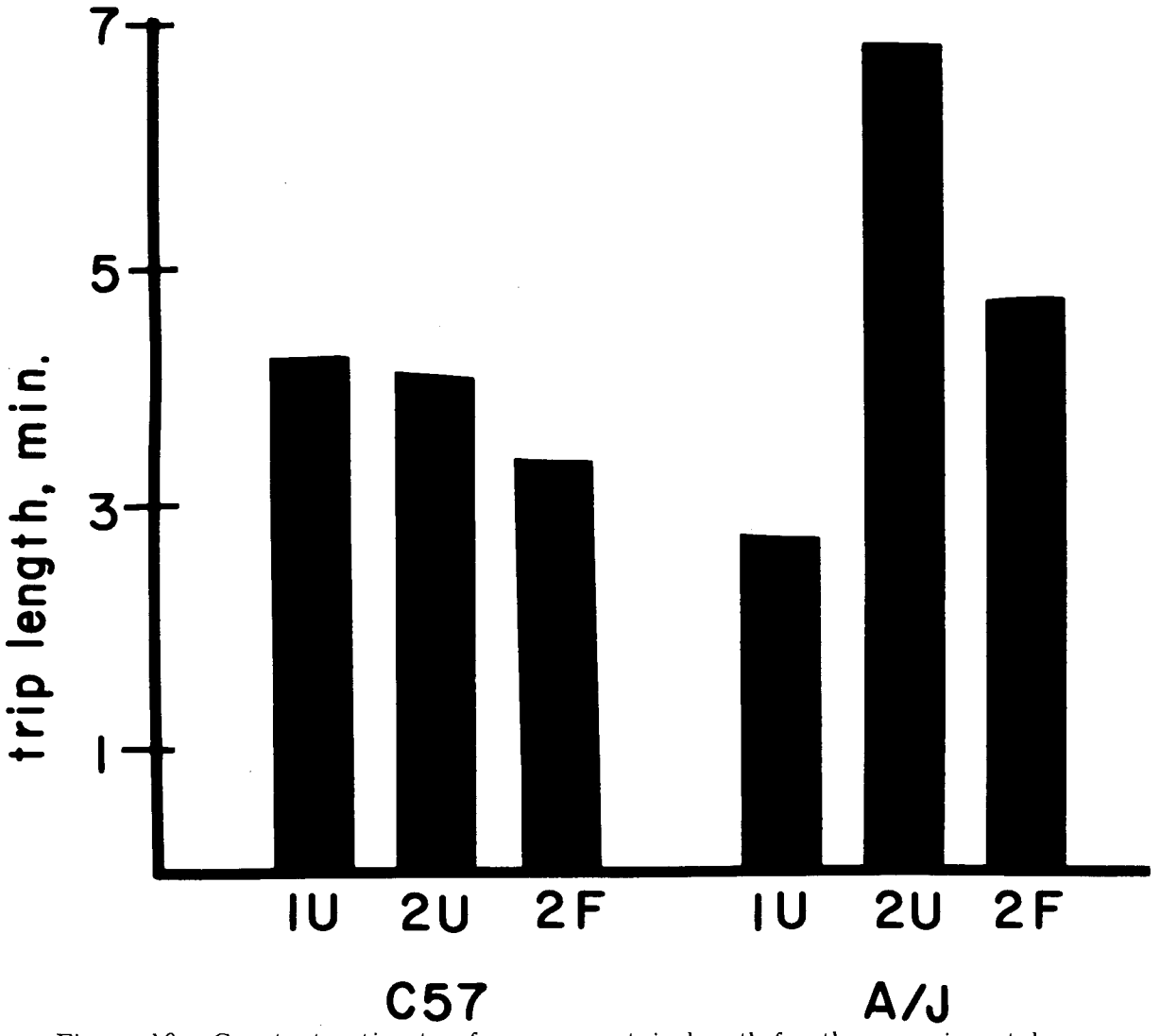



Figure 10. Constant estimates for average trip length for the experimental groups within each inbred line.

extreme in the A/J line. The patterns differed in the two lines. In the C57 line the fostered group was clearly different from the unfostered groups. Thus in the two unfostered groups, the greater number of trips in the generation 1 group was a reflection of the greater amount of time away from the litter per day; the average trip length was the same for the two unfostered groups. In the A/J line all three groups were distinct, and the extreme values were found in the two unfostered groups.

In both lines, significant differences between individuals in trip length were found. Again, individual effects were more pronounced in the A/J line.

## Effect of Litter Size and Pup Weight on Maternal Behavior

Because litter sizes were not standardized, it was possible that some of the results noted in the previous sections were due to stimuli from litters of various sizes. The difference in reproduction between the two lines was marked. At 10 days the average litter size of the C57 line was 6.12 and the average weight was 5.63 grams per pup; standard deviations were 1.56 and 0.84, respectively. In contrast, the average litter size of the A/J line at 10 days was 4.17 and the average pup weight was 4.63 grams, with standard deviations of 2.26 and 1.03, respectively.

These differences do not necessarily reflect a great disparity in average litter size at birth. Indeed, published records for litter sizes at birth for these two lines are quite similar; average litter size for the C57 line was 5.9 and for the A/J line it was 5.7 (Green, 1968). The litter size and weight at birth were not recorded to avoid disturbing the mother and possibly influencing her maternal behavior. However, the incidence of dead and partially cannibalized pups in the first day after parturition was high in the A/J line and practically nonexistent in the C57 line. Many of the A/J litters did not survive long enough to start the test. Testing procedures were initiated on 34 A/J mothers. Of these only 18 still had at least one pup alive at 10 days. In contrast, of the 30 C57 mothers started on test, 26 raised litters to 10 days of age.

From this study it cannot be determined whether the low litter size at 10 days of the A/J mothers is due to poor fecundity, poor maternal care or poor survivability of the pups or some combination. As an indication, however, the five pairs of crossfostered litters may be compared. Of these, two pairs represented second parturitions and all pups of these four litters (20 A/J, 17 C57) survived to 10 days. The remaining three crossfostered pairs were first parturitions. A total of 21 A/J pups were born in these three litters; five were dead before crossfostering, five died after fostering to a C57 mother and 11 survived to 10 days. Seventeen C57 pups were in the counterpart

litters; all were alive at the time of fostering and 16 survived to 10 days under A/J maternal care. Thus both maternal care and survivability are involved: all of the A/J litters raised by C57 mothers had at least two pups alive at 10 days but the mortality of A/J pups was higher than C57 pups.

Although line differences in litter size and weight cannot be linked to behavior differences, it is feasible to compare individuals within a line to see if stimuli from various litter sizes affected maternal behavior.

First, the hypothesis that there was no difference in litter size or litter mass among the three experimental groups of each line was tested. In the A/J line the experimental groups had different average litter sizes, but the variation within groups was great and these differences were not significant (Table 12). No differences in average pup weight at 10 days were found among the three experimental groups in this line although the average for the generation 2 unfostered group was somewhat larger (Table 13). The average pup weight at 10 days did not depend on litter size; the correlation between the variables was +0.14, which was nonsignificant.

In the C57 line differences among the experimental groups in litter size were significant at the 10% level (Table 14). Differences among the C57 groups in 10 day pup weight were highly significant (Table 15). In this line, a small, nonsignificant positive correlation (+0.10) was found between litter size and pup weight.

Table 12. Analysis of variance of 10 day litter size, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 17 | | | |
| Error | 15 | 5.56 | | |
| Between Groups | 2 | 1.55 | 0.28 | - |

Model:

$$Y = 4.14 - 0.54(IU) + 0.01(2U) + 0.53(2F)$$

Table 13. Analysis of variance of average pup weight at 10 days, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 17 | | | |
| Error | 14 | 1.18 | | |
| Litter size | 1 | 0.33 | 0.28 | - |
| Between Groups | 2 | 0.64 | 0.55 | - |

Model:

$$Y = 4.36 + 0.06(\text{litter size}) - 0.26(IU) + 0.36(2U) - 0.10(2F)$$

Table 14. Analysis of variance of 10 day litter size, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 25 | | | |
| Error | 23 | 2.14 | | |
| Within Groups | 2 | 5.76 | 2.70 | .10 |

$R^2$ = 19%

Model:

$Y = 6.02 - 0.02(IU) - 0.77(2U) + 0.80(2F)$

Table 15. Analysis of variance of average pup weight at 10 days, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 25 | | | |
| Error | 22 | 0.417 | | |
| Litter Size | 1 | 2.803 | 4.99 | .05 |
| Between Groups | 2 | 4.115 | 9.85 | .005 |

$R^2$ = 47.8%

Model:

$Y = 4.41 + 0.21(\text{litter size}) - 0.43(1U) + 0.88(2U) - 0.45(2F)$

Among the experimental groups, however, the 10 day pup weight appears to have an inverse relationship to litter size. The constant estimates (Tables 14 and 15) show the second generation, unfostered group to have the smallest litter size and the largest 10 day pup weight.

Litter size and litter mass at 10 days of age were recorded on the litters of each of the females tested. These two measures were then included in the model as continuous variables to determine whether or not they would alter the findings of the previous sections. The initial model for each line included litter size and total litter weight at 10 days, generation effect, fostering effect and day effect. The residuals from this analysis were then examined by means of a one-way analysis of variance to determine if a significant individual effect remained. These two analyses were then combined into one analysis of variance table.

Tables 16, 17 and 18 present the results for time away from the nest per day, number of trips per day and average trip length, respectively, for the A/J line. Comparable analyses which do not include litter variables are found in Tables 4, 7 and 10.

Litter variables were found to significantly influence the total time away from the nest per day and the average trip length. For each increase in litter size of one pup, the female spent 0.28 hours per day more away from the nest and the average trip length was increased by 0.15 minutes. For each gram increase in litter weight at 10 days, the

Table 16. Analysis of variance of time away from the nest with litter characteristics included in the model, A/J line.

| Sourcw | df | MS | F | P |
|---|---|---|---|---|
| Total | 147 | | | |
| Error | 119 | 0.989 | | |
| Regression | 28 | 20.144 | 20.37 | .005 |
| Litter Parameters | 2 | 69.88 | 70.68 | .005 |
| Generation | 1 | 13.01 | 13.15 | .005 |
| Fostering | 1 | 23.06 | 23.32 | .005 |
| Day | 9 | 19.47 | 19.69 | .005 |
| Individuals | 15 | 12.66 | 12.80 | .005 |

$R^2 = 82.7\%$

Model:

$$Y = 2.49 - 1.89(D1) - 1.61(D2) - 0.61(D3) - 0.37(D4) + 0.11(D5) + 0.30(D6) + 0.71(D7) + 0.48(D8) + 1.33(D9) + 1.55(D10) + 0.28(\text{litter size}) + 0.034(\text{litter mass}) + 0.40(G1) - 0.40(G2) + 0.50(U) - 0.50(F)$$

Table 17. Analysis of variance of number of trips per day with litter characteristics included in the model, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 147 | | | |
| Error | 119 | 861 | | |
| Regression | 28 | 19991 | 23.22 | .005 |
| Litter parameters | 2 | 1400 | 1.63 | .25 |
| Generation | 1 | 78785 | 91.50 | .005 |
| Fostering | 1 | 9407 | 10.93 | .005 |
| Day | 9 | 1848 | 2.15 | .025 |
| Individual | 15 | 20239 | 23.50 | .005 |

$R^2 = 84.5\%$

Model:

$$Y = 111.5 - 11.9(D1) - 11.6(D2) + 7.9(D3) + 17.1(D4) + 10.6(D5)$$
$$- 7.9(D6) + 13.1(D7) + 0.2(D8) - 0.43(D9) - 13.2(D10)$$
$$-2.7(\text{litter size}) + 0.1(\text{litter weight}) + 45.9(G1) - 45.9(G2)$$
$$- 10.2(U) + 10.2(F)$$

Table 18. Analysis of variance of average trip length per day with litter characteristics included in the model, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 147 | | | |
| Error | 119 | 6.03 | | |
| Regression | 28 | 94.18 | 15.61 | .005 |
| Litter parameters | 2 | 462.38 | 76.64 | .005 |
| Generation | 1 | 173.22 | 28.71 | .005 |
| Fostering | 1 | 95.39 | 15.81 | .005 |
| Day | 9 | 77.42 | 12.83 | .005 |
| Individual | 15 | 32.28 | 5.35 | .005 |

$R^2 = 87.6\%$

Model:

$$Y = -0.18 - 2.80(D1) - 2.62(D2) - 1.46(D3) - 1.31(D4) - 0.58(D5) - 0.01(D6) + 0.50(D7) + 0.74(D8) + 2.62(D9) + 4.54(D10) + 0.15(\text{litter size}) + 0.189(\text{litter weight}) - 1.45(G1) + 1.45(G2) + 1.03(U) - 1.03(F)$$

time away from the litter was increased by 0.034 hours and the average trip length was increased by 0.129 minutes. The litter variables had little influence on the number of trips made per day.

For all three variables, the $R^2$ values, the size of the mean square error and mean square regression were the same as in the previous analyses. Thus, the significant litter effects did not account for any previously unexplained variation but only reallocated portions of the regression variation.

The mean square terms for individual effects for time out per day and average trip length have been reduced by half and two-thirds, respectively, when litter parameters are included in the model. This suggests that a large part of the individual variation reflected differences associated with the size and mass of the offspring. It is noteworthy that significant individual differences still remained; mothers did not differ in behavior solely because of the characteristics of the litter they were raising.

The effect of generation on total time out per day was much greater if litter variables were included in the model. Previously generation 1 individuals were away from their litters 0.4 hours longer per day; when a correction for litter differences was included, the estimated effect for generation 1 was 1.1 hours per day greater than for generation 2.

The effect of generation on average trip length was reduced when litter variables were included in the model. The mean square for generation was less than half the value previously obtained, although the effect was still highly significant. The estimate for generation 1 was about three minutes per trip less instead of four minutes per trip as was previously found. This suggested that a difference in litter characteristics between generation 1 and generation 2 mice did exist and was not detectable statistically because of small sample sizes and large variation within each group.

The fostering effect in both time out per day and average trip length was unchanged when litter parameters were included.

Tables 19, 20 and 21 present the results for time away from the nest per day, number of trips per day and average trip length, respectively, for the C57 line with litter parameters included in the model. Comparable analyses which did not include litter variables are found in Tables 5, 8, and 11.

Litter characteristics were found to be a significant influence on all three measures of behavior in the C57 line. Unlike the results in the A/J line, however, increasing litter size appeared to have an opposite effect to increasing litter weight. An increase in the size of the litter by one pup decreased the estimate for time out per day by 0.14 hours, increased the estimate for number of trips per day by seven, and decreased the estimate for trip length by 0.55 minutes.

Table 19. Analysis of variance of time away from the nest per day with litter characteristics included in the model, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 240 | | | |
| Error | 204 | 0.860 | | |
| Regression | 36 | 19.781 | 23.01 | .005 |
| Litter parameters | 2 | 59.47 | 69.19 | .005 |
| Generation | 1 | 7.58 | 8.81 | .005 |
| Fostering | 1 | 1.62 | 1.88 | .25 |
| Day | 9 | 47.18 | 54.88 | .005 |
| Individuals | 23 | 6.82 | 7.93 | .005 |

$R^2 = 80.2\%$

Model:

$$Y = 2.94 - 2.05(D1) - 1.56(D2) - 1.34(D3) - 0.66(D4) - 0.22(D5)$$
$$+0.45(D6) + 0.63(D7) + 0.89(D8) + 1.74(D9) + 2.11(D10)$$
$$- 0.14(\text{litter size}) + 0.093(\text{litter weight}) + 0.30(G1) - 0.30(G2)$$
$$+ 0.14(U) - 0.14(F)$$

Table 20. Analysis of variance of number of trips per day with litter characteristics included in the model, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 240 | | | |
| Error | 204 | 820.8 | | |
| Regression | 36 | 7763.0 | 9.46 | .005 |
| Litter parameters | 2 | 2645 | 3.22 | .05 |
| Generation | 1 | 3254 | 3.96 | .05 |
| Fostering | 1 | 5142 | 6.26 | .025 |
| Day | 9 | 9017 | 10.99 | .005 |
| Individuals | 23 | 7255 | 8.84 | .005 |

$R^2 = 62.5\%$

Model:

$$Y = 105.2 + 29.5(D1) + 26.1(D2) + 20.2(D3) + 3.6(D4) - 3.5(D5)$$
$$- 6.5(D6) - 9.0(D7) - 16.1(D8) - 21.1(D9) - 23.2(D10)$$
$$+ 6.9(\text{litter size}) - 1.3(\text{litter weight}) + 6.2(G1) - 6.2(G2)$$
$$- 8.0(U) + 8.0(F)$$

Table 21. Analysis of variance of average trip length with litter characteristics included in the model, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 240 | | | |
| Error | 204 | 1.662 | | |
| Regression | 36 | 28.665 | 17.67 | .005 |
| Litter Parameters | 2 | 59.99 | 36.99 | .005 |
| Generation | 1 | 11.15 | 6.78 | .01 |
| Fostering | 1 | 3.24 | 2.00 | .25 |
| Day | 9 | 70.03 | 43.18 | .005 |
| Individual | 23 | 10.07 | 6.21 | .005 |

$R^2 = 75.7\%$

Model:

$$Y = 2.36 - 2.25(D1) - 1.89(D2) - 1.77(D3) - 0.91(D4) - 0.15(D5)$$
$$+ 0.23(D6) + 0.61(D7) + 1.29(D8) + 2.19(D9) + 2.65(D10)$$
$$- 0.55(\text{litter size}) + 0.143(\text{litter weight}) + 0.36(G1) - 0.36(G2)$$
$$+ 0.20(U) - 0.20(F)$$

Since litter size was included in the analysis, an increase in litter mass could be interpreted as an increase in pup weight with litter size held constant. An increase of one gram in litter mass at 10 days increased the estimate for time out by 0.093 hours (Table 19), decreased the estimate for the number of trips out by 1.3 (Table 20), and increased the estimate for average trip length by 0.143 minutes (Table 21).

As in the A/J line analysis, the $R^2$ values and the mean square regression and mean square error terms were comparable to the preceding analyses. Because of the significant differences in litter characteristics among the experimental groups in the C57 line, it could have been expected that the reallocation of variation would affect estimates for the experimental groups.

While some reduction in variation attributable to individual effects was noted, the reduction was not as dramatic as in the A/J line. The largest decrease occurred in average trip length; the mean square term was about a third smaller. In all three analyses, significant individual effects remained after inclusion of litter parameters in the model. These results suggested that individual differences among C57 mothers did not necessarily reflect differences in their litter size or weight.

In the previous analyses, fostering was found to be a significant factor influencing the number of trips per day and average trip length

but not time away from the litter. When corrected for litter variables, the difference between fostered and nonfostered groups was significant only for number of trips per day and the size of the difference was reduced. When litter variables were included in the analysis, it was estimated that unfostered individuals made 16 fewer trips per day than did fostered individuals (Table 20) vs 22 fewer trips estimated from the previous analysis (Table 8). The previous estimate of 0.73 minutes per trip longer for unfostered individuals (Table 11) was reduced to an estimated difference of 0.40 minutes (Table 21) which was no longer significant.

The estimated differences between the two generations were increased for time away from the litter and average trip length, but the estimated difference in number of trips was reduced. This model estimated that generation 1 individuals spent 0.6 hours longer per day away from the litter (Table 19), made 12 more trips per day (Table 20), and averaged 0.72 minutes longer per trip (Table 21). All of these differences were found to be statistically significant. Estimates of differences from the previous analysis were found to be 0.4 hours per day (Table 5), 18 trips (Table 8) and 0.13 minutes per trip (Table 11), respectively. Only the first two were found to be statistically significant.

To summarize, differences in the size and weight of the litter were reflected in the behavior of the female. The trends were

different for the two lines. In the A/J line, an increase in litter size and weight was associated with more time spent away from the litter and longer trip length. In the C57 line an increase in litter size was associated with a decrease in the time out per day and trip length, and an increase in number of trips. Increasing litter mass influenced the behavior measures in the opposite direction.

In the A/J line large differences between experimental groups remained; correction for litter variables mainly reduced the size of the individual differences between mothers. In the C57 line there were fewer differences between groups and individuals in the previous analyses, and the litter parameters differed among experimental groups. The magnitude but not the direction of the behavior differences between experimental groups was altered. In general, fostering effects became less important and generation effects became more important.

## Day versus Night Behavior Patterns

The most striking difference in behavior between the two lines was in the temporal occurrence of the three behaviors studied. Activity of A/J mothers was limited to hours of darkness while C57 mothers left their litters during both light and dark intervals.

To measure these differences, the records for each day were divided into lights-on period, i.e., day, and lights-off period, i.e.,

night. Since each 24 hour measure started with noon, the night measure was considered to be that portion of the measured behavior occurring between 2000 hours and 0600 hours. The day measure was considered to be that portion falling in the intervals 1200 hours to 2000 hours and 0600 hours to 1200 hours. The measure of behavior was used as the dependent variable in a one-way analysis of variance using as the division criterion Day 1, Night 1, Day 2, Night 2, etc. The residuals from this analysis were then used to evaluate the differences between the three groups tested and the individual effects. The individual effect in this analysis was considered to be the individual's repeatable deviation from the mean daytime value, and separately, the mean nighttime value of the group to which it belonged.

Table 22 presents the analysis of variance tables for time away from the litter for the A/J and C57 lines. Significant differences among time intervals were found in both lines.

Figures 11 and 12 illustrate the subclass means of time away from the nest based on time intervals for the A/J and C57 lines respectively. Figure 11 shows that almost all of the time away from the nest in A/J mothers occurred in darkness. The A/J mothers averaged only 0.5 hours away from their litters during light intervals while the average for dark intervals was about 3.8 hours. The day effect increase noted in the previous sections represented an increase in nocturnal activity in the A/J line.

Table 22. Analysis of variance of time away from the litter based on light and dark intervals.

| Source | df | MS | F | P |
|---|---|---|---|---|
| | | A/J line | | |
| Total | 311 | | | |
| Time Interval | 19 | 50.39 | 38.72 | .005 |
| Error | 292 | 1.30 | | |
| | | C57 line | | |
| Total | 480 | | | |
| Time Interval | 19 | 11.47 | 11.47 | .005 |
| Error | 461 | 1.00 | | |

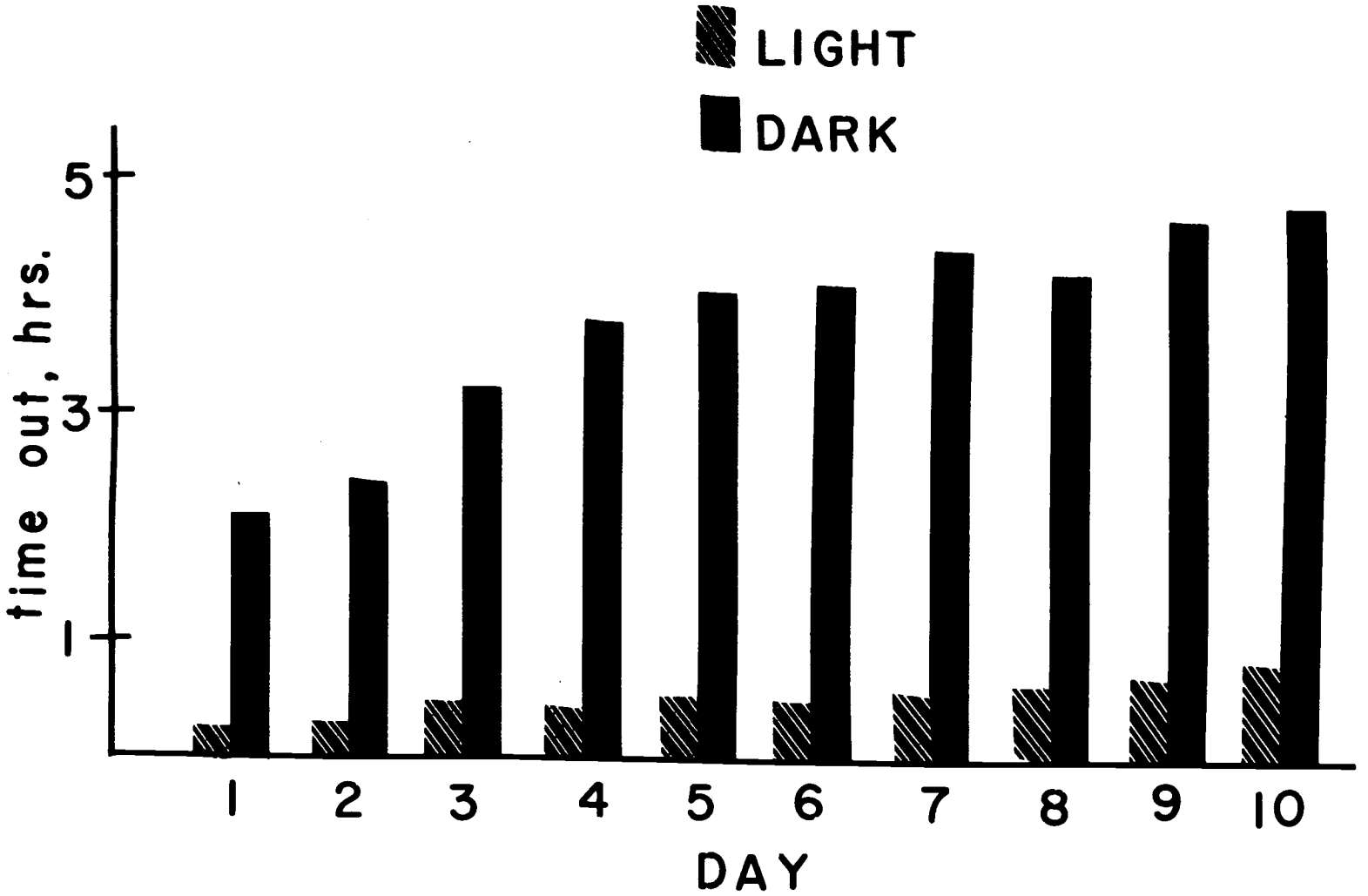



Figure 11. Time away from the nest during light and dark intervals for the 10 days following parturition in the A/J line.

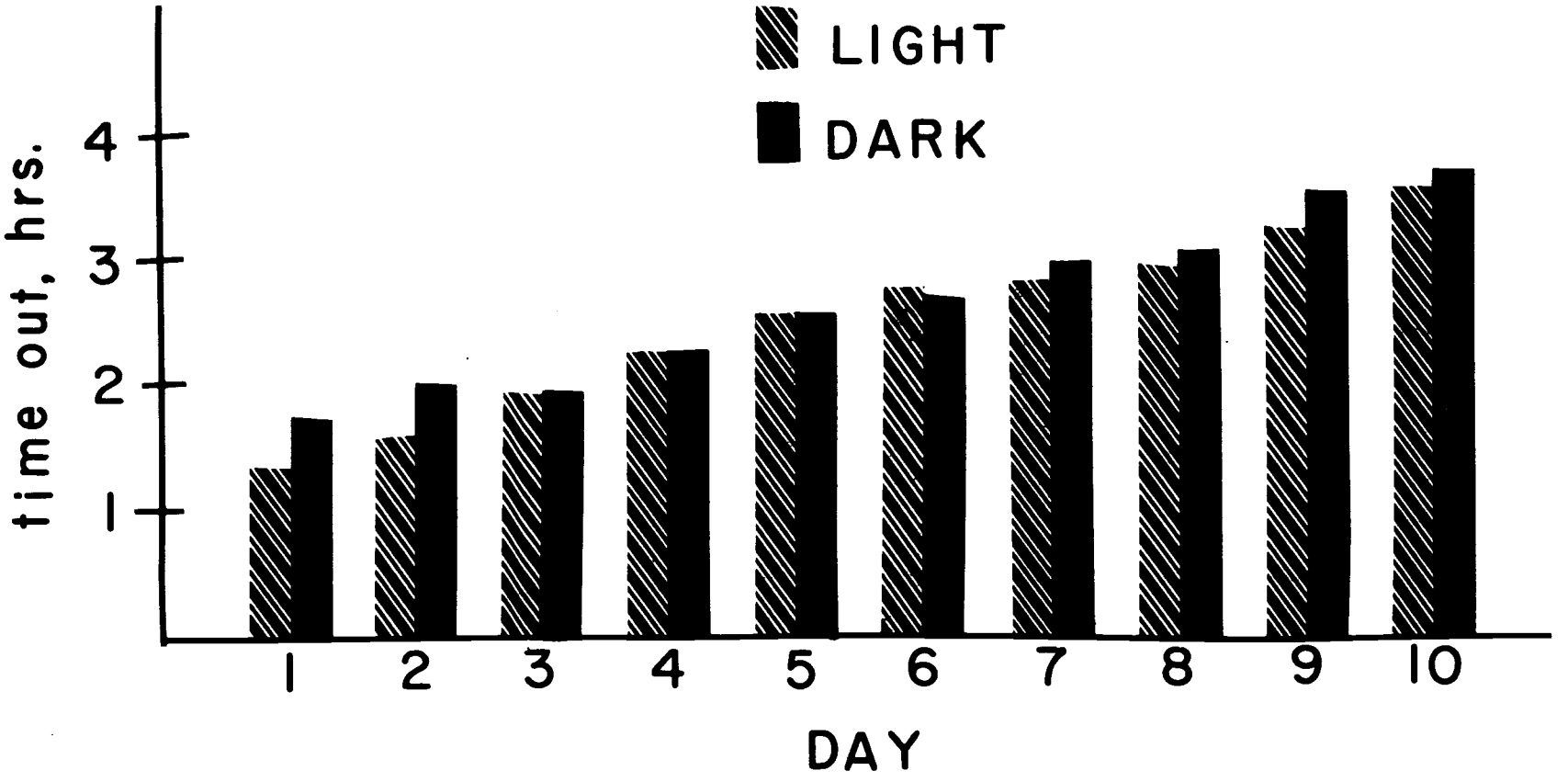



Figure 12. Time away from the nest during light and dark intervals for the 10 days following parturition in the C57 line.

In the C57 line it appears that the actual difference between light and dark periods of the same day was small (Figure 12), and most of the time interval effect in the analysis of variance table (22) was due to the 24 hour increases. The day effect increase noted in previous sections was apparently distributed fairly evently between light and dark intervals. It should be noted that equality in the two measures of activity implied some nocturnal tendency, since light and dark intervals were of unequal length. The light period was 14 hours so if the activity was distributed without regard to light, one could have expected approximately 58% to occur during light intervals. This was clearly not the case.

Table 23 presents the analysis of variance tables for number of trips away from the litter for the A/J and C57 lines. Significant differences were found among time intervals in both lines.

Figures 13 and 14 illustrate the subclass means for number of trips per time interval for the A/J and C57 lines, respectively. In the A/J line the number of trips per 24 hours primarily reflected nocturnal activity. During light intervals, A/J mothers averaged only eight trips, whereas during dark intervals the average number of trips was 72.

In the C57 line, the number of trips away from the litter was about the same for the light and dark portions of each 24 hour period. This, like in total time away from the nest, reflected some nocturnal

Table 23. Analysis of variance of number of trips away from the litter based on light and dark intervals.

| Source | df | MS | F | P |
|---|---|---|---|---|
| | | A/J line | | |
| Total | 311 | | | |
| Time Interval | 19 | 18234 | 9.22 | .005 |
| Error | 292 | 1977 | | |
| | | C57 line | | |
| Total | 480 | | | |
| Time Interval | 19 | 2210 | 4.11 | .005 |
| Error | 461 | 538 | | |

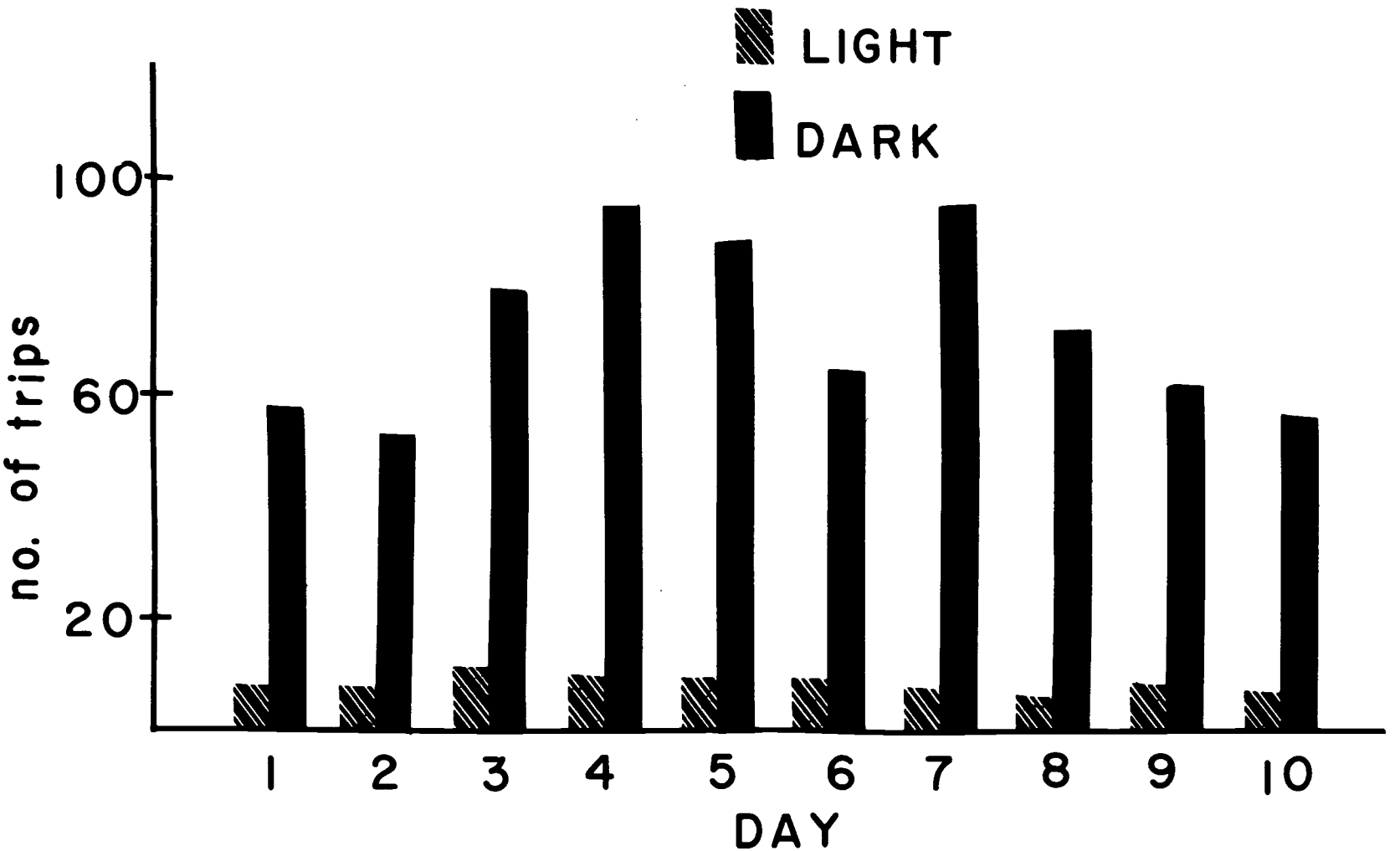



Figure 13. Number of trips away from the nest during light and dark intervals for the 10 days following parturition in the A/J line.

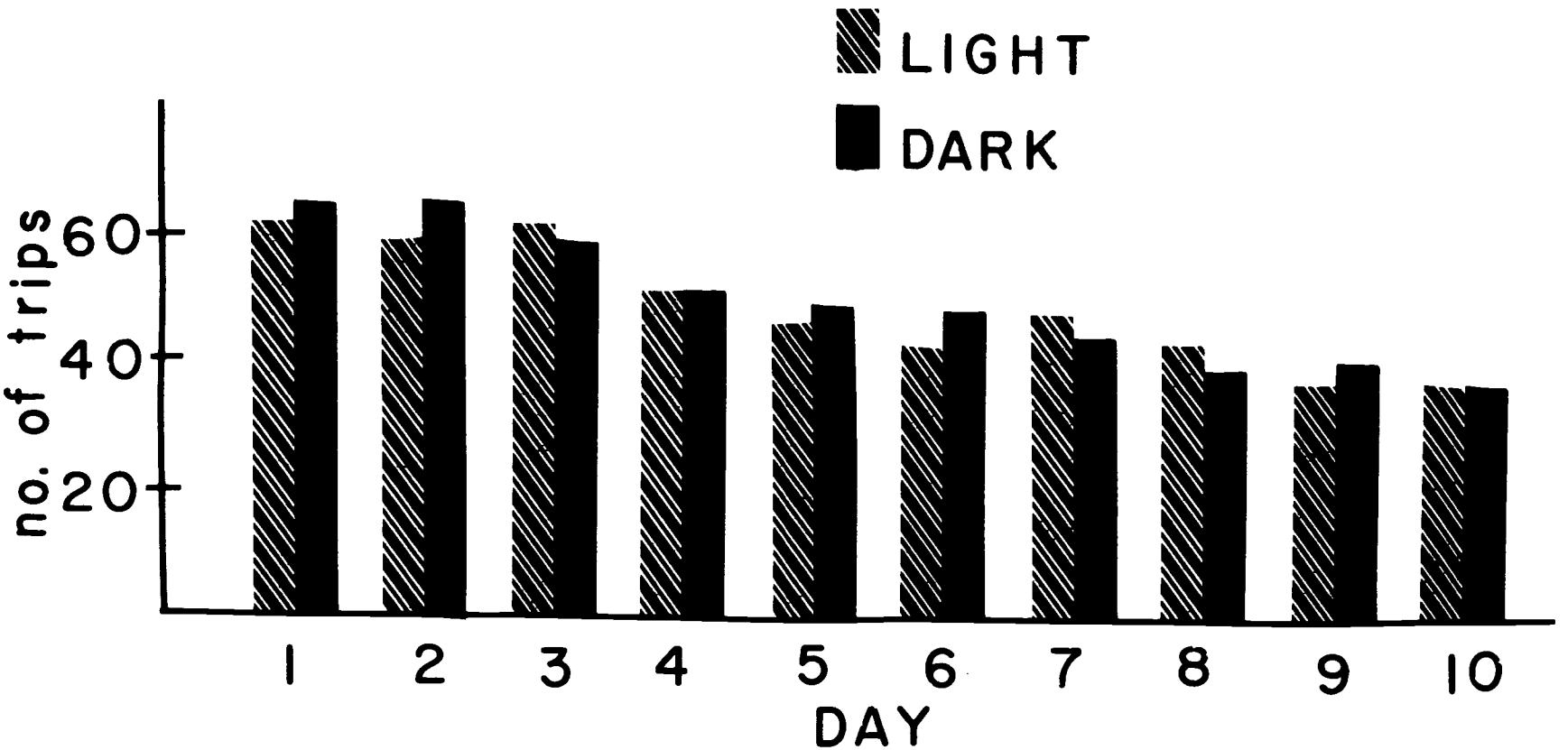



Figure 14. Number of trips away from the nest during light and dark intervals for the 10 days following parturition in the C57 line.

tendencies. The decrease in number of trips as the litter matured was evently distributed between the light and dark intervals.

Table 24 presents the analysis of variance table for trip length for the A/J and C57 lines. Significant differences were found for the C57 but not the A/J line.

Figures 15 and 16 illustrate the subclass means for trip length per time interval for the A/J line and the C57 line, respectively. The average trip length in the A/J line was only slightly less during light periods than dark periods. Much of this decrease probably resulted from the definition of the quantity: 0 hours out ÷ 0 trips out = 0 minutes per trip, which was necessary to perform the analysis. Thus, in spite of the small amount of time spent away from the litter during light intervals, the average trip length was probably not greatly shortened.

In the C57 line, the average trip length for day and night intervals was quite similar. With the exception of day 6, the average trip is slightly longer during the dark interval as compared to the corresponding day interval.

The average values for the behavioral measures for each experimental group were also obtained from the residuals of the one-way analysis based on time intervals. However, much of the disparity between these groups was a reflection of total day (day + night) differences previously described. In order to compare the influence of

Table 24. Analysis of variance of trip length based on light and dark intervals.

| Source | df | MS | F | P |
|---|---|---|---|---|
| | | A/J line | | |
| Total | 311 | | | |
| Time Interval | 19 | 70.45 | 1.87 | - |
| Error | 292 | 37.60 | | |
| | | C57 line | | |
| Total | 480 | | | |
| Time Interval | 19 | 69.46 | 16.38 | .005 |
| Error | 461 | 4.24 | | |

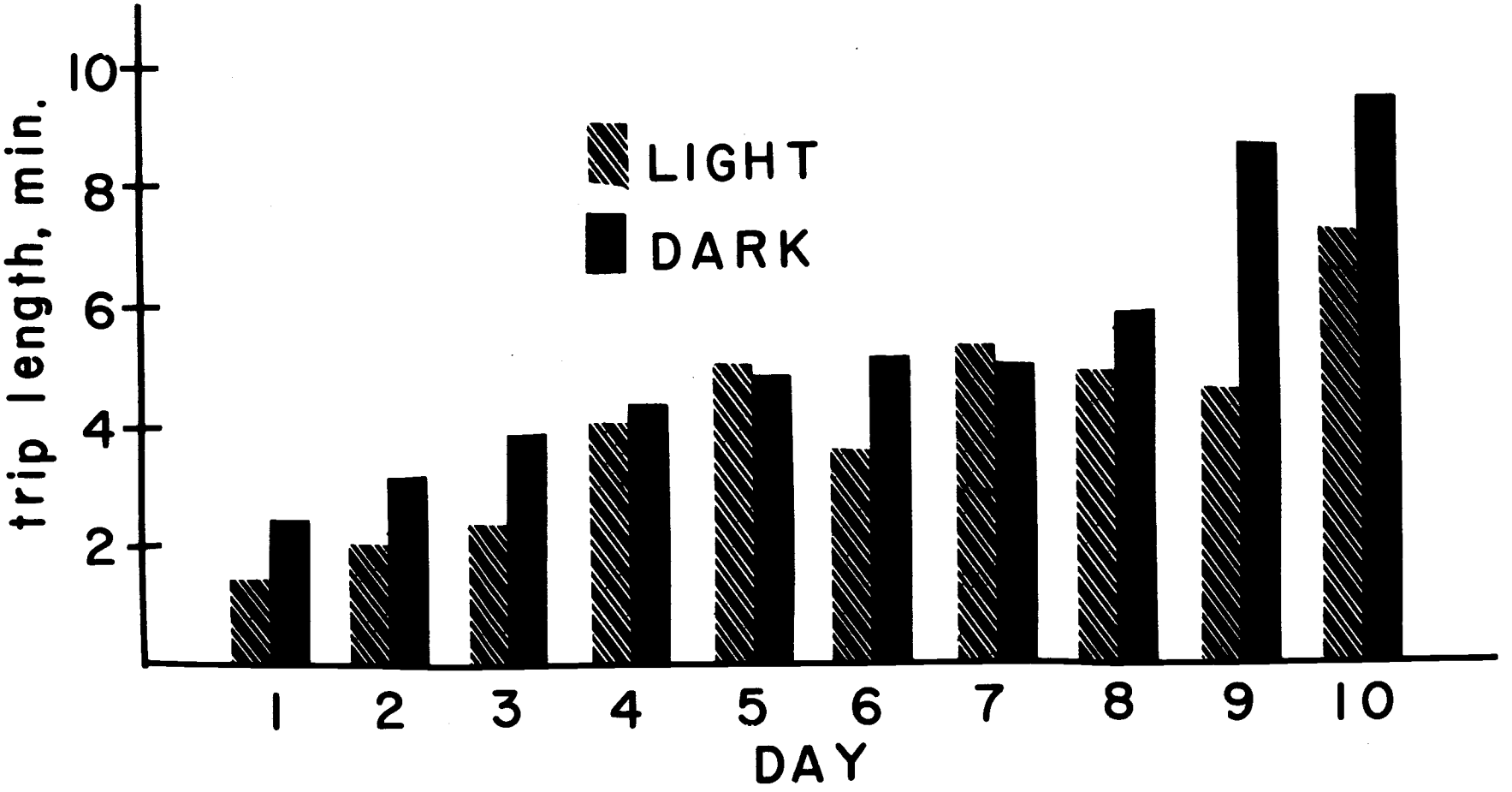



Figure 15. Average trip length during light and dark intervals for the 10 days following parturition in the A/J line.

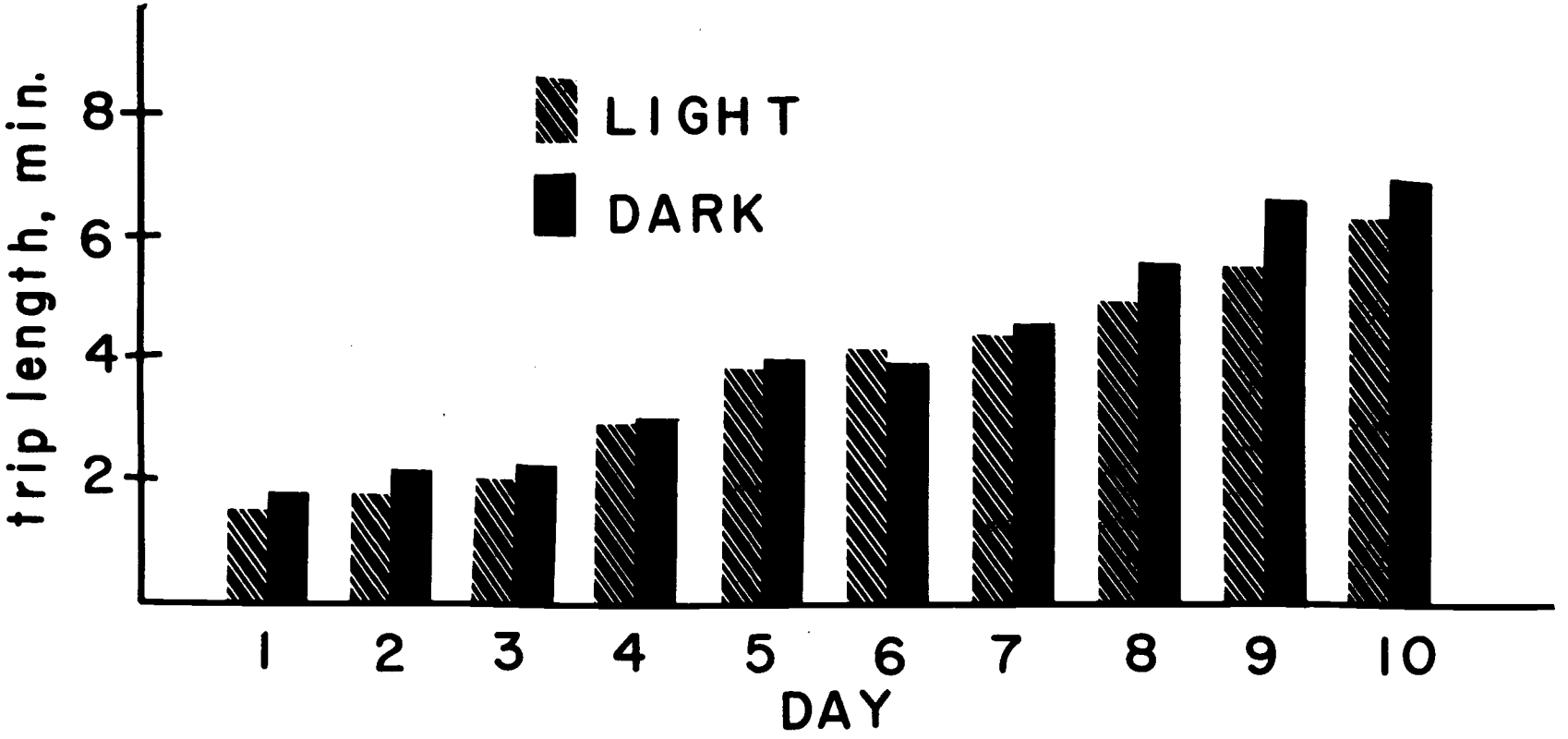



Figure 16. Average trip length during light and dark intervals for the 10 days following parturition in the C57 line.

light-dark cycles on these experimental groups, percentages based upon the totals were also calculated. Table 25 presents the analysis of variance for time away from the nest and Figure 17 illustrates the percent of the time spent away from the nest which occurred in the lighted interval for each of the experimental groups. The relative ranking of the three groups was about the same in both lines. The generation 1, unfostered group and the generation 2, fostered group had about the same proportion of the time away from the litter occurring in light, while the proportion for the generation 2, unfostered group was somewhat greater. Differences in the A/J line were small and nonsignificant. In this group in the C57 line, 59% of the total time out occurred during the light periods, implying that light did not inhibit activity in this group.

Table 26 presents the analysis of variance for number of trips and Figure 18 illustrates the percent of the number of trips which occurred in the lighted interval for each of the experimental groups. In this measure of behavior, the relative rankings in the two lines differed. In the A/J line, the fostered individuals had a greater proportion of the trips away from the litter during light than did the two unfostered groups. In the C57 line, the generation 2, unfostered group had the largest portion of trips (55%) during the light intervals. This lack of preference for nocturnal activity again contrasted with

Table 25. Analysis of variance of time away from the litter in light and dark, corrected for time interval.

| Source | df | MS | F | P |
|---|---|---|---|---|
| | | A/J line | | |
| Group | 4 | 11.62 | 1.62 | - |
| Individual/Group | 30 | 7.17 | 15.51 | .005 |
| Error | 257 | 0.46 | | |
| | | C57 line | | |
| Group | 4 | 18.18 | 3.70 | .025 |
| Individual/Group | 46 | 4.91 | 12.38 | .005 |
| Error | 411 | 0.40 | | |

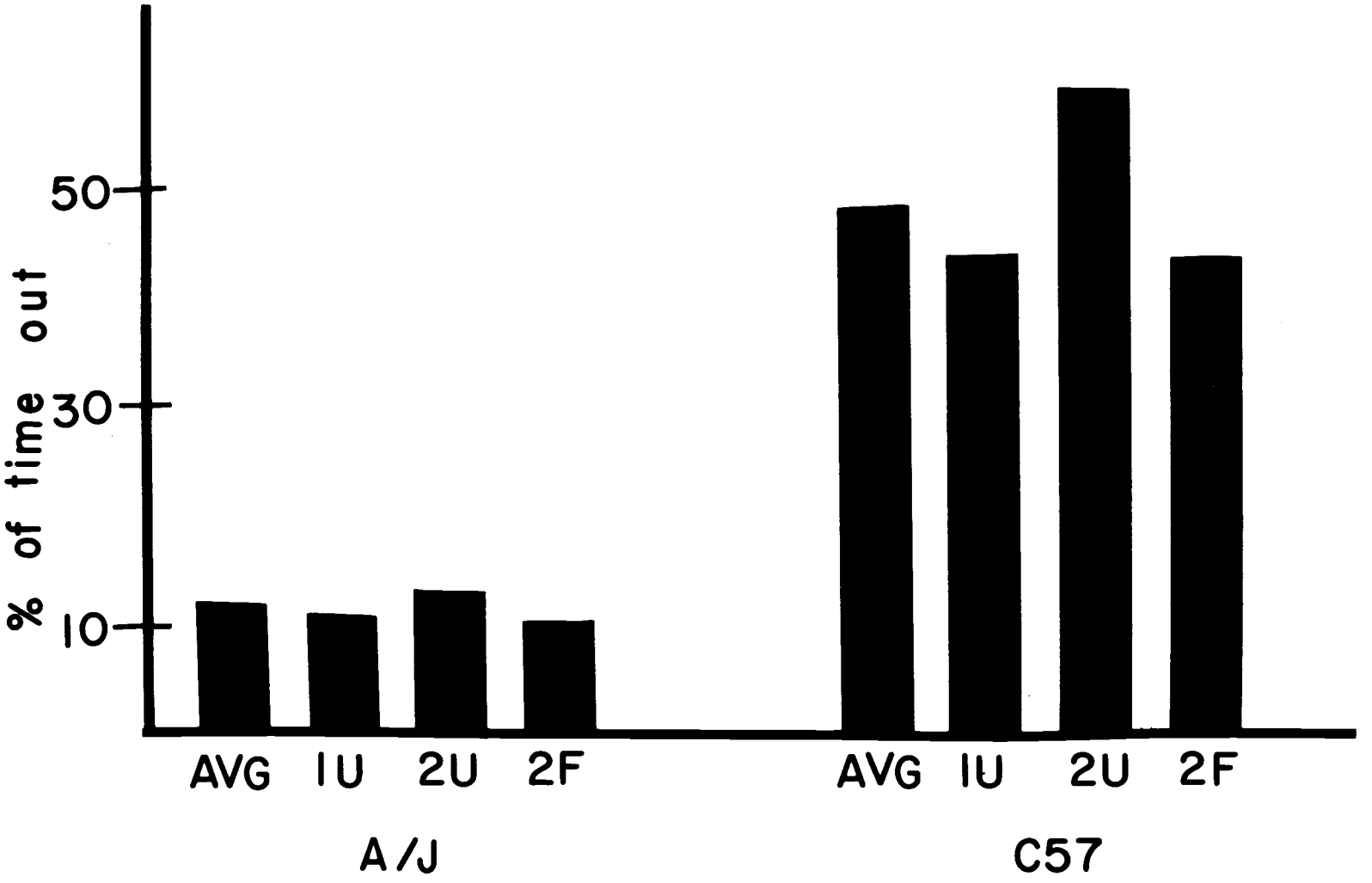



Figure 17. Percent of estimated time away from the litter occurring in the light part of the day in each experimental group of each line.

Table 26. Analysis of variance of trip length in light and dark, corrected for time interval.

| Source | df | MS | F | P |
|---|---|---|---|---|
| | | A/J line | | |
| Group | 4 | 244.8 | 1.10 | - |
| Individual/Group | 30 | 222.8 | 17.28 | .005 |
| Error | 258 | 12.9 | | |
| | | C57 line | | |
| Group | 4 | 36.16 | 1.98 | .25 |
| Individual/Group | 46 | 18.25 | 9.46 | .005 |
| Error | 411 | 1.93 | | |

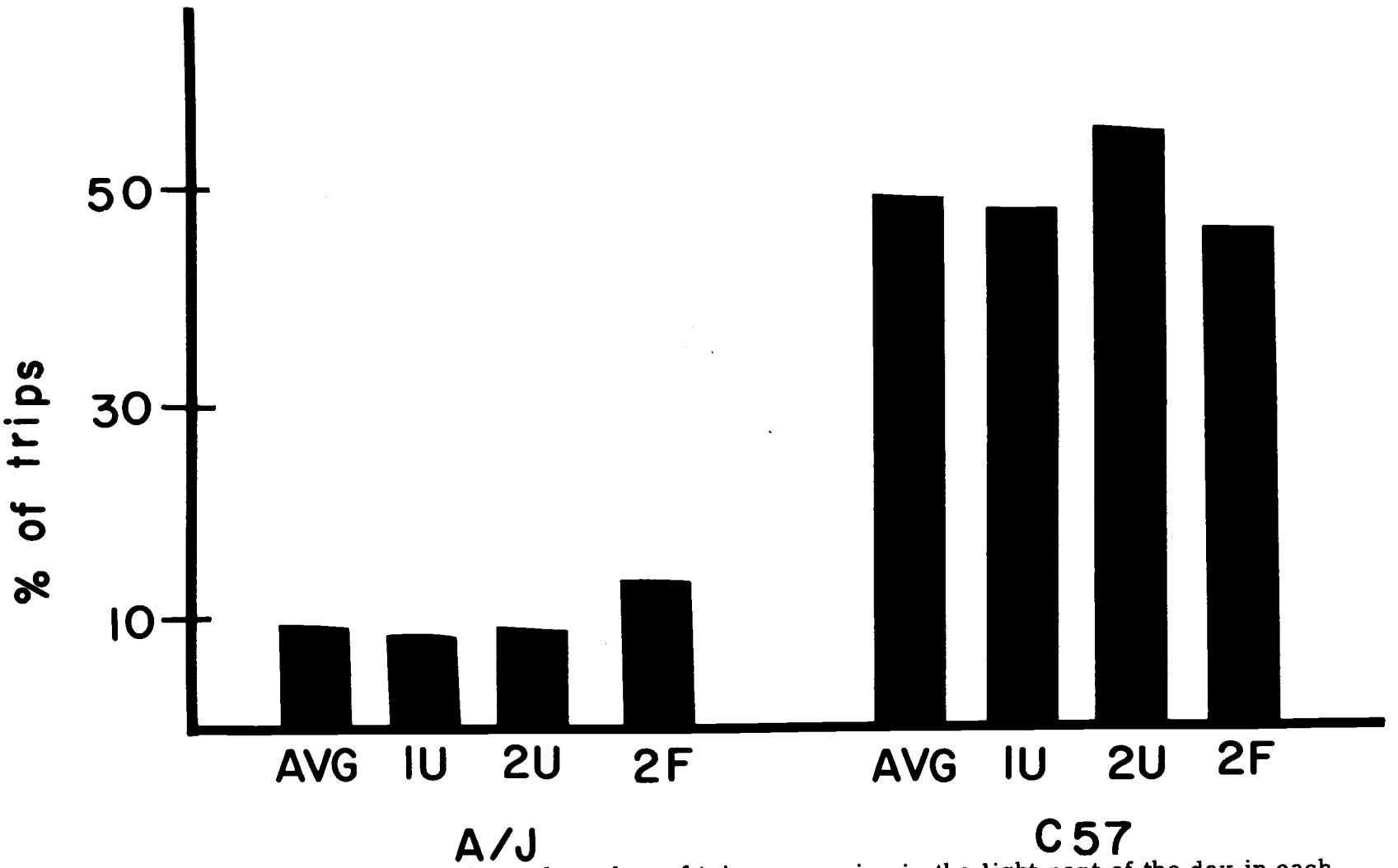



Figure 18. Percent of estimated number of trips occurring in the light part of the day in each experimental group of each line.

the other C57 groups in which less than half of the trips out were during day.

Table 27 presents the analysis of variance of trip length and Figure 19 illustrates the ratio of the trip length during light to the trip length during darkness for each of the experimental groups. In the A/J line, the average trip length of the generation 1, unfostered group was the same during light and dark intervals. The average trip length for the fostered group was greatly reduced, which reflected the larger proportion of trips during day without a concomitant increase in the portion of the time away.

In the C57 line, the generation 2, unfostered group averaged a longer trip length during light intervals than dark intervals. The reverse was true in the other two C57 groups.

## Circadian Rhythms

### Regularity of the Ten Day Patterns

In order to examine the circadian patterns in these two lines of mice, the behavioral measures were tabulated at hourly intervals for each mouse. Thus, if no data were missing, each mouse would have had 240 observations. A one-way analysis of variance was performed within each of the six experimental groups. The results for time out per hour per day are presented in Tables 28 and 29.

Table 27. Analysis of variance of number of trips away from the litter in light and dark, corrected for time interval.

| Source | df | MS | F | P |
|---|---|---|---|---|
| | | A/J line | | |
| Group | 4 | 46902 | 4.90 | .005 |
| Individual/Group | 30 | 9564 | 23.99 | .005 |
| Error | 258 | 399 | | |
| | | C57 line | | |
| Group | 4 | 4526 | 1.94 | .25 |
| Individual/Group | 46 | 2323 | 7.75 | .005 |
| Error | 411 | 300 | | |

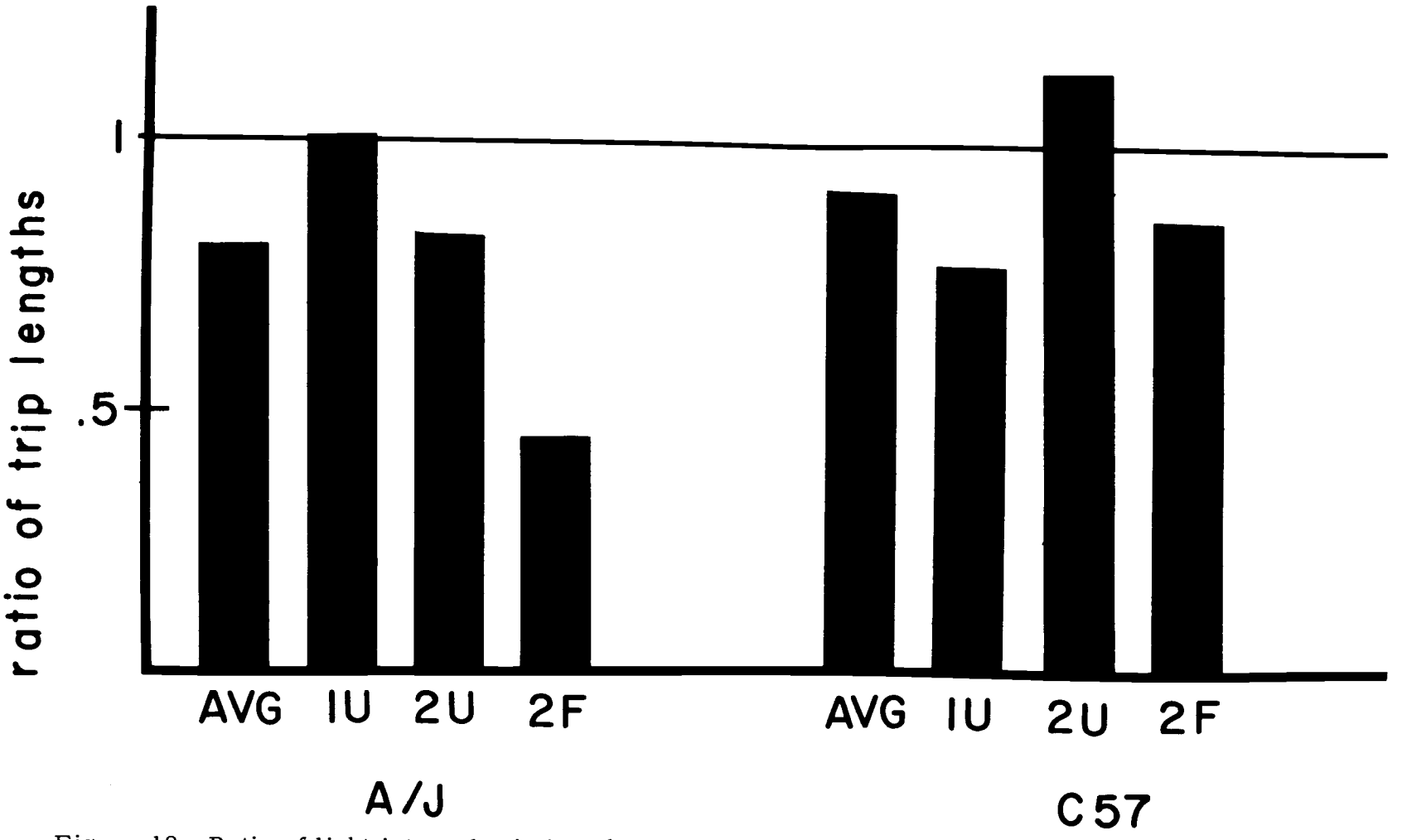



Figure 19. Ratio of light interval trip length to dark interval trip length for each experimental group of each line.

Table 28. Analysis of variance of time away from the nest per hour per day, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| | Generation 1, Unfostered | | | |
| Total | 1099 | | | |
| Hour | 239 | 0.2562 | 6.01 | .005 |
| Error | 860 | 0.0426 | | |
| | | $R^2$ = 62.6% | C = 1.02 | |
| | Generation 2, Unfostered | | | |
| Total | 1485 | | | |
| Hour | 239 | 0.3299 | 8.07 | .005 |
| Error | 1246 | 0.409 | | |
| | | $R^2$ = 60.7% | C = 1.09 | |
| | Generation 2, Fostered | | | |
| Total | 1207 | | | |
| Hour | 239 | 0.1852 | 6.42 | .005 |
| Error | 968 | 0.0289 | | |
| | | $R^2$ = 61.3% | C = 1.19 | |

Table 29. Analysis of variance of time away from the nest per hour per day, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| | Generation 1, Unfostered | | | |
| Total | 1615 | | | |
| Hour | 239 | 0.1459 | 2.61 | .005 |
| Error | 1376 | 0.0559 | | |
| | $R^2$ = 31.2% | | C = 1.04 | |
| | Generation 2, Unfostered | | | |
| Total | 1806 | | | |
| Hour | 239 | 0.1628 | 4.50 | .005 |
| Error | 1567 | 0.0362 | | |
| | $R^2$ = 40.7% | | C = 0.89 | |
| | Generation 2, Fostered | | | |
| Total | 2474 | | | |
| Hour | 239 | 0.1976 | 4.55 | .005 |
| Error | 2235 | 0.0434 | | |
| | $R^2$ = 32.7% | | C = 0.99 | |

Coefficients of variation are also given. The results were all highly significant. This indicated that females of an experimental group shared a common pattern of activity, and tended to be away from the litter during the same hours of the day. In the A/J line (Table 28) the difference in time out between hourly intervals accounted for about 60% of the variation in time away from the litter per hour. The $R^2$ values were similar for the experimental groups. These values were especially impressive in light of the differences between females in total time spent away from the litter per day (Table 4).

The 10 day pattern was more poorly defined among C57 mothers (Table 29). Hourly intervals accounted for only 30-40% of the variation in time out per hour; the F values for the C57 experimental groups were lower than those in the A/J groups.

The analysis of variance tables for number of trips out for each hour of each day are given in Tables 30 and 31. All were significant. However, in each case the $R^2$ value and F value were lower than in the companion analysis for time away from the litter per hour. This indicated that mice within an experimental group were more dissimilar in the 10 day pattern in trips away from the nest. In the A/J line 35-52% of the variation was explained by time intervals (Table 30) while in the C57 line only 22-30% of the variation was explained (Table 31).

Table 30. Analysis of variance of number of trips per hour per day, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| | Generation 1, Unfostered | | | |
| Total | 1099 | | | |
| Hour | 239 | 228.0 | 3.32 | .005 |
| Error | 860 | 68.6 | | |
| | $R^2$ = 48.0% | | C = 1.41 | |
| | Generation 2, Unfostered | | | |
| Total | 1485 | | | |
| Hour | 239 | 38.42 | 5.73 | .005 |
| Error | 1246 | 6.70 | | |
| | $R^2$ = 52.4% | | C = 1.27 | |
| | Generation 2, Fostered | | | |
| Total | 1207 | | | |
| Hour | 239 | 61.37 | 2.15 | .005 |
| Error | 968 | 28.55 | | |
| | $R^2$ = 34.7% | | C = 2.04 | |

Table 31. Analysis of variance of number of trips per hour per day, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| | Generation 1, Unfostered | | | |
| Total | 1615 | | | |
| Hour | 239 | 56.71 | 1.60 | .005 |
| Error | 1376 | 35.41 | | |
| | $R^2$ = 21.8% | | C = 1.39 | |
| | Generation 2, Unfostered | | | |
| Total | 1806 | | | |
| Hour | 239 | 43.18 | 2.86 | .005 |
| Error | 1567 | 15.11 | | |
| | $R^2$ = 30.4% | | C = 1.10 | |
| | Generation 2, Fostered | | | |
| Total | 2474 | | | |
| Hour | 239 | 88.58 | 2.99 | .005 |
| Error | 2235 | 29.58 | | |
| | $R^2$ = 24.3% | | C = 1.22 | |

Also, the amount of variation present differed among the experimental groups. In the A/J line the first generation unfostered group was the most variable, and the second generation unfostered was the least variable. The coefficients of variation indicated that the amount of variation present might depend on the size of the mean in the first generation, unfostered group which had the highest estimate for number of trips away from the nest per day and the second generation, unfostered group which had the lowest estimate.

In the C57 line the differences in the amount of variation present among the experimental groups were not as great as among the A. J groups. However, the second generation, unfostered was clearly the least variable, and this group also had the lowest estimated number of trips away from the litter per day. Coefficients of variation were similar.

## Circadian Rhythms

In previous sections, it was shown that the time spent away from the litter each day increased steadily from day 1 to day 10. In order to define the circadian rhythms present and determine which parts of the daily activity cycle were affected by the increasing time away from the nest, a simple linear regression was performed on the subclass means determined in the previous section. For each hour within each experimental group the model assumed for the linear

regression was:

$$Y = \alpha + \beta \text{ (day)} + \varepsilon$$

The estimated time out for that hourly interval was evaluated for day 1 and day 10. The results are plotted in Figures 20-25.

In the A/J line, Figures 20-22, most time away from the litter occurred during the dark interval, from 2000 to 0600 hours. For all three groups, the estimated cycles for day 1 were similar. All showed the greatest time away from the nest to occur between 2000 to 2200 hours. In the fostered (Figure 22) and generation 2, unfostered groups (Figure 21), the female spent over 70% of the hour between 2000 to 2100 hours away from the litter; in the generation 1, unfostered group (Figure 20), activity was spread over a three hour interval, with less than 50% of each hour away from the litter. The onset of the nightly activity was abrupt. This was interpreted as a direct response to the light cut off at 2000 hours.

A secondary peak of activity in day 1 was seen in the early hours of the morning for all three experimental groups. This activity period generally fell between 0200 and 0400 hours and activity had generally declined by 0500 hours, which was an hour before the start of the light interval. So the A/J mouse did not directly respond to lights on, but reacted to the anticipation of lights on.

In the A/J line the bulk of the increase in time spent away from the litter from day 1 to day 10 was added to the dark part of the cycle.

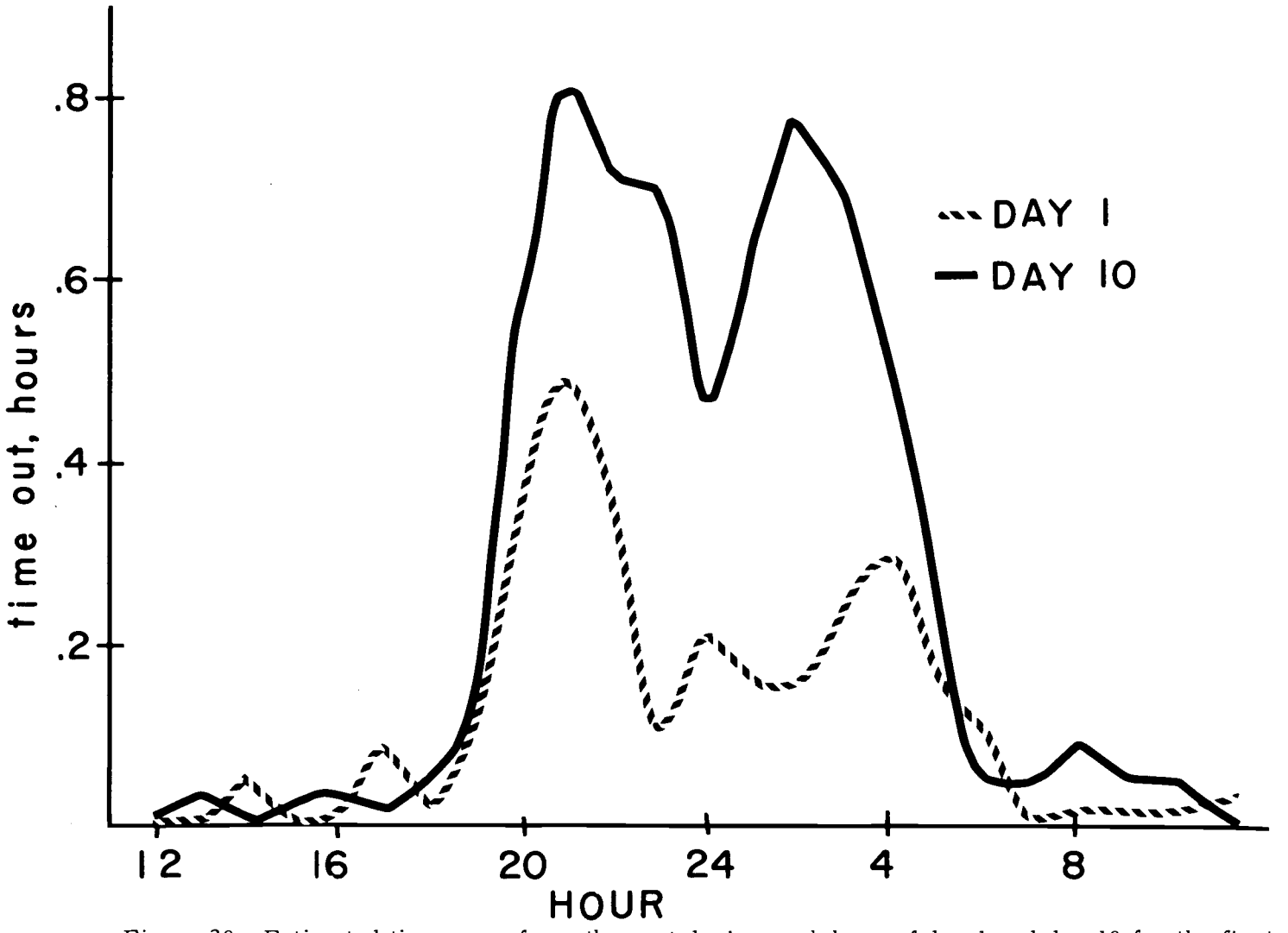



Figure 20. Estimated time away from the nest during each hour of day 1 and day 10 for the first generation unfostered of the A/J line.

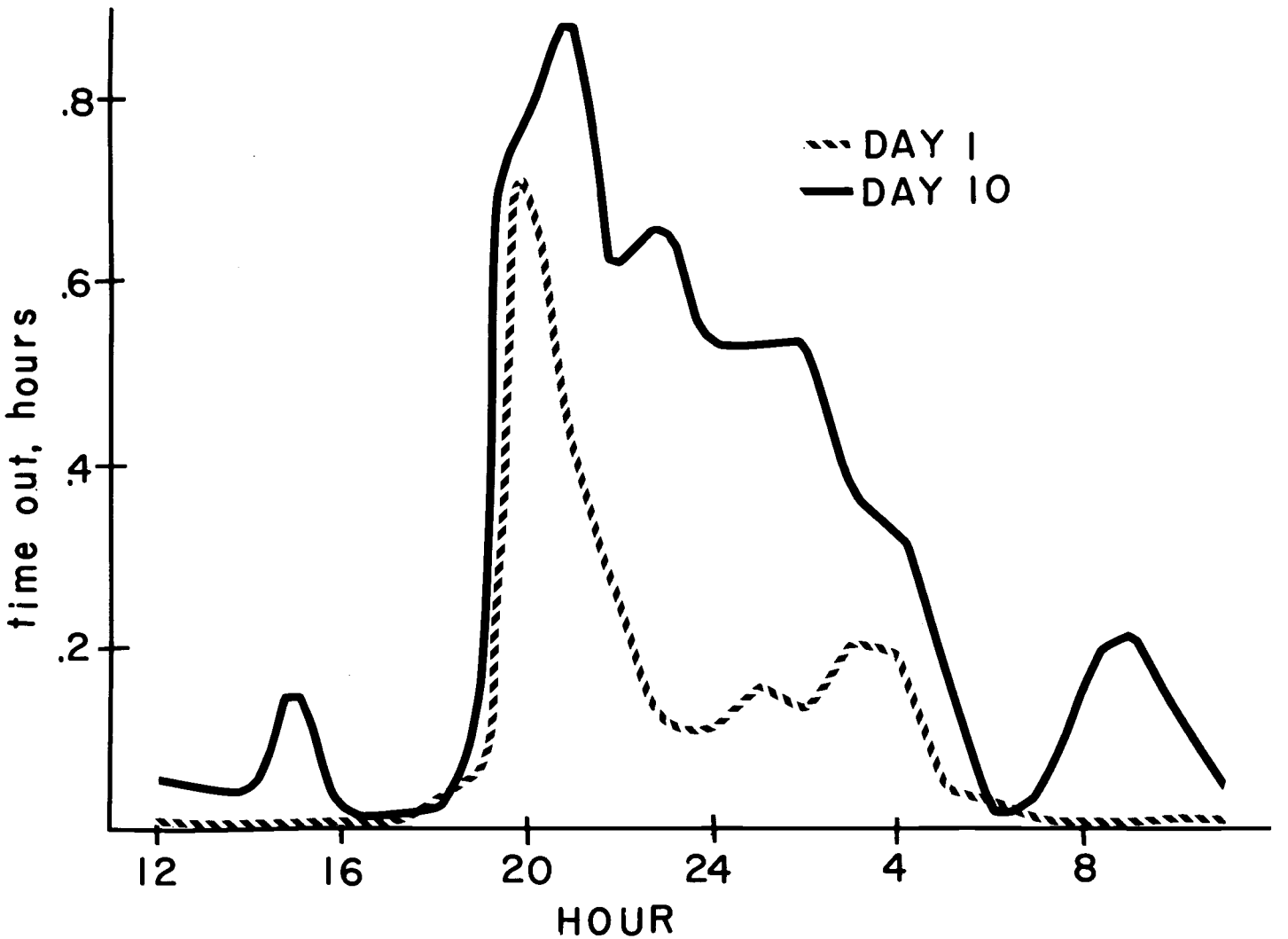



Figure 21. Estimated time away from the nest during each hour of day 1 and day 10 for the second generation unfostered group of the A/J line.

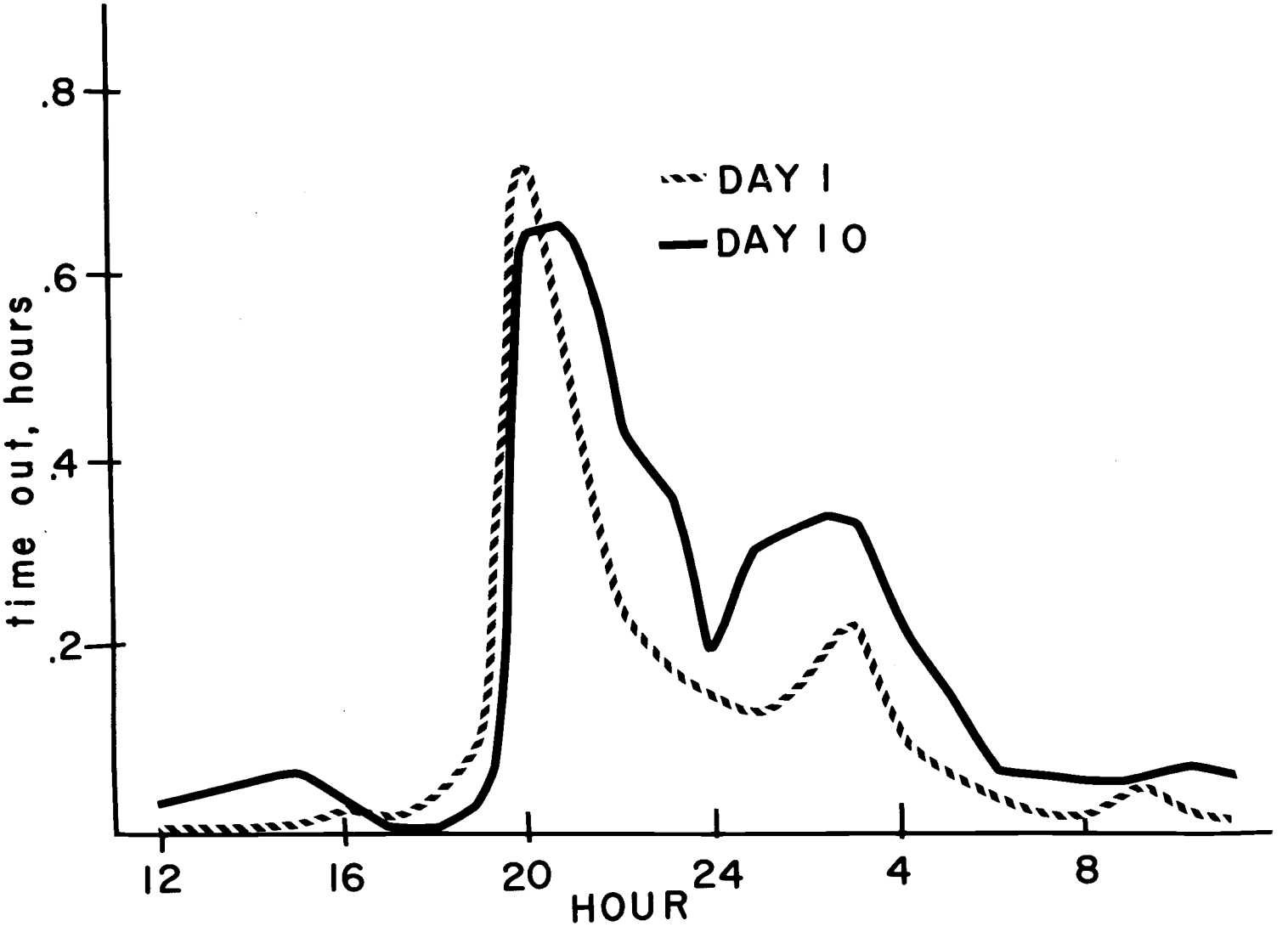



Figure 22. Estimated time away from the nest during each hour of day 1 and day 10 for the second generation fostered group of the A/J line.

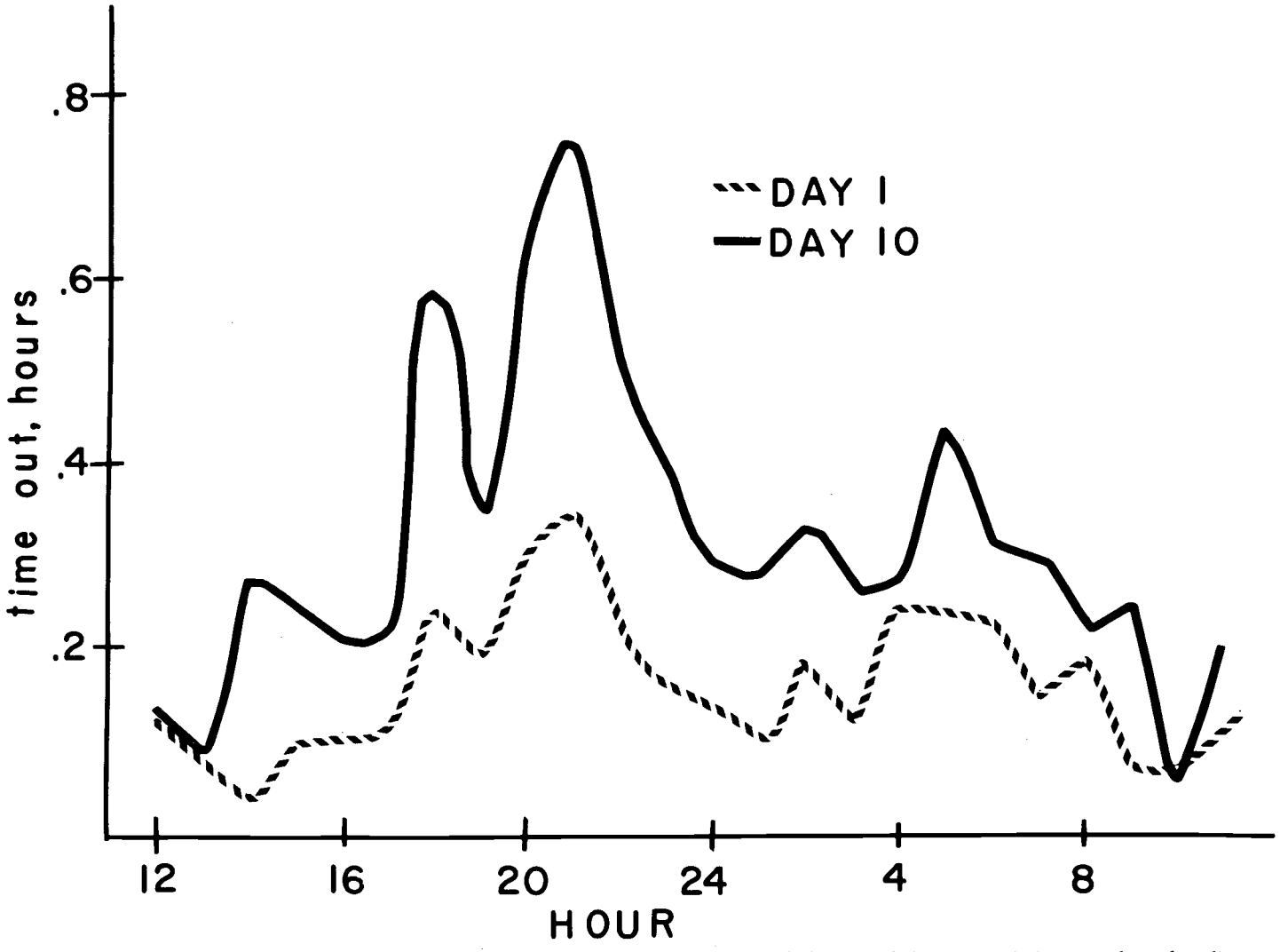



Figure 23. Estimated time away from the nest during each hour of day 1 and day 10 for the first generation unfostered group of the C57 line.

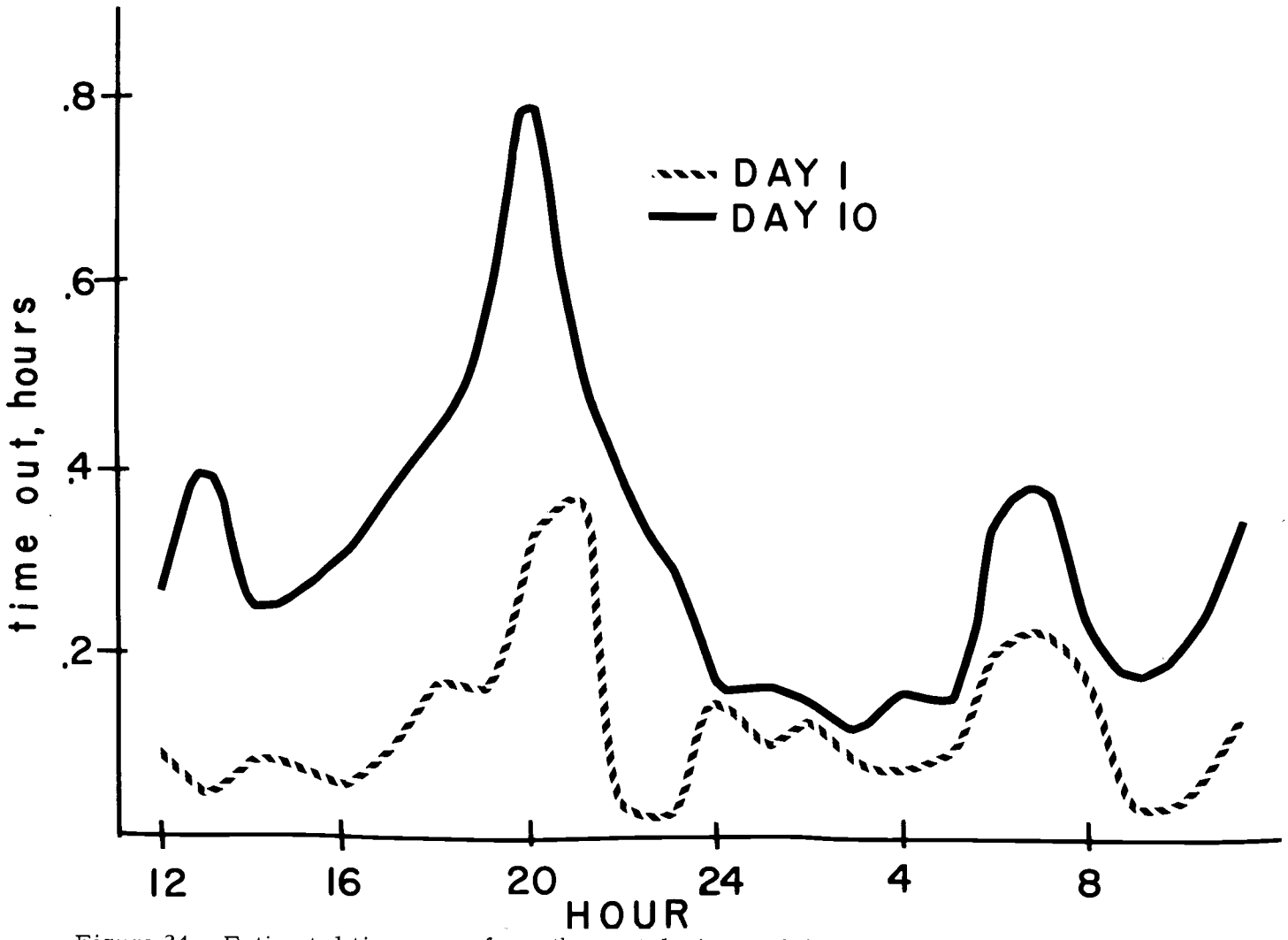



Figure 24. Estimated time away from the nest during each hour of day 1 and day 10 for the second generation unfostered group of the C57 line.

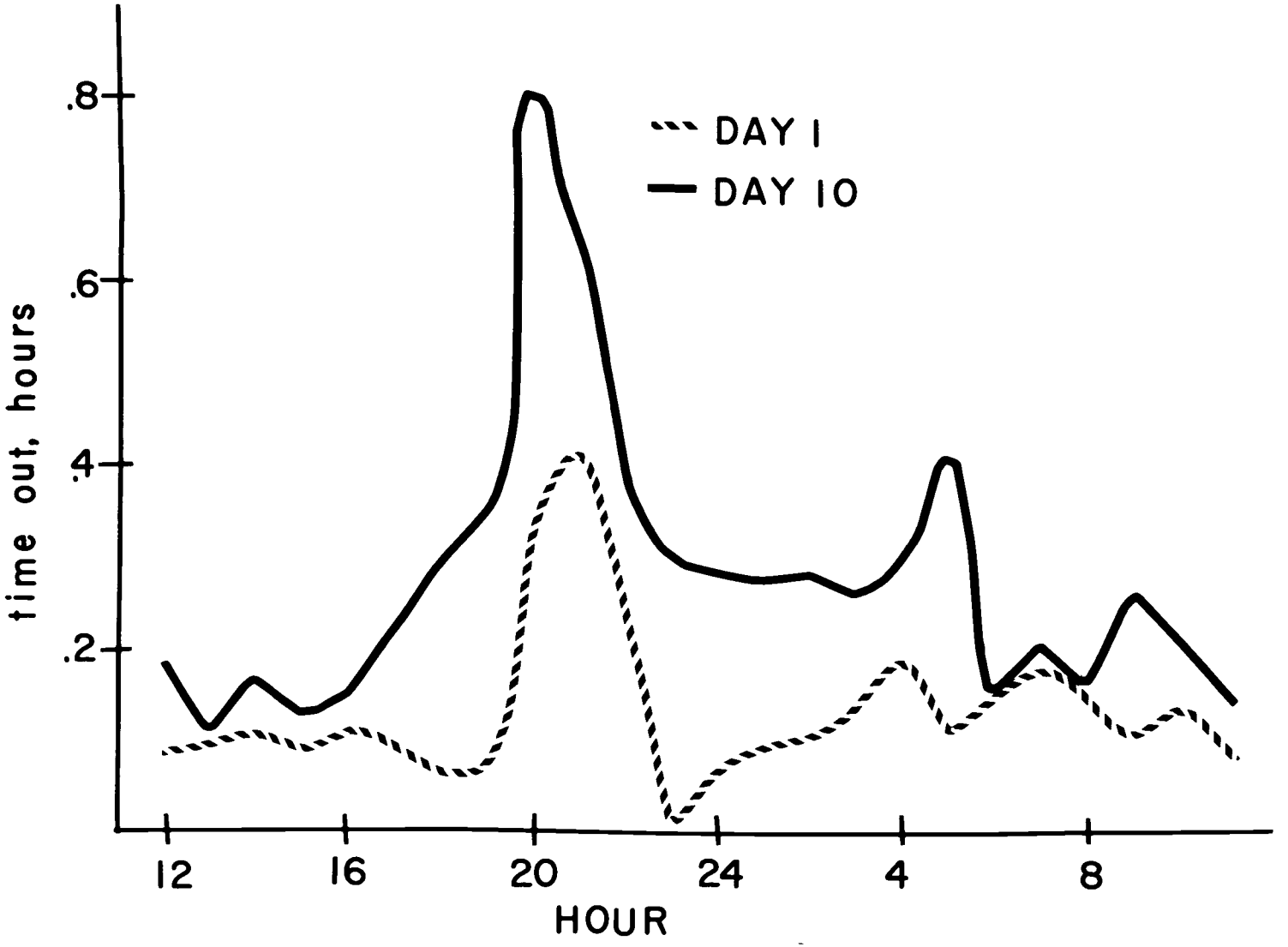



Figure 25. Estimated time away from the nest during each hour for day 1 and day 10 for the second generation fostered group of the C57 line.

This was true in all three experimental groups. In previous sections it was shown that the fostered group spent less time away from the litter per day than the other two groups. This can also be seen in Figures 20-22. The pattern and total amount of time spent away from the litter appeared similar in all three groups for day 1. However, the fostered group (Figure 22) showed less increase in time spent away from the litter which was apparent in comparisons of the day 10 cycles.

The generation 1, unfostered group (Figure 20) and the fostered group (Figure 22) increased time spent away from the litter in the same parts of the cycle. In the day 10 cycles, both the peak after lights off (2000-2300 hours) and the early morning peak (0200-0500 hours) were still recognizable. Both peaks had generally increased in intensity; that is, a greater part of each hour was spent away from the litter. The length of each activity period had expanded about an hour with the expansion occurring in the darkness hours. Thus at 10 days the peak after lights off extended from 2000 to 2400 hours, and the early morning peak extended from 0100 to 0500 hours.

The pattern for day 10 for the second generation unfostered group (Figure 21) was somewhat different than that of the other two groups. There was no recognizable early morning peak. The peak after lights off still had a pronounced onset, but activity generally declined from that level throughout the night, with a lesser portion of

each successive hour spent away from the litter. A smaller peak of activity was also seen in the light interval between 0800 and 1100 hours. This peak was not present in the other two groups.

The estimated time out per hour for day 1 and day 10 of the three experimental groups of the C57 line are plotted in Figures 23-25. The general patterns were much less regular than in the A/J line. The C57 line tended to maintain a higher level of general activity than the A/J line. That is, a portion of each hour, independent of the time of day, was spent away from the litter rather than having all time away from the litter concentrated in certain times of the day.

The most repeatable and recognizable peak of activity in all groups occurred around 2000 hours, or the beginning of the dark period. This peak was not as pronounced as in the A/J line; the onset of activity was not abrupt, and a steadily increasing amount of time in the hours before lights off was spent away from the litter. This indicated that C57 females responded to the anticipation of lights off, but did not necessarily wait for lights off to initiate activity as in the A/J line.

Another period of activity occurred in the morning hours in each group. It was much less striking in the C57 line than in the A/J line. In the generation 1, unfostered group (Figure 23) and the fostered group (Figure 25) the activity was centered at 0500-0600 hours, and activity decreased with the onset of light at 0600 hours. In the second

generation unfostered group (Figure 24) this peak occurred after lights on, between 0600-0800 hours.

It was previously noted that the second generation unfostered group spent a larger proportion of the time away from the litter during the light interval. This peak of activity which occurred during light was part of the reason. However, it was also observed in the day 10 pattern that the level of general activity between the two peaks was higher in the light part of the day than in the dark part. In both the generation 1, unfostered and the fostered groups the reverse was true; a higher level of general activity was seen in the dark period.

Even though the generation 1 unfostered (Figure 23) and generation 2 fostered (Figure 25) groups both tended to be more active at night, there were apparent differences in the two groups in the time intervals in which the increased time spent away from the litter in day 10 vs day 1 was added. The general activity increase in the generation 1 unfostered was fairly constant, independent of time of day. However, the fostered group added more time out during the darkness hours than the light hours. Thus, the presence of light had the most inhibitory effect in activity in the fostered group and the least inhibitory effect in the second generation unfostered group.

## Open Field Testing

Representative animals from the two lines were tested in the

open field; ambulation and defecation scores were recorded. Striking differences between the two lines were found. Individuals of the A/J line averaged 33.4 squares entered in the five minute test period; individuals of the C57 line had an average ambulation score of 342. Differences in defecation scores were also large: A/J individuals averaged 4.1 fecal boluses in the five minute period while members of the C57 line averaged only 0.8. The correlation between ambulation and defecation was negative in both lines; it was -0.38 for the A/J line and -0.31 for the C57 line. Because larger variances were associated with the larger means, data from the two lines were analyzed separately.

Ambulation Scores

The analysis of variance tables for ambulation scores are presented in Table 32 for the A/J line and Table 33 for the C57 line. There are common trends; in both lines females had higher ambulation scores than males. The difference was significant only for the C57 line (Table 33), in which the females had an average score of 367 squares entered *vs* a score of 300 for males. In the A/J line the females entered an average of 26.9 squares *vs* 19.9 for males (Table 32).

Members of both lines yielded higher ambulation scores when tested under red rather than white lighting. Although the actual

Table 32. Analysis of variance of ambulation scores, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 178 | | | |
| Sex | 1 | 2153 | 1.69 | .25 |
| Fostering | 1 | 109 | 0.08 | - |
| Age | 1 | 10195 | 8.01 | .01 |
| Light | 1 | 79776 | 62.67 | .005 |
| Round | 1 | 150 | 0.12 | - |
| Trial | 2 | 16832 | 13.22 | .005 |
| Error | 171 | 1273 | | |
| | | $R^2$ = 36.1% | | |

Model:

Y = 23.4 + 3.5(female) - 3.5(male) - 1.3(U) + 1.3(F) + 16.6(young - 16.6(old) - 21.1(white light) + 21.1(red light) + 0.9(round 1) - 0.9(round 2) + 31.1(trial 1) - 14.8(trial 2) - 16.3(trial 3)

Table 33. Analysis of variance of ambulation score, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 188 | | | |
| Sex | 1 | 199767 | 32.00 | .005 |
| Fostering | 1 | 394 | 0.06 | - |
| Age | 1 | 155 | 0.02 | - |
| Light | 1 | 66519 | 10.65 | .005 |
| Round | 1 | 197468 | 31.62 | .005 |
| Trial | 2 | 569 | 0.09 | - |
| Error | 181 | 6245 | | |
| | | $R^2$ = 29.2% | | |

Model:

Y = 333.9 + 33.5(females) - 33.5(males) + 2.2(U) - 2.2(F) + 2.2(young) - 2.2(old) - 18.8(white light) + 18.8(red light) + 32.5(round 1) - 32.5(round 2) + 4.2(trial 1) + 1.4(trial 2) - 5.6(trial 3)

numerical difference was about the same the effect of the change in lighting was much greater in the A/J line because of the low mean score. A/J individuals tested under red light had an average score of 44.5 while under white light the average score was only 2.3 (Table 32). For the C57 line the estimated score was 353 for red light vs 315 for white light (Table 33).

Another indication that members of the A/J line might be more sensitive to changing test conditions was the finding of significant differences between trials in this line (Table 32). The scores from the first trial differed from the scores obtained in the other two trials; this corresponded to the change in the room location of the test. No indication of such an effect was found in the C57 line.

Significant differences in scores were found between the two rounds for the C57 line (Table 33) but not the A/J line (Table 32). This could be interpreted to mean that in the A/J line the change in light color altered the perceived stimuli enough so that the two rounds were independent tests. In the C57 line the rounds were not independent and the second could not be considered exploration in a novel situation.

The fostered individuals were indistinguishable from the unfostered individuals in both lines. In the A/J line the older animals were found to have lower scores than the younger animals. Because of confounding this difference cannot be attributable either to age or

to factors associated with the generation 1 group in previous sections.

The basic model was then expanded to include a large number of interaction terms. Residuals from this analysis were then used to evaluate the individual differences between mice.

In the expanded model, only one interaction term, light x round, was significant at the 2.5% level for both lines. This interaction indicated that testing order was important. The estimated effects of the two treatments with interaction are presented in Figure 26. The trends differ between the two lines. In the C57 line, presentation of white light in the first round stimulated greater ambulation than did red light; presentation of red light on the second round stimulated greater ambulation than white light. In other words, mice tested in the order white light then red light had average test scores 14 points higher than those tested in the reverse order.

In the A/J line the opposite was true; mice tested in the red light then white light had higher average scores than mice tested in white then red light. This is another example of line specific responses to a particular test situation.

Significant differences between mice for individual effects were found in both lines. These differences were significant at the 5% level in the A/J line and at the 0.5% level in the C57 line.

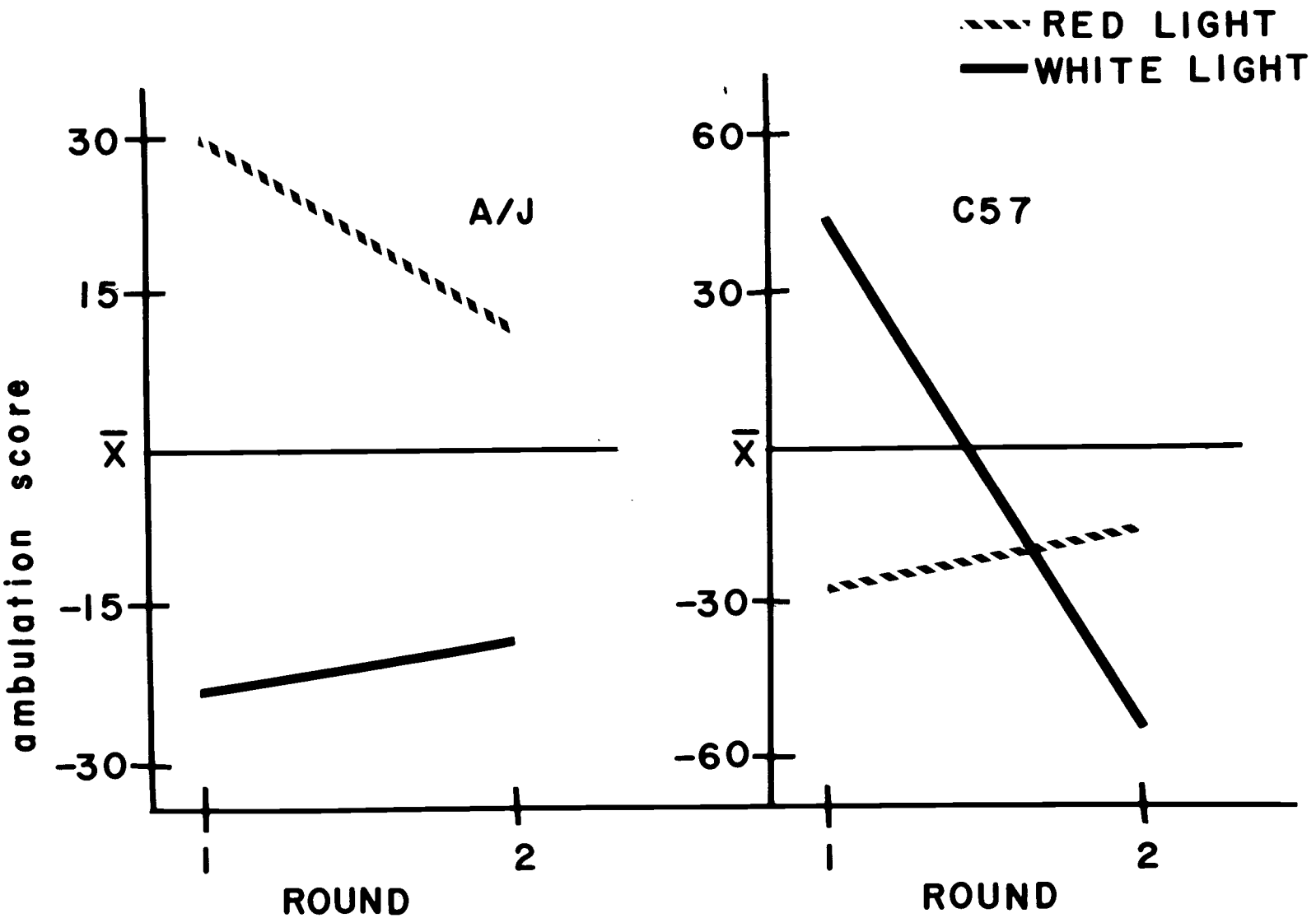



Figure 26. Light x round interaction in open field ambulation scores.

Defecation Scores

The analysis of variance tables for defecation scores are presented in Table 34 for the A/J line and Table 35 for the C57 line. The only factor significant for both lines was round, with both showing increased defecation scores in the second round.

In the A/J line (Table 34) several other factors were found to be significant. Young mice deposited an average of 1.6 fecal boluses more than the old mice. An average of one bolus more was dropped under white light than red light. A difference in results between trials was noted, with results from the first trial averaging less than the other two. With the exception of round, the above factors were also found to significantly influence ambulation scores in this line.

In the C57 line (Table 35) the only factor besides round found to be significant was sex, with males depositing an average of 0.9 fecal boluses more than females. In this line defecation scores were generally quite low, with the average score being only 0.8. For this reason, very little variation was present to partition into causative factors, and the validity of the test can be questioned.

The basic model was expanded to include interaction terms, residuals were obtained, and these were used to evaluate individual effects within a line. No repeatable difference was found in the A/J line. However, individual differences in defecation were found in the

Table 34. Analysis of variance of defecation score, A/J line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 178 | | | |
| Sex | 1 | 1.0 | 0.20 | - |
| Fostering | 1 | 1.5 | 0.32 | - |
| Age | 1 | 24.3 | 5.09 | .05 |
| Light | 1 | 47.6 | 10.00 | .005 |
| Round | 1 | 54.8 | 11.50 | .005 |
| Trial | 2 | 21.6 | 4.53 | .025 |
| Error | 171 | 4.8 | | |

$R^2$ = 32.0%

Model:

Y = 3.68 - 0.07(female) + 0.07(male) + 0.15(U) - 0.15(F)
+0.81(young) - 0.81(old) +0.52(white light) - 0.52(red light)
- 0.55(round 1) + 0.55(round 2) - 0.95(trial 1) + 0.71(trial 2)
+ 0.24(trial 3)

Table 35. Analysis of variance of defecation score, C57 line.

| Source | df | MS | F | P |
|---|---|---|---|---|
| Total | 188 | | | |
| Sex | 1 | 34.96 | 29.25 | .005 |
| Maternal | 1 | 1.30 | 1.09 | - |
| Age | 1 | 0.33 | 0.27 | - |
| Light | 1 | 0.05 | 0.04 | - |
| Round | 1 | 5.00 | 4.18 | .05 |
| Trial | 2 | 2.05 | 1.71 | - |
| Error | 181 | 1.20 | | |

$R^2$ = 17.6%

Model:

Y = 0.74 - 0.44(female) + 0.44(male) - 0.13(U) - 0.13(F)
+ 0.10(young) - 0.10(old) + 0.2(white light) - 0.02(red light)
- 0.17(round 1) + 0.17(round 2) - 0.06(trial 1) + 0.22(trial 2)
- 0.16(trial 3)

C57 line; these differences were significant at the 0.5% level. A negative correlation was found between the residuals for ambulation and the residuals for defecation in both lines. In the A/J line this correlation was -0.41, and in the C57 line it was -0.19. This indicated that a part of the overall negative correlation between ambulation and defecation was at the level of the individual.

# DISCUSSION

Infant mice are quite helpless, and all mother infant interactions must be initiated by the approach of the female. Under natural conditions, the female is free to leave the nest at any time for any length of time. Indeed, from an evolutionary standpoint, the amount of time necessary for adequate maternal care must be balanced by the need for foraging. Thus maternal care, which necessarily involves mother-infant contact, must be accommodated with the regular daily cycle of activity. In an attempt to simulate more naturalistic conditions, the nest area for this experiment was a small, enclosed room and the larger wire mesh room contained food and water supplies. Superimposed on the study of maternal behavior was the experimental design. Two strains of mice known to differ in their behavioral measures, particularly those involving activity, were compared.

Thus discussion of maternal activity and rhythms must involve four levels of organization. They are: facets of behavior common to both lines of mice which can be related to behavior trends of the entire species; behavior patterns which differ between the two inbred lines and which may be construed to be rooted in underlying genetic differences; behavioral differences within an inbred line which have been induced by environmental factors, such as generation and rearing by a mother of the opposite line; and individual differences.

## Similarities between Lines

In the amount of time spent away from the nest each day (Table 2) and the average length of each trip (Table 9) mothers of both lines responded to the maturation of their litters in the same way. In both lines the time spent away from the litter steadily increased from day 1 to day 10; at the same time, mothers of both lines displayed increasing trip length with day. Grota and Ader (1969) reported a comparable trend in lactating rats. They found that the day after parturition, rat mothers spent 85% of the time with their litters; by day 10 the portion of the day with the litter was about 53%, and by day 17 the figure had fallen to 30%. In this study with mice, females spent a somewhat larger proportion of the time in the nest: 89% on day one declining to 76% on day 10. Grota and Ader (1969) further reported the same trend of increasing average trip length found in this study. They reported that the median duration of periods when the lactating female rat remained away from the litter was less than 3.0 minutes during the first week postpartum and 12.5 minutes during the third week. In this study, trip length increased from a day 1 average of 1.3 minutes to a day 10 average of 7.2 minutes; the overall average for the first week was 2.8 minutes.

Such findings cannot be interpreted as waning responsiveness to the young since maternal response in other measures such as retrieving

remain high throughout this period (Rosenblatt, 1969). Rosenblatt (1970) used the concept of synchrony to refer to the fact that the mother's behavior is adapted to the needs and behavioral capacities of the young and that the mother's behavior changes as the capacities of the young develop. He further stated that the relationship between suckling and maternal lactation is synchronized throughout the entire period of maternal care, that the synchrony is progressive with respect to the amount of suckling stimulation the mother receives and the amount of milk she produces, and apparently the nipples undergo a gradual change which enables them to withstand the more vigorous suckling of the young as they grow. Thus the amount of time a female spends nursing her young is not necessarily an accurate measure of how much milk they receive.

The increasing amount of time away from the nest would be expected because a greater food intake was necessary to provide increasing amounts of milk for a growing litter. This fails to account for any discretionary time, however. It assumes that the female is mainly engaged in maternal care when in the nest and consumatory behavior when outside the nest. This concept is supported by the work of Priestnall (1972) who reported a steady decline in nest attendance during the first two weeks of lactation in mice. The behavior most frequently observed in the nest was nursing, while eating was the most frequent out of nest activity. When food supplies

were placed over the nest, females spent significantly more time in the nest, although the number of observations recording nursing did not change.

Grota and Ader (1974) observed the behavior of lactating rats in a dual chambered cage for one minute periods at hourly intervals. They found that the probability of observing nursing when the mother and litter were together remained constant at about 87% throughout the period of lactation. The bulk of the other in nest observation consisted of manipulation of nesting material. Even though food was available in both chambers, almost all of the consumatory behavior occurred in the chamber away from the litter. When the female was away from the litter consumatory behavior was noted in 28% of the observations for the first week of lactation and 38% during the second week. Thus, eating was not the most prevalent behavior when the female was away from the nest. Other behaviors tabulated by Grota and Ader (1974) when the female was away from the litter included: activity, 52% of observations during week 1 of lactation and 19% in week 2; lying still in 27% of observations during week 1 and 56% during week 2. Thus, while the time spent with the litter reflected interaction with the litter, much of the time away from the litter could be considered discretionary, since it did not involve foraging behavior.

Results from studies by Ader and Grota are not precisely comparable to this one. Rats in their study were tested during the second

lactation, while mice in this study were tested during the first lactation. The implications of this difference are difficult to assess. Carlier and Noirot (1965) have reported that some of the improvement noted in retrieving behavior during the first lactation period was maintained during the second lactation period. However, Grota (1973) reported no significant difference in the amount of time spent with the litter for rats in first vs second parturitions.

The cage design of the two studies was quite different. Those used by Ader and Grota consisted of two identical lucite compartments, each containing food, water and nesting material. Placement of food should have little effect since nursing and eating tended to occur in separate compartments. Major differences were found in the environment of the nest area. The lucite cage was larger and the nest more visible than the small enclosed nest area of this study. Due to the size restrictions of the room, it could be expected that most general activity would occur away from the litter room, as in the study by Grota and Ader. However, it is probable that the incidence of lying still in the room away from the litter would be reduced, since no bedding material was available in that room and the litter area was more isolated. Such a shift could easily account for the greater amount of time spent in the litter room in this study. Another possibility is that the difference was a species difference.

Thus the overall trend in lactating females to increase the amount of time spent away from the litter as it matures can be construed as a reflection of the synchrony in the mother-yount interaction. However, the amount of time spent with the litter vs away from the litter is not solely determined by either the care requirements of the young or the nutritional requirements of the female; the amount of time spent in nonconsumatory activity and resting are potential sources of variation.

The time spent away from the nest was not distributed at random during the day; demonstrable circadian rhythms existed in both inbred lines. Ader and Grota (1970) reported that in lactating rats the female spent the greatest amount of time with its litter approximately midway through the period when the lights were on and the least amount of time with the litter during the period of darkness. In this study also the probability of finding the female away from the litter was greater during darkness, and the probability of finding the female with the litter was greater during the light period. However, the magnitude of these probabilities differed markedly between the two lines, which will be discussed later.

There were several similarities in the circadian rhythms between the two lines. Both tended to have two activity peaks per 24 hour interval. An activity peak in this study can be defined as a period of time when the lactating female had a higher than average

probability of being away from the nest room. The most prominant peak corresponded to lights off time; the highest probability of the female being away from the nest occurred between 2000 and 2200 in both lines (Figures 20-25). This agrees with the work of Evans (1971) in rats, who found the greatest output of activity to be associated with the onset of the dark phase of the light-dark cycle. The secondary peak of activity was less well defined both in magnitude and time scale, and differed between the two lines.

These data are consistent with the hypothesis that the timing of maternal care, as represented by the presence of the female in the nest, is a reflection of the circadian activity rhythm in rodents (Ader and Grota, 1970). It had been reported in rats that there is a strong tendency for all forms of activity, both regulatory such as eating and drinking, and nonregulatory, such as grooming and mating, to reach maxima and minima during the same half hour periods (Beagley and Gallistel, 1970). Thus the conclusion that maternal care replaced periods of inactivity is consistent with the functional grouping of activity into certain periods of the day. Furthermore, rhythms found in time away from the nest conform to observations concerning activity cycles in general. Lighting changes acted as phase setters with activity periods having a consistent relationship with light cycles. In mice, as would be expected of a nocturnal species, the initiation of activity bore a more constant relationship to cessation of light than

cessation of activity to light onset, as predicted by Hinde (1970).

## Differences between Lines

In an open field test, mice of the C57 line have consistently been reported to have much higher ambulation scores than the A/J line; in most tests there has been no overlap between scores of the two lines (Thompson, 1953, 1956; Goodrick, 1971). In addition, the lines have shown situational consistency in that the lines have had the same relative ranking in different tests designed to measure movement in novel situations (McClearn, 1959). These conclusions were supported by results in this study; the average C57 ambulation score was over 10 times greater than the A/J average (Tables 32 and 33).

Defecation scores measured concurrently with ambulation have generally been negatively correlated (Archer, 1973). In this study, the low ambulating A/J line had higher defecation scores and the high ambulating C57 line had lower defecation scores (Tables 34 and 35). A negative correlation between the two measures was also found within lines.

These two lines have also been found to differ in spontaneous wheelrunning activity, with the C57 line being the more active (Bruell, 1962, 1964b). Each of these observations indicates that innate differences in general level of activity probably exist between the lines.

Significant differences between the two lines were found in the amount of time spent away from the nest per day (Table 2). The less active A/J line spent over 1.2 hours more per day in the nest than the C57 line. It is likely that this reduction in time away from the nest reflected a decrease in nonconsumatory activity in the room away from the litter.

Differential light responses between these two lines were noted in the open field test. Under bright, white light members of the A/J line averaged only 2 squares entered, while under red light to simulate darkness the A/J line averaged 44.5 squares (Table 32). This is an increase of over 2000%. While the C57 line also showed a significant increase in ambulation under red light, the increase was from 315 squares to 353 squares, or only about 10%. Similar results for these two inbred lines have been reported previously (McClearn, 1960; Goodrick, 1971).

Some of this difference in light response is due to the fact that the A/J line is albino and the lack of eye pigmentation causes increased sensitivity to light while the C57 line is pigmented. However, the change to red light did not eliminate the strain differences. Other workers have concluded that albino inbred strains are not inactive in the open field solely because of differential sensitivity to light, but that genes at other loci are involved (DeFries, Hegmann and Weir, 1966; DeFries, 1969; DeFries and Hegmann, 1970).

Differences in the level of activity in light vs dark intervals were found in these two inbred lines. These can in part be interpreted on the basis of differential response to light. The pattern of activity shows the A/J line to be decidedly nocturnal. Time away from the nest occurred almost exclusively in darkness (Figure 11). By contrast, in the C57 line nearly 50% of the time away from the litter occurred in light (Figure 12). Since the lighting schedule was such that there were four more hours of light than dark per day, this percentage still reflects some nocturnal tendency, which would be expected in mice. However, it was clear that light did not have the same inhibitory effect in the C57 line as in the A/J line.

As in the open field test, the strong nocturnal tendency in the A/J line was not necessarily a consequence of only the albino gene. Low scores in the open field test have often been assumed to be an indicator of either fear or emotionality (Archer, 1973). It is possible that high fear states in open areas could be associated with the highly nocturnal daily rhythms in this line.

Two other behavior differences tended to support the theory that the A/J line was more timid than the C57 line. First, a difference in the type of nest built was noted. The nesting area contained sawdust and each female was also provided a facial tissue. Members of the C57 line tended to mat the tissue and nest and nurse on top of it. They were thus visible through the top of the litter room. In contrast,

members of the A/J line tended to shred the tissue and nest and nurse under the pile. They could not be seen when viewed through the top of the litter room. Secondly, these 2 lines have been reported to differ in aggressiveness, with the C57 line the more aggressive (Green, 1968).

The highly nocturnal nature of the A/J line vs the lesser influence of light in the C57 line was also reflected in the circadian activities of these inbred lines. In both lines an increase in time away from the litter was observed as the pups matured from infancy to day 10. In the A/J line the increased time away from the nest was added during the dark interval (Figures 20-22); in the C57 line the added time away from the litter was divided between light and dark intervals (Figures 23-25).

As stated previously, the primary peak of activity occurred between 2000 and 2200 hours in both lines. However, the relationship between cessation of light and onset of activity differed between the two lines. In the A/J line activity began abruptly at 2000 hours with the beginning of the dark phase (Figures 20-22). In the C57 line the onset of activity was more gradual, with portions of the hours preceeding lights off spent outside the nest area (Figures 23-25). This again indicates that a discrepancy in response to light existed between the lines. The A/J line apparently responded to darkness per se and did not leave the nest area until after darkness onset. The C57 line

apparently responded to the anticipation of the onset of darkness with increasing levels of activity until the absolute peak of activity occurred after darkness onset.

While each line displayed a smaller secondary peak of activity, the time at which it occurred was different. In the A/J line the increase in activity was generally noted between 0100 and 0500 hours; the activity increase was well contained in the darkness hours and the female had returned to the litter room by lights on at 0600. In the C57 line increased activity was noted sometime between 0400 and 0800. Whether the bulk of the activity occurred before or after light onset depended apparently upon the previous experiences of the female, but in all cases it was much closer to lights on time in the C57 line than in the A/J line.

The difference in nocturnal tendencies between the two inbred lines was to some degree independant of the circadian rhythm of activity. Much of the time away from the litter, particularly during the second week of lactation, occurred at times other than during activity peaks. We may then speak of general levels of activity apart from peaks of activity. In the C57 line, females tended to spend a portion of each hour outside the litter room, whether lights were on or off. The observation that a larger percentage of time was spent away from the litter during darkness was probably due to the activity peak at light cessation. In the A/J line, however, the general level

of activity was very high at night and very low during light.

Such a difference in the nocturnal tendencies and activity distribution necessarily reflected a difference in maternal care strategies. In the C57 line, there was a time balance of maternal presence in the nest and activity for each hour. In the A/J line, the bulk of the maternal care occurred in light intervals. During the dark interval, maternal attendance and care were minimal.

## Effects Attributable to Experimental Groups

The intent of this study was to compare maternal behavior between inbred mothers and crossfostered mothers and to partition total variation into that attributable to maternal effects and line effects. The variation due to line effects was expected to measure genetic differences between the two lines. The maternal effect was expected to measure the degree to which learning from the dam affected subsequent maternal behavior.

When 24 hour behavioral measures were analyzed, only 2 line differences were found. The C57 line spent more time away from the nest per day than the A/J line (Table 2). This was probably a reflection of either the higher activity level of the C57 line or the stimulus of larger litters in the C57 line. The other line difference was in the day to day pattern in number of trips (Table 6).

There was no indication in the fostered groups that their maternal behavior was more like that of their foster mother's line than their own genetic line, even though significant maternal and line x maternal line interaction effects were found (Table 2, Table 6, Table 9). In both lines the fostered mothers averaged less time away from the nest per day (Tables 4 and 5), a larger number of trips to and from the nest per day (Tables 7 and 8), and a shorter average trip length (Tables 10 and 11) than did unfostered members of their own line. These differences must be interpreted as an effect of the fostering procedure instead of an influence learned from the maternal line.

A fostering effect could be induced in the offspring either by the disruption of maternal care due to the agitated state of the female or by a difference in the maternal-young interaction when the offspring are fostered. Beach and Jaynes (1956a) reported that female rats appeared to discriminate between their own and alien young; alien young of the same age (6 days) were retrieved more slowly than the mother's own offspring.

Although no difference in ambulation or defecation between fostered and unfostered individuals were found in this study (Tables 32-35), some studies have reported that fostering can influence later behavior in the open field. The direction of the reported influence on response was not consistent. Hockman (1961) reported a decrease in

activity of prenatally stressed rats if they had been fostered, either to experimental mothers or to controls, but no decrease in activity was found if they were reared by their own mothers. Thompson, Watson and Charlesworth (1962) reported that crossfostering and fostering did interact significantly with prenatal treatments. Rearing by a foster mother of the same treatment group increased activity of both experimentals and controls at 30-40 days, but fostering increased the activity of experimentals and decreased that of controls at 130-140 days. Thus, it has been reported that the fostering procedure can affect later behavior, and apparently the maternal behavior of fostered individuals in this study was affected.

Comparisons between fostered and unfostered individuals were difficult because of the behavior differences between generations of unfostered individuals. This generation difference prevented characterization of normal behavior levels. In unfostered groups of both lines the first generation averaged more time away from the litter per day (Tables 4 and 5) and more trips away from the litter per day (Tables 7 and 8). In the C57 line, the larger number of trips was mainly a reflection of the greater amount of time away, because the trip length was the same for both generations (Table 10). In the A/J line, however, this was not the case and the trip length for the generation 1 group was much shorter than for generation 2 (Table 11).

Even though both generations were raised by dams of their own inbred line, the remainder of the early life stimuli were quite different. Besides the possible differences in environments in the two laboratories, the generation 1 group, at an age of approximately 8 weeks, underwent the stress of crating and air transport.

It has been shown that various forms of experimental stress can influence later life behavior, and several types of early stimulation tend to influence later behavior similarly (Denenberg, 1969; King, 1970; Williams and Russell, 1972). It has been suggested that the diverse types of early stimulation act by producing a common mediation effect which then produces the behavioral consequencies, and since stimulation affects responses to novel or fear producing situations, the response is likely to involve the adrenal-pituitary axis (Hinde, 1970). It has been established that the infant rat responds with rises in adrenal and plasma corticosterone to a variety of stressors, including the standard handling procedures used in many of these experiments (Hinde, 1970). It has also been suggested that handling stress causes variation in the concentration of adrenal steroids in the infant and that this in turn modifies the control system governing their secretion. Thus the concentration of steroids can vary in a graded manner in the adult (Levine and Mullins, 1966).

This generation effect, coupled with the fostering effect, in essence produced an experiment with three treatment groups:

Generation 1, unfostered; Generation 2, unfostered; and Generation 2, fostered. The similar environmental factors produced more extensive behavior modification in one inbred line than the other. In the A/J line the range between means for the three experimental groups was 1.4 hours per day away from the litter, 92 trips and 4.13 minutes per trip. In the C57 line the difference between the highest and lowest estimates of the three measures of behavior was 0.4 hours per day, 22 trips and 0.86 minutes per trip. In the C57 line the day effect accounted for a greater proportion of the total variation, and individual effects accounted for a lesser portion of the total variation than in the A/J line. Thus, the C57 line was apparently more phenotypically stable than the A/J line; variation in environment, both that common in a treatment and that peculiar to the individual did not modify behavior as much in the C57 as in the A/J line. The finding of significant differences between individuals conflicts with the study by Grota and Ader (1969) which reported a great deal of consistency in time away from the litter per day between rats.

In the three experimental groups, the second generation unfostered group would be expected to display a "normal" behavior pattern for a line since it underwent the least environmental change. This assertion would be based on the assumption that environmental fluctuations would act as a stressor, and many stressors tended to influence later behavior in the same direction.

However, there was no consistent ranking among the experimental groups in these three behavioral measures. Previous studies on the effect of prenatal stress have shown that these two lines differ in response, with the A/J line showing an increase and the C57 line showing a decrease in later open field ambulation (Thomspon and Olian, 1961). In that study, the behavior of the A/J line was affected to a greater degree than the C57 line.

The second generation unfostered group did appear to differ from the other two in that it was less nocturnal. This was true in both lines although the difference was more apparent in the C57 line. In both lines, a larger proportion of the time away from the litter occurred during the light in this group (Figure 17). In this group in the C57 line 58% of the time away from the litter occurred during light, which was the expected value if there were no nocturnal tendency. Secondly, in this group in both lines the average trip length was as long or longer during light intervals as dark intervals (Figure 19).

Activity cycles for the second generation unfostered groups differed from those of the other two groups. In the A/J line, this group showed a peak of activity during the light interval, between 0800 and 1100 (Figure 21), which was not present in the other groups (Figures 20 and 22). In the C57 line, the secondary peak of activity occurred prior to lights on, between 0400 and 0600 in the first generation unfostered (Figure 23) and fostered groups (Figure 25) but between

0600 and 0900, after lights on, in the second generation unfostered group (Figure 24). Also in this group, the general activity was higher during the hours of darkness than light. This was not observed in the other C57 groups.

## Individual Differences

Individual mothers were found to have repeatable differences in maternal behavior in both lines. Individual differences were more pronounced in the A/J line. Individual estimates in the A/J line for time out per day, number of trips and average trip length ranged from 2.4 to 8.1 hours, 32 to 273 trips and 0.4 to 15.8 minutes per trip respectively. Comparable values for the C57 line were 3.0 to 7.5 hours, 61 to 182 trips and 1.7 to 6.8 minutes per trip. Although part of the individual differences could be explained by differing litter characteristics, a significant portion remained unexplained.

Since both lines were highly inbred and therefore highly homogeneous, it can be assumed that the individual differences were induced by environmental factors. Likewise, from this study, it cannot be determined whether these represent permanent differences or differences maintained only for this lactation. Temporary but stable differences could arise from such factors as season effects and slight differences in age. Permanent differences could be induced by such factors as preweaning environment and size of the female. No

pertinent literature on the effect of such environmental factors on maternal behavior was found.

## Time Spent Away from the Nest as an Index of Maternal Care

Grota and Ader (1974) have reported that the amount of time a mother spent in the chamber or nest with her litter could be taken as a valid reflection of the maternal behavior. Support for this statement was derived from two lines of reasoning. The first was their finding that when the female was in the same cage as the offspring, she was probably nursing. The second line of evidence came from comparing patterns in other measures of maternal behavior, in particular those derived by Sietz (1958), with patterns of nest attendance.

Grota and Ader (1974) reported that the score for "quality of the nest" showed decreases over days which were comparable to changes in total time spent with the litter. However, other measures of maternal behavior such as defense of the young, initiation of nest building, and retrieving, showed an inconsistent relationship with nest attendance. In particular, the percentage of females that retrieved either the first pup presented or the entire litter within a three minute period remained high until day 15 then decreased rapidly.

All measures of maternal behavior have been found to depend on litter size. Sietz (1958) divised a maternal behavior index and tested rat mothers with litter sizes of 3, 6, 9, and 12; he reported that for each increase in litter size there was a corresponding stepwise decrease in maternal behavior. Grota (1973) reported that for the first 10 days of lactation, females with litters of four spent more time in the nest than females with litters of eight. Priestnall (1972) also reported that during the first two weeks of lactation, female mice rearing smaller litters recorded the highest nursing and nest attendance times.

The mother rearing a larger litter might be expected to spend more time eating because of the increased lactation demands. Priestnall (1972) did find that females raising larger litters did spend significantly more time eating and drinking and significantly less time engaged in grooming and resting than did females rearing smaller litters. When food and water were supplied over the nest, all females spent significantly more time in the nest, but females with two pups continued to spend significantly more time in the nest than females with litters of eight pups (Priestnall, 1973). Thus the increased time spent away from the nest of females raising larger litters was not necessarily a reflection of increased food requirements.

In this study, it was found that the A/J line, which had the smaller average litter size, also spent more time in the nest than the

C57 line. However, the between line difference in litter size may be a function of genetic difference in activity rather than the environmental influence of litter size altering activity. In a selection experiment for high and low wheelrunning in rats, after seven generations of selection the reduction of litter size and percent bred in the inactive group was apparent (Rundquist, 1933), and the inactive strain could not be continued after the 25 generation due to the lack of offspring (Brody, 1942). Griesel (1964) also reported that fewer inactive mothers bore litters and that their litters were significantly smaller.

In the A/J line it was found that mothers rearing larger, heavier litters spent more time away from the nest (Table 16). The C57 line was not consistent: larger litters were associated with a slight decrease in time away from the litter per day, while heavier litters were associated with increased time away from the litter per day (Table 20).

Thus the amount of time a mother spends with the litter may be positively associated with other maternal behavior measures and may reflect the amount of time spent nursing, but it is not a stable indicator of maternal productivity. In this study the relationship between nest attendance and litter variables was not consistent between lines. Furthermore, the amount of time spent in the nest per day was highly influenced by previous environmental influences of the female, as evidenced by significant fostering and generation effects

which remained after correction for litter size and mass. Even after correction for litter and treatment effects, significant repeatable individual effects remained. Thus the amount of time a female spent with the litter reflected previous environmental conditions and individuality as well as the level of stimulation from the litter.

# SUMMARY AND CONCLUSIONS

In order to study maternal behavior in mice, a cage of two compartments, a nest area and a larger wire cage, was designed such that the room location of the subject could be continuously recorded. The female was placed in the cage several days before littering; her movement was recorded for 10 days after parturition. The subjects belonged to two inbred lines, A/J and C57BL/6J. Within each inbred line three groups of subjects were tested: the original generation, their unfostered offspring, and their offspring which had been cross-fostered to females of the opposite inbred line.

The continuous recording of the room location of the female was divided into time intervals and within each time interval three measures of behavior were computed: time away from the litter, number of trips and average trip length.

When the time interval considered was 24 hours, trends in maternal behavior could be examined. In both lines, the time spent away from the litter increased almost linearly with day after parturition (b = 25 minutes per day). Mothers of the C57 line spent about 1.1 hours per day more away from the nest than mothers of the A/J line. This was a reflection of either the higher activity level, the larger average litter sizes of the C57 line or both. The average trip length also increased in a linear fashion with day; the average trip

length for day 1 was 1.3 minutes and for day 10 it was 7.2 minutes. No difference between lines was found. The day to day patterns for number of trips differed between the inbred lines. In the C57 line the number of trips showed a decrease with day, while in the A/J line an initial increase was followed by a decrease to day 1 levels.

In the C57 line a larger proportion of the variation was attributable to day effect, and a lesser portion was attributable to experimental group and individual effects than in the A/J line. The environmental factors common to the experimental groups were similar in both lines, but more extensive behavior modification was produced in the A/J line, as evidenced by the larger estimated differences.

There was no indication in the fostered females that their maternal behavior was more similar to that of their foster mother's line than their own genetic line. Instead, examination of the line x maternal line interaction indicated the presence of a fostering effect. In the A/J line, fostered individuals spent less time away from the nest, made more trips away from the nest and had a shorter average trip length. In the C57 line the fostered group made significantly more trips per day.

Differences between the two generations were found in both lines. In both lines, members of generation 1 were found to average more time per day away from the nest and more trips per day. In the C57 line these two differences were proportional such that the average trip

length was about the same in both generations, 3.8 minutes. In the A/J line this was not the case; the first generation had an average trip length of 1.7 minutes, while the second generation averaged 5.8 minutes.

A striking difference between the two lines was found in activity relative to the light-dark cycles. In the A/J line, only 13% of the time away from the nest occurred in light, while in the C57 line 47% of the time away from the litter occurred during the 14 hours of light per day. While the circadian rhythms of both lines were characterized by a prominant peak of activity stimulated by darkness onset, the stimulus strength apparently differed between the lines. In the A/J line, activity began abruptly after darkness onset; in the C57 line activity increases were noted in the hours prior to lights off, with the peak after darkness onset. This differential sensitivity to light was also noted in open field ambulation scores. The change from white to red light resulted in a greater percentage increase in A/J scores than C57 scores.

Differences in the experimental groups in circadian patterns were also noted, with the second generation unfostered group showing the least nocturnal tendency in each line. This is interesting since the second generation unfostered group experienced the most constant pre and post weaning environment. The difference between groups is particularly pronounced in the C57 line. The second generation

unfostered group spent a larger proportion of the time away from the litter during light than the other two groups. Also, the average trip length was longer during light than dark, while the reverse was true in the other two groups. In the C57 generation 2 unfostered group the secondary peak of activity occurred prior to light onset and the level of general activity was the same or lower in light than dark. The most apparent manifestation of decreased nocturnal tendency in the A/J second generation unfostered group was a third peak of activity occurring in light between 0800 and 1000 hours, which was not present in the other groups.

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