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Variations in maternal care in the rat as a mediating influence for the effects of environment on development

Frances A. Champagne, Darlene D. Francis, Adam Mar, Michael J. Meaney*

Developmental Neuroendocrinology Laboratory, Douglas Hospital Research Centre, McGill University, 6875 LaSalle Boulevard, Montreal, Quebec, Canada H4H 1R3

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Abstract

Variations in maternal care have been widely considered as a critical influence in development. In the rat, variations in maternal behavior, particularly in licking/grooming, regulate the development of endocrine, emotional and cognitive responses to stress. These studies form the basis of a potentially useful model for the study of maternal effects in mammals. In this paper we provide a detailed methodological investigation into this model of maternal behavior, providing an analysis of the frequency, temporal dynamics, and transmission of maternal licking/grooming in several large cohorts. Frequency data indicate that licking/grooming is normally distributed across dams. The peak in licking/grooming occurs in the first few days postpartum and gradually declines. Dams designated as High or Low LG mothers differ in this behavior only during the first week postpartum. Observations over Days 2 to 5 postpartum are essential for the reliable assessments of individual differences in maternal behavior. Individual differences in licking/grooming behavior are stable across multiple litters, and are not associated with differences in litter size, weaning weight of pups, or gender ratio of the litter. We also observed no significant differences in the amount of licking/grooming received by individual pups within a litter, though variation does exist. Finally, maternal licking/grooming is transmitted to female offspring, though there is considerable within-litter variation in the expression of this behavior. Overall, these findings indicate considerable, normal variations in licking/grooming in the rat that are a stable, individual characteristic of rat dams.

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Keywords: Maternal behavior; Rat; Individual differences; Maternal effects

1. Introduction

Despite the common assumption in both psychology and biology that parental behavior exerts a pervasive influence on phenotype, the ability to clearly establish such effects has been troublesome. The unambiguous demonstration of parental effects in mammals is thwarted by the absence of experimental models that can reliably distinguish parental effects that are nongenomic in nature from those associated with genetic transmission. The issues here concern the transmission of traits from parents to offspring. Ideally, we need to effectively describe the similarity between parent and offspring in the expression of a given trait, as well as the mechanism of transmission.

In mammalian species, experimental evidence for the role of maternal care is commonly derived from deprivation studies. Thus, in both primates and rodents, infants deprived of maternal care for extended periods of time exhibit dramatically increased fearfulness, inappropriate and often excessively aggressive patterns of social behavior, and impaired cognitive development (e.g., Refs. [1–4]). The decreased mother–infant contact resulting from extended periods of maternal separation seems likely to be critical for the effects on development. But does this imply that under normal conditions maternal care actively contributes to the development of specific neural systems, or simply that the absence of the mother is so disruptive to pup physiology that it affects the development of these systems? If maternal care is indeed critical under normal conditions, then what are the relevant features of mother–pup interactions, and how do they influence neural development? Are variations in maternal behavior within a normal range a relevant source of individual differences in development? Could such variations serve as

* Corresponding author. Tel.: +1-514-761-6131; fax: +1-514-762-3034.

E-mail address: michael.meaney@mcgill.ca (M.J. Meaney).

the basis for a nongenomic transmission of traits from parent to offspring?

One obvious approach to such questions is to simply examine the relationship between variations in maternal behavior under normal conditions and development of specific traits in the offspring. Indeed, within developmental psychology parental care has often been considered as a major influence over the development of the brain and behavior [4,5]. In humans, however, evidence for the importance of parental care is often compromised by the inherently correlational nature of studies in child development. Similar issues prevail in the primate literature, where large-scale cross-fostering studies that could define the influence of infant-directed behaviors at a particular time in development are extremely difficult. The answer would seem to lie in the use of species with a shorter life span, where the potential consequences of variation in parent–offspring interactions could be examined within a far shorter time period. However, with the exception of the primate literature, maternal behavior in mammalian species has often been characterized as the stereotyped, invariant or “innate” behavior patterns in that emerge from the combination of endocrine signals associated with the later stages of pregnancy and the stimulus properties of the young. The assumption has been that animals are either maternal or not, and such conditions are defined by endocrine events and the presence of young. Indeed such endocrine signals are of unquestionable importance in triggering the onset of maternal behavior, and for many species they are obligatory events [6–10]. Likewise, stimuli derived from the neonates are a necessary feature of the conditions that support the expression of maternal care [7,10]. But do these findings necessarily imply invariance?

Reports of individual differences in maternal behavior in several species would seem to contradict the idea that maternal behavior is necessarily the inevitable consequence of gestational hormones in combination with the presence of the young. In old world monkeys, for example, individual differences in maternal behavior are stable and are commonly transmitted from mother to daughter [11,12]. However, while nonhuman primates offer a fascinating opportunity for the study of behavioral development, invasive studies that clearly establish causal relationships or the opportunity to study underlying mechanism are usually very difficult or virtually impossible. The question is whether we can readily identify reliable individual differences in a nonhuman species in which both experimental manipulations of rearing conditions and studies of mechanism are feasible.

In the studies reported here, we have expanded on the results of an earlier report of Myers et al. [13] describing naturally occurring variations in maternal behavior in the Long–Evans rats over the first week postpartum. The focus of both the previous and current report is on licking/grooming and arched-back nursing, two behaviors implicated in many aspects of developmental regulation in the rat (e.g., Refs. [13,14]). As with the primates, such individual differences in maternal behavior are stable across litters and are reliably

transmitted from mother to female offspring. We think that this model will prove useful in the study of the influence of maternal care on neural and behavioral development, in a species whose life span permits more definitive longitudinal analysis as well as greater potential for intervention. Finally, the development of such models also permits studies on the functional importance of parental effects on phenotype.

2. General methods

2.1. Subjects

Adult, Long–Evans rats derived from animals obtained from Charles River Canada (St. Constant, Quebec) were used in these studies. For most studies, the adult females used to characterize maternal behavior were born in our colony. However, in other cases, adult females were obtained directly from the breeder. These animals also served to replenish the colony. For all studies, females and their litters were the only animals housed in the colony room. Food and water was permitted ad libitum, and the animals were maintained on a 12:12 light/dark schedule with lights on at 0800 h. Litters were left intact, and on Day 10, following observations, the litters were counted and sexed. Otherwise, animals were left undisturbed. Cage maintenance was performed every 3 days beginning only on Day 11. Animal care staff independently determined that air quality (i.e., ammonia levels) were maintained within an acceptable range for all animals in the colony.

The females were mated with adult males drawn at random from a group of “stud” males. Stud males were placed, one per cage, with two females for 7–10 consecutive days. This procedure produced a 90–95% success rate for mating. Following mating, the females were individually housed in maternity cages (46 × 30 × 30 cm) that permitted a clear view from all sides. No litters of <5 pups were included in the study.

2.2. Observations of maternal behavior

We examined maternal behavior using a version of the procedure described by Myers et al. [13]. The behavior of

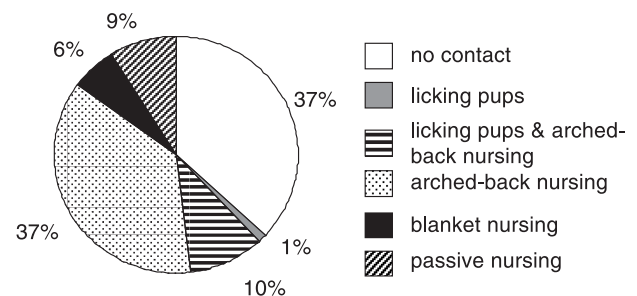


Fig. 1. Illustration of cumulative time Long–Evans rat dams spent engaging in each category of observed maternal behavior.

each dam was observed for five, 72-min observation periods daily for the first 6–8 days postpartum, depending upon the study. Observations occurred at regular times each day with three periods during the light phase (1000, 1300, 1700 h) and two periods during the dark phase of the light/dark cycle (0600 and 2100 h). The distribution of the observations was

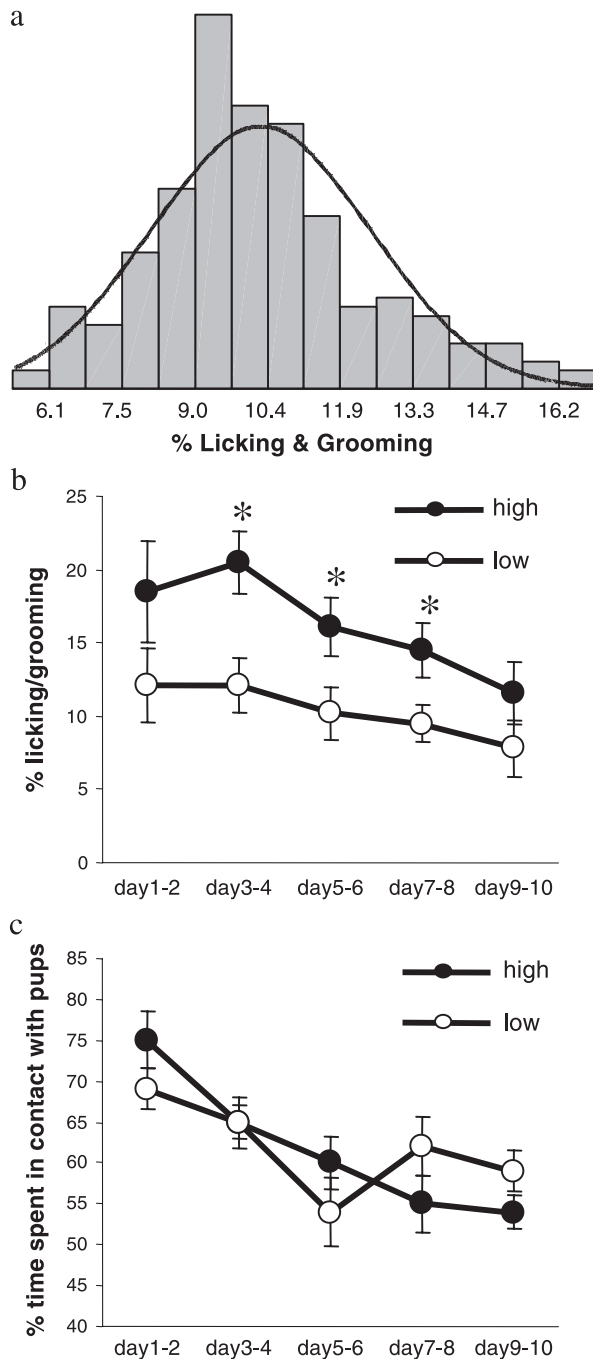


Fig. 2. (a) Frequency distribution of cumulative licking/grooming during the first 6 days postpartum. Superimposed is a computer-generated normal distribution. (b) Mean \pm S.E.M. frequency of licking/grooming in High and Low LG dams across the first 10 days postpartum. (c) Mean \pm S.E.M. percentage of observations in which the mother was in contact with pups in High and Low LG dams over the first 10 days postpartum.

based on the finding that nursing in rats occurs more frequently during the light phase of the cycle. Within each observation period the behavior of each mother was scored every 3 min (25 observations/period \times 5 periods per day = 125 observations/mother/day). Each observation period begins with animals observed, in sequence, and with the observer recording the ongoing behavior at the instant of the observation. The following behaviors were scored: (1) mother off pups, (2) mother licking and grooming any pup, (3) mother nursing pups in either an arched-back posture, (4) a “blanket” posture in which the mother lays over the pups, or a (5) passive posture in which the mother is lying either on her back or side while the pups nurse. A detailed description of these behaviors is provided in Myers et al. [13]. Note, behavioral categories are not mutually exclusive. For, example, licking and grooming often occurred while the mother was nursing the pups. The frequency of time dams were observed engaging in each of these categories of behavior is illustrated in Fig. 1.

The selection of animals as High or Low licking/grooming–arched-back nursing (LG–ABN) mothers is based on the mean and standard deviation for these measures for the maternal cohort (Fig. 2a). The characterization of individual mothers thus depends upon the reliability of the cohort data set. To provide more reliable estimates of individual differences in maternal behavior we observe cohorts of approximately 40 mothers/litters. The size of this cohort is also determined by the observational procedure. One individual can accurately observe the behavior of as many as 40 animals with sequential observations within a 3-min time span. Larger cohorts become more challenging with the same protocol. High licking/grooming mothers were defined as females whose frequency scores for both licking/grooming and arched-back nursing were greater than 1 S.D. above the mean. Low licking/grooming mothers were defined as females whose frequency scores for both licking/grooming and arched-back nursing were greater than 1 S.D. below the mean.

3. Results

3.1. Naturally occurring variations in maternal behavior

3.1.1. Frequency distribution

Lactating female rats differ in their frequency of licking/grooming and arched-back nursing of pups over the first 6–8 days postpartum (Fig. 2a). We first examined the frequency distribution for these behaviors to determine whether mothers which exhibit high or low levels of these behaviors form distinct populations, or simply lie at opposite ends on a single, continuous population. To examine this question 115 females were mated, allowed to give birth and maternal behavior was observed over the first 10 days postpartum. The animals were pooled over six different breeding cohorts, with 25–35 females per cohort.

The behavioral data for each female were analyzed as a percentage of the total number of observations for that female over the entire observation period. Percentage scores were used to account for occasional disruptions of observations resulting in a slightly unequal number of observations per day. The focus here was on licking/grooming and arched-back nursing, however, because of the close correlation between the two behaviors, we present only the data for licking/grooming.

The mean percentage frequency for licking/grooming over the entire sample was 10.6% with scores ranging from 4.9% to 19.7% (greater than a fourfold degree of variation). A normal probability density function was estimated based on the computed mean and S.D. and is superimposed on the actual histogram in Fig. 2a. A Kolmogorov–Smirnov test indicated that the observed distribution of licking/grooming scores did not differ significantly from a normal distribution. This unimodal distribution is not skewed or kurtotic. There were also no differences in the distribution of these scores as a function of the source of the animals (colony vs. commercial breeder: $t=0.24$, $df=336$, $P<.81$; Table 1).

We then selected samples of High and Low LG–ABN mothers based on the mean and standard deviation of both the licking/grooming and arched-back nursing scores for the respective cohort. High and Low LG–ABN mothers were defined as animals whose scores on both behaviors fell 1 S.D. above (High) or below (Low) the mean. ANOVA indicated a significant effect of group (High vs. Low, $F=110.01$, $P<.001$) and a main effect of day ($F=7.04$, $P<.001$) on licking/grooming. A representative data set for licking/grooming is provided in Fig. 2b and, as previously reported [15], shows that licking/grooming was significantly higher in High LG–ABN mothers for the first 3–8 days of lactation ($P<.01$), but not on Days 9–10.

High and Low LG–ABN mothers do not differ in contact time with pups. The data from these same animals was then analyzed for total time in contact with pups to ensure that differences in licking/grooming did not emerge simply because some mothers spent more time actively interacting with their pups. Contact was defined as any behavior that involved physical contact or close proximity to pups and almost invariably implied nursing and/or licking/grooming. As seen in Fig. 2c, there was no difference in the percentage

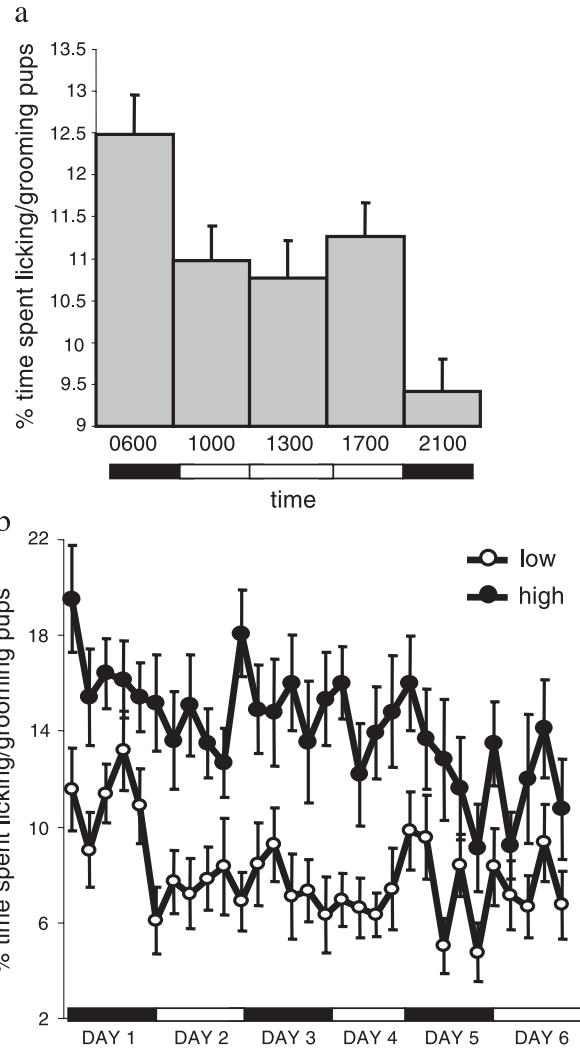


Fig. 3. (a) Mean \pm S.E.M. frequency of licking/grooming at different times across the light/dark cycle (Note: lights on at 0800 h, lights off at 2000 h) for all animals. (b) Mean \pm S.E.M. frequency of licking/grooming in High and Low LG dams across 30 observation sessions (5 per day for 6 days).

of total observations in which High or Low LG–ABN mothers were in contact with their pups. Thus, the data presented in Fig. 2b are unchanged if licking/grooming scores are presented as a function of percent of total observations in which mothers were in contact with pups, instead of simply total observations.

3.1.2. Licking/grooming: variability within days

The frequency of licking/grooming and arched-back nursing decreases over the first week postpartum. In addition to this variability over time, these behaviors show considerable circadian variation. Overall, this licking/grooming occurs most frequently during the 0600 dark cycle observation just prior to the beginning of the light cycle (Fig. 3a). The difference in magnitude between High and Low LG dams is at its peak during the 0600 observation and decreases over the course of the day (Fig. 3a). There is an additional peak just before the dark cycle begins. Despite

Table 1

Mean, S.D. and range of licking/grooming scores derived from females reared at the Charles River Facility vs. the Douglas Hospital Facility indicating no differences in the distribution of this behavior as a function of source

	Females reared at Charles River Facility	Females reared at Douglas Hospital Facility
<i>n</i>	169	169
mean % LG	10.88	10.81
S.D.	3.17	2.01
range	13.8	13.3
minimum	4.9	5.6
maximum	19.7	18.9

the combination of circadian variability, and decrease in licking/grooming over consecutive days (see Fig. 3b), High and Low LG–ABN dams remain distinct groups at most of the observation time points, particularly those from Days 2 to 4 postpartum.

3.1.3. Licking/grooming: frequency vs. duration

The scores presented must be considered as the percentage of observations in which mothers were observed to be licking/grooming pups. These scores do not necessarily reflect either the number of licking/grooming bouts or the length of these bouts, but emerge as a function of both frequency and duration. To assess group differences in the duration of single bouts of licking/grooming, individual High and Low LG–ABN mothers ($n=8/\text{group}$) were observed continuously for 1 h between 0900 and 1300 on Day 5 of lactation. The observer noted the onset and offset of each individual bout of pup licking/grooming providing a direct measure of duration. Overall, bout lengths ranged from 2 s to over 4 min. The data in Fig. 4a show that there were significant group differences in the mean duration, with bouts of High LG–ABN mothers persisting for more than four times as long as those for Low LG–ABN dams ($P<.001$). It is noteworthy, that none of the Low LG–ABN dams were observed to engage in a bout greater than 60 s in duration. In addition, High LG–ABN dams licked more frequently than Low LG–ABN dams ($P<.01$, Fig. 4b). High LG–ABN dams were observed to engage in both long (>1 min) and short (<1 min) duration bouts.

3.2. Stability of individual differences in maternal behavior over time

To examine the stability of the differences in maternal behavior, the behavior of a cohort of animals ultimately defined as High or Low was examined across the first 8 days of lactation (Fig. 2b). The behavior of the mothers within

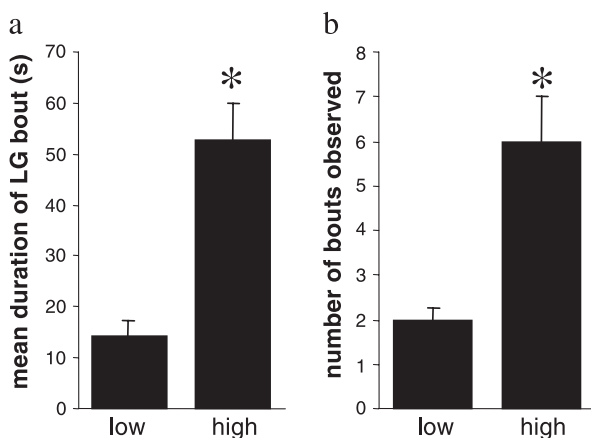


Fig. 4. (a) Mean \pm S.E.M. duration and (b) frequency of licking/grooming bouts in High and Low LG dams measured during a 1-h focal observation sample on postpartum Day 5 ($n=8/\text{group}$).

Table 2

Correlations between cumulative Day 8 licking/grooming scores and cumulative Days 1–7 scores indicated that Day 6 cumulative scores predict Day 8 scores with a high degree of accuracy

Consecutive days of observation	Pearson correlation with total LG after 8 consecutive days
1	.60
2	.68
3	.74
4	.86
5	.89
6	.97
7	.98

the first day following birth was highly variable, but hereafter differences between mothers were stable, suggesting that the group differences are expressed somewhat uniformly across days over the first week of lactation.

The stability of the individual differences in maternal behaviour over time suggests a certain measure of redundancy in the consecutive days of observations. We thus examined the degree to which shorter periods of observation predicted the results obtained with the full observational schedule. The results (Table 2) suggest that data obtained after six consecutive days of observations is sufficient to reliably predict behavior of the mother across the first 8 days of lactation.

3.3. Growth and survival of the offspring of High and Low LG–ABN mothers

The male and female offspring of High and Low LG–ABN mothers did not differ in weaning weights, taken on Day 22 of life. For Low LG–ABN litters, mean pup weight was 48.7 g (S.E.M. \pm 2.49) and for High LG–ABN litters, mean pup weight was 47.8 g (S.E.M. \pm 2.5). There was a trend for female offspring to weigh less than males, regardless of maternal status. This very basic indication of growth and survival of the litter, suggests that gross measures of reproductive success do not differentiate High and Low LG mothers.

3.4. Effect of litter size and gender composition

We then examined the frequency of licking/grooming and arched-back nursing as a function of the size and gender composition of the litter. Litter size was estimated as the number of pups counted at Day 10 of age. Gender composition was established at the same time as the simple ratio of males/females. Across a wide range in litter size (5 to 18 pups) there was no significant correlation between size of litter and either licking/grooming ($r=.06$) or arched-back nursing ($r=.09$). Likewise, gender composition, which ranged from 0.33 to 2.75, was not significantly correlated with either licking/grooming ($r=.07$) or arched-back nursing ($r=.23$). These findings suggest that neither the size nor

gender composition of the litter significantly influences the frequency of maternal licking grooming or arched-back nursing.

3.5. Licking/grooming directed towards individual pups

Lactating female rats show stable individual differences in pup licking/grooming and arched-back nursing. Such differences in maternal behavior are apparently independent of litter size. Nevertheless, it is not clear whether all pups under the care of a High LG–ABN mother receive a comparable level of licking/grooming. This is an important issue, since previous studies of the offspring of High and Low LG–ABN mothers reveal a surprising level of within-litter variability on behavioral and neuroendocrine measures (and see below). While such variations are unlikely to be associated with differences in the nursing posture, since pups are usually nursed as a group, differences in behaviors directed towards individual pups could be a factor influencing such within-litter variability. In this study we examined licking/grooming in 20 litters of 12 pups, in which 4 of the pups were individually marked.

Within 12 h of birth, all litters were culled to 12 pups. Litters with less than 12 pups were not used in the study to avoid cross-fostering effects. At this time four pups in each litter were marked with Codman pens (Johnson and Johnson, Brunswick, NJ) as follows: (1) marked on the left hind leg, (2) marked on the right hind leg, (3) marked on both hind legs, (4) marked on the tail. The markings were renewed on Day 5. Behavioral observations were performed as described above and the licking/grooming directed towards marked vs. unmarked pups was recorded, and this included noting the individual marked pup. If the target pup could not be identified, the observation was left unscored.

The data were analyzed over the first 8 days postpartum and are presented for each of the 20 litters. We first calculated the amount of licking/grooming directed towards the four marked pups by comparison to unmarked peers. The results (Fig. 5a) show that the observed licking/grooming to marked pups did not differ significantly from that of unmarked pups over all 20 litters ($t=0.6$, $P=.55$). Thus, it seems reasonable to conclude that the pen markings had no effect on maternal licking/grooming. Although there was considerable variability among the marked pups, there was generally a correspondence between the amount of “expected” (based on equal licking towards each pup) and the actual licking/grooming received by each pup. As illustrated in Fig. 5b, in 70% of the observed litters, actual licking deviated by less than 2% from expected licking. Since there is no meaningful way to evaluate how much variation is biologically significant, we present these data largely as a descriptive effort to provide the reader with an impression of the variation within litters in maternal licking/grooming. It is our impression that there is rather little evidence for a systematic bias in licking/grooming towards any individual pup on the part of the mother.

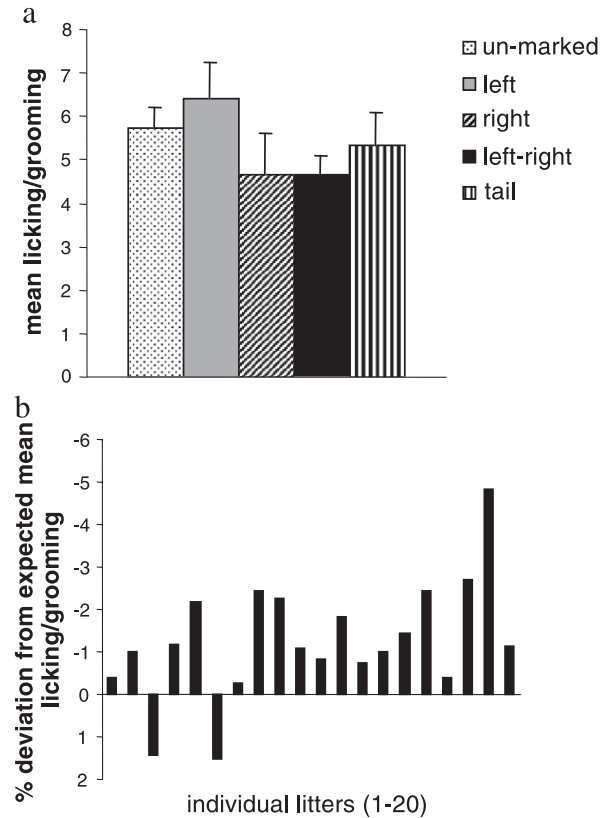


Fig. 5. (a) Mean \pm S.E.M. licking/grooming toward individual marked (left, right, left–right, tail) and unmarked pups in litter (b) Licking/grooming toward individual pups expressed as deviation from expected licking/grooming predicted to be received.

In addition to assessing the amount of licking received by marked and unmarked pups, we were able to measure frequency of licking toward male and female pups within the marked pup group for each litter. As is illustrated in Fig. 6, there were no gender differences in licking/grooming received during the first 8 days postpartum ($t=1.337$, $P<.2$).

3.6. Stability across litters

The results of section 3.1 as well as earlier reports suggest considerable variation in maternal behavior in the rat. In other species, notably in primates, maternal behavior has been reported to change with parity. Typically, females become more competent with experience in rearing offspring to weaning. In this study we examined the stability of the variations in maternal behavior as a function of parity.

The animals were selected and bred as described above. Following behavioral observations, the mothers were permitted to rear their litter to weaning (Day 21). Three weeks later the animals were again bred and allowed to rear a second litter to weaning. A subset of the mothers were then mated a third time to make comparisons across three litters. Behavioral observations were performed as described above.

Analysis of the maternal behavior revealed a highly significant correlation across the first two litters for pup

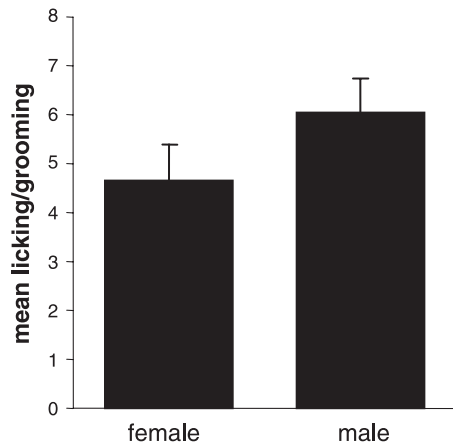


Fig. 6. Mean \pm S.E.M. licking/grooming toward individual male and female pups during the first 6 days postpartum (group difference is not significant).

licking/grooming ($r=.80$; $P<.0001$, Fig. 7a). As apparent in the scattergram, there were outlying females, and it should not be assumed that continuity is without exception. Nevertheless, the stability of the individual differences in maternal licking/grooming was also apparent when we examined maternal behavior across litters 2 and 3 ($r=.87$; $P<.001$; Fig. 7b) as well as from litter 1 to litter 3 ($r=.72$; $P<.01$, see Fig. 7c). Further analysis of litter 1 vs. litter 2 data indicated that there was no differences in licking/grooming scores between primiparous and multiparous females. Taken together, the data suggest a considerable degree of stability in the naturally occurring individual differences in maternal behavior and that the absolute frequency of licking/grooming across mothers is not influenced by parity.

The issue of stability of maternal behavior is also a methodological concern in cross-fostering studies. To determine the impact of this manipulation of licking/grooming, we characterized 10 High and 10 Low LG dams, rebred these dams 2 weeks postweaning, and cross fostered four pups from each litter within 12 h of birth. Maternal behavior was observed until Day 6 postpartum. Analysis indicated no difference in licking/grooming as a function of whether dams were given pups from High vs. Low dams ($t=0.66$, $P=.55$). There were also no observed changes in maternal licking/grooming between litters 1 and 2, suggesting that cross fostering has no effect on licking/grooming ($t=0.46$, $P=.64$).

3.7. Intergenerational transmission

Individual differences in maternal behavior are reliably transmitted from mother to her female offspring [16–18]. Thus, as adults, the female offspring of High LG–ABN mothers exhibit significantly higher levels of licking/grooming than do the offspring of Low LG–ABN dams. Moreover, the results of a cross-fostering study revealed that the maternal behavior of the biological offspring of Low LG–ABN mothers reared by High LG–ABN mothers was

indistinguishable from that of the normal offspring High LG–ABN females [17]. The reverse was also true, such that the maternal behavior of the biological offspring of High LG–ABN mothers reared by Low LG–ABN mothers was indistinguishable from that of the normal offspring Low LG–ABN females.

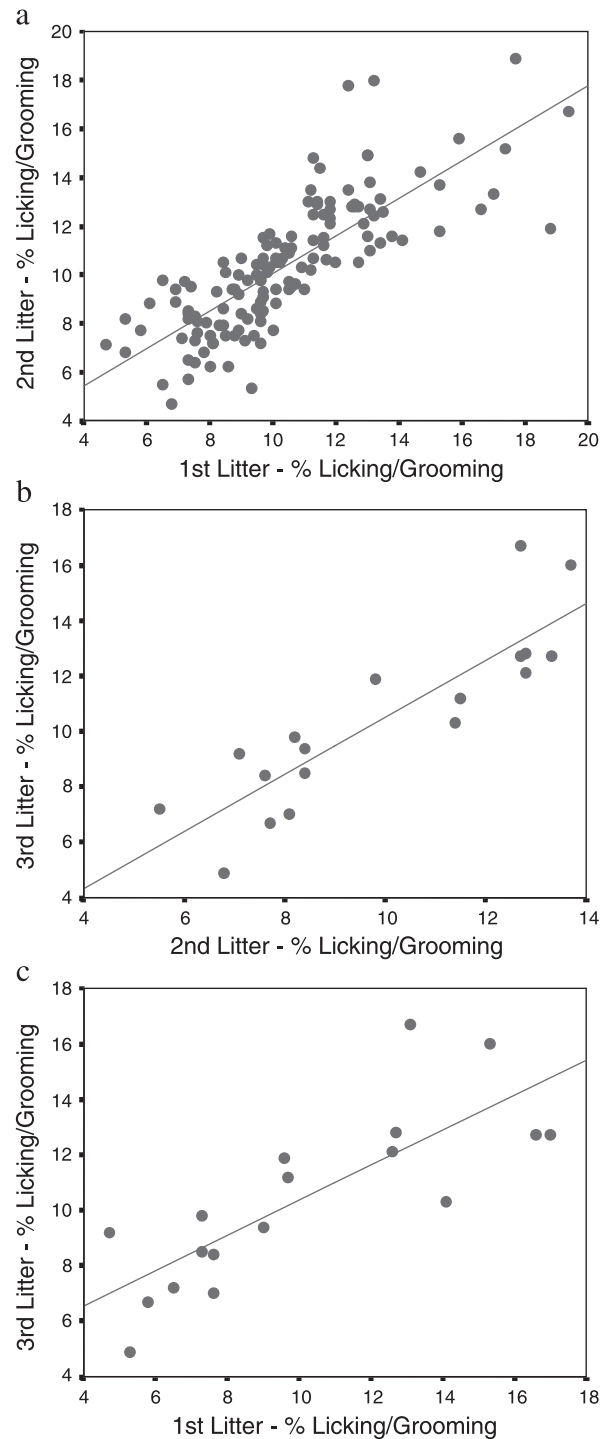


Fig. 7. (a) Scattergram of licking/grooming of individual mothers toward first and second litters ($r=.80$, $P<.0001$), (b) second and third litters ($r=.87$, $P<.001$), and (c) first and third litters ($r=.72$, $P<.01$).

The results of these studies suggest that individual differences in maternal behavior can be transmitted through a behavioral mode of transmission. In the current study, we replicated the early findings with a focus on the reliability of such effects at the level of individual female offspring within litters. The female offspring 20 Low, 20 Mid, and 20 High LG–ABN mothers were mated and allowed to give birth. There were at least three female offspring mated per litter (range 3–10). Females were then observed with their pups over the first 8 days postpartum as described above.

The female offspring of High LG–ABN mothers exhibited significantly higher levels of pup licking/grooming than did the female offspring of Low and Mid LG–ABN mothers ($F=27.29$, $P<.001$, Fig. 8a), replicating our pre-

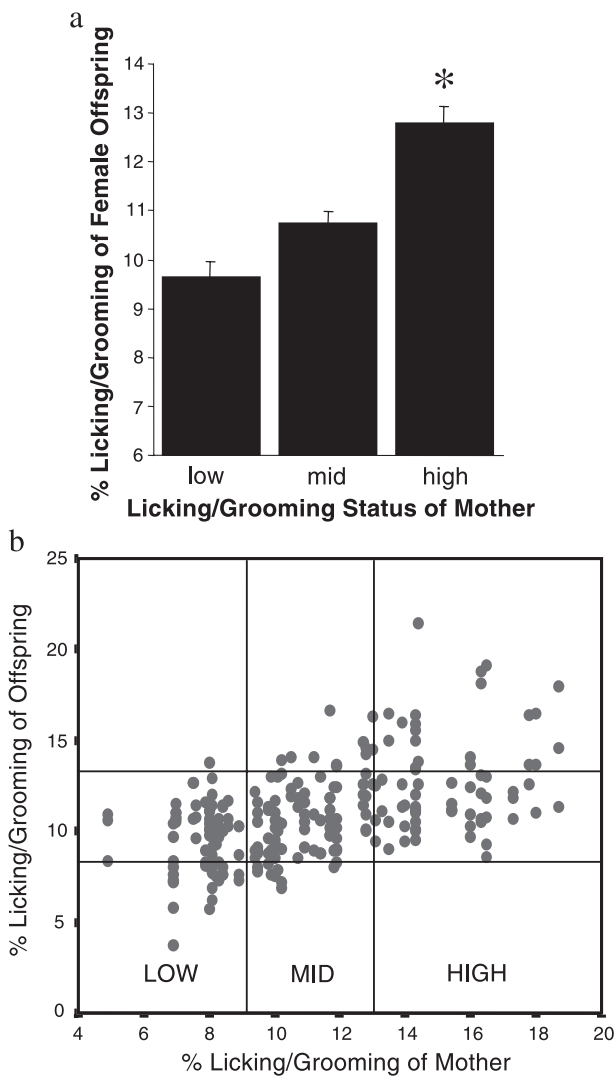


Fig. 8. (a) Mean ± S.E.M. licking/grooming of adult female offspring of High, Mid, and Low LG dams. (b) Scatterplot illustrating the relationship between the mean frequency of licking/grooming of mothers over the first 8 days postpartum and that of individual female offspring. The horizontal lines depict the mean ± 1 S.D. for the entire cohort of offspring. Values falling above the upper line indicate High LG females, while those falling below the lower line indicate Low LG mothers.

Table 3
Classification of the female offspring of High, Mid, and Low LG dams based on maternal behavior of the offspring (%)

Licking/Grooming status of offspring			
Low	Mid	High	
37.7	61	1.3	Low
26.1	58.7	15.2	Mid
1.3	61	38.7	High

vious finding [17]. In Fig. 8b we have plotted the scores for individual females within each of the 60 litters. Consistent with the overall statistical analysis, note that the mean score for each litter derived from High LG–ABN mothers is higher than that of any of the litters from Low LG–ABN mothers, and there is a significant linear correlation between mother and offspring licking/grooming scores ($r=.53$, $P<.001$). Nevertheless, there is considerable variation, even within individual litters. We then developed the mean and standard deviation for the entire population and classified the female offspring as High (scores > mean + 1 S.D.) or Low (scores < mean – 1 S.D.). Table 3 shows the results of this classification. While it was rare for a female offspring of a High LG–ABN mother to emerge as a Low LG–ABN mother (1/74) or the female offspring of Low LG–ABN mothers to become High LG–ABN dams (1/78), it was not uncommon for female offspring from either group to be classified as “Mid” (i.e., females whose licking/grooming scores fell within the mean ± 1 S.D.). While the transmission of individual differences is clearly a reliable phenomenon, there is considerable variation that likely reflects the importance of the postweaning environment.

4. Discussion

Blaffer-Hrdy [18] summarized evidence from several species for variation in maternal care. This work has clearly questioned the idea that maternal behavior emerges as an invariant property of the endocrine events accompanying pregnancy and parturition. In nonhuman primates, the amount of time mothers spend in physical contact with infants exhibits natural variation and this trait is transmitted to female offspring [12,19]. Likewise, in macaques, there are stable individual differences in the rates of infant

rejection as well as infant abuse [11,20]. The results from studies of the rat clearly support the existence of variation in maternal care, and suggest the existence of stable individual differences in specific forms of maternal behavior. Perhaps the most compelling evidence in favor of such stable individual differences in maternal behavior emerges from studies showing that mothers tend to behave in a comparable way across birth cohorts (Fig. 7). Indeed, despite variability in litter size and gender composition of litters, lactating rats show a very high degree of stability in licking/grooming behavior across multiple litters.

The distinction between variation in mother–infant contact and other aspects of maternal behavior in the rat, particularly licking/grooming, is important when considering the potential mechanisms determining these naturally occurring variations. Recent studies suggest that oxytocin receptor levels are functionally related to differences in licking/grooming [21,22]. Central infusion of an oxytocin receptor antagonist (OTA) on Day 3 postpartum, significantly reduces licking/grooming in High, but not Low LG dams. Indeed, OTA treatment eliminates group differences in licking/grooming [21]. However, these same infusions have no effect on amount of time spent in contact between mother and pups. The prevailing hypothesis in the study of maternal behavior in the rat, stated that oxytocin receptors are involved in the initiation, but not maintenance of maternal behavior [23]. This idea was derived from findings showing that central OTA infusion when performed following the day of birth did not modify maternal behavior [23]. However, these results may be limited to measures of the amount of mother–infant contact. Indeed, the findings of Champagne et al. [21] are consistent with these earlier reports such that, as in previous reports, central OTA infusion on Day 3 postpartum did not affect time spent in contact in either High or Low LG–ABN mothers. However, this treatment did dramatically reduce licking/grooming in the High LG–ABN mothers. It would appear that licking/grooming and mother–infant contact are behaviorally and, in part at least, mechanistically distinct [10]. We propose a revision to the existing hypothesis to suggest that, in the rat, oxytocin is essential for the initiation of maternal behavior and *can* subsequently serve to modify the expression of specific forms of maternal care over the first week postpartum.

An obvious question is whether the naturally occurring variations in maternal behavior are dependent upon pup-related stimuli; do High LG–ABN pups produce High LG–ABN mothering? Cross-fostering studies seem to indicate that this is unlikely to be the case [17]. Interestingly, the variations in maternal care reported here are independent of many, basic litter characteristics. For example, litter size apparently has little effect on either the frequency of licking/grooming or arched-back nursing. This suggests that the culling of litters is not necessary to maintain stability in maternal care. Likewise, the male/female composition of the litter did not influence frequency of licking/grooming or arched-back nursing, although within litters, males did

receive slightly more licking/grooming than did females. This finding might surprise those familiar with the research of Moore [24–26] showing greater anogenital maternal licking/grooming towards male pups. However, such gender differences appear only by the beginning of the second week of life, while our observations focus on the first 8 days of life. Moreover, we score all forms of licking/grooming, including both anogenital licking/grooming, which ultimately depends upon the gender of the pup, as well as body licking, which does not [24]. With respect to outcome measures of gene expression and HPA development, we have observed no gender differences in the effects of these naturally occurring differences in maternal care.

It is important to note that the variations in maternal care should not be considered as a case of “good” and “bad” mothers. Differences in licking/grooming lie within the functional range of parental care and are unrelated to basic measures of reproductive success. The data suggest that Low LG–ABN mothers provide adequate maternal care. The offspring of Low and High LG–ABN mothers do not differ in survival to weaning or in weaning weights. There are no differences in the rates of pup killing, such that the loss of litters due to cannibalism is uniformly low in both groups. Moreover, there are no differences in fecundity in the female offspring of Low and High LG–ABN mothers, although we admit this issue has been examined under the rather unexacting circumstance of colony breeding. Nevertheless, under such conditions the breeding success is unrelated to the differences in maternal care. To the extent that these factors reflect the adequacy of maternal care, we would conclude that the behavior of the Low and High LG–ABN mothers falls within a normal range of adequate care. Obviously there is no established criteria for “normal” vs. “abnormal” parental care in nonhuman species. Most would probably agree that the patterns of apparent abuse observed in studies with primates [20] lie outside the normal range of behavior. However, this is simply a matter of consensus. To date, there is little evidence that such patterns of behavior in nonhuman species produce differences in survival or reproductive success in the offspring. Our findings on reproductive outcomes with the rat model are consistent with the idea that the variations in licking/grooming and arched-back nursing described across females do indeed lie within a normal range.

Variations in maternal licking/grooming and arched-back nursing have been associated with effects (summarized in Table 4) on the development of neural systems that mediate hypothalamic–pituitary–adrenal and behavioral responses to stress as well as certain forms of learning and memory [15,18,27–29]. The magnitude of these effects is impressive, often associated with greater than twofold changes in gene expression. What is perhaps surprising here, is that developmental effects of such magnitude derive from variations in parental care that appear to lie within a normal range for the species. Why would these developmental effects exist? We think this question is best considered

Table 4
Summary of differences between offspring of High and Low LG dams

Measure	Maternal LG–ABN
ACTH response to acute stress	High < Low
CORT response to acute stress	High < Low
Hippocampal GC receptor	
mRNA expression	High > Low
Protein expression	High > Low
PVNH CRF mRNA expression	High < Low
GC negative-feedback sensitivity	High > Low
Open-field exploration	High > Low
Morris water maze learning latency	High < Low
Novelty-suppression of feeding	High < Low
CBZ receptor	
Central nucleus of the amygdala	High > Low
Lateral nucleus of the amygdala	High > Low
Locus ceruleus	High > Low
Nucleus tractus solitarius	High > Low
Hippocampus	High = Low
Frontal cortex	High = Low
Medial prefrontal cortex	High = Low
CRF receptor	
Locus ceruleus	High < Low
NMDA receptor (hippocampus)	
NR2A subunit	High > Low
NR2B subunit	High > Low
α_2 adrenoreceptor	
Locus ceruleus	High > Low
Nucleus tractus solitarius	High > Low
PVNH	High = Low
Synaptophysin	
Hippocampus	High > Low
N-CAM	
Hippocampus	High > Low
Acetylcholine Levels	
Dorsal hippocampus	High > Low
Vassopressin receptor (V1a)	
Central nucleus of the amygdala	High > Low
Oxytocin receptor binding (females)	
Central nucleus of the amygdala	High > Low
Bed nucleus of the stria terminalis	High > Low
Medial preoptic area	High > Low
Lateral septum	High > Low
V. nucleus of the hypothalamus	High = Low
Estrogen receptor α (females)	
Medial preoptic area	High > Low
V. nucleus of the hypothalamus	High < Low

within an evolutionary context. Studies on the long-term effects of maternal care in the rat are comparable to those performed in evolutionary biology examining “maternal effects” [30–32]. Within evolutionary biology maternal or parental effects are defined as those effects that refer to any component of phenotype expression that is derived from either the mother or the father, apart from nuclear genes [30,31]. These effects have been studied across a variety of different species and clearly indicate that environmentally induced modifications of the parental phenotype can be transmitted to offspring. In many invertebrates, maternal effects have reported with inducible defensives [32,33]. Such defensives, as opposed to constitutive defensives, emerge or develop to full strength in response to signals

from environmental threats, such as those associated with predators. For example, in response to chemosignals, or kairomones, from aquatic predators, water fleas (*Daphnia*) form impressive, helmetlike growths on their necks and spines along their tails [32,34]. These morphological changes make the animals less likely to be captured and ingested [34]. One might immediately wonder why, if so effective, are such defensives not permanently maintained (i.e., constitutive). The answer may lie in the simple fact that while these defenses are effective they, like the HPA response of the mammal, are metabolically costly; hence, use only as required. Thus, in *Daphnia* helmet sizes roughly doubles in response to the kairomone signals—an inducible defense [32]. Moreover, there is evidence for transgenerational effects, comparable to those reported in the behavioral and endocrine responses to stress in the rat [17]. In the rat, Low LG–ABN mothers are more fearful, and beget more fearful, stress reactive offspring. The mechanism for this transgenerational effect involves the variations in maternal behavior described here. In *Daphnia*, the mechanism is less clear, but the evidence for intergenerational transmission is no less compelling. The F1 and F2 generations of mothers exposed to kairomones up until pregnancy, and clean water thereafter, exhibited significantly larger helmets than do those of mothers from clean water environments [34].

In the rat, we have argued that variations in maternal behavior are associated with the quality of the maternal environment [29]. Perhaps the most compelling evidence for this process emerges from the studies of Rosenblum and Andrews [37] and Coplan et al. [35,36] where Bonnet macaque, mother–infant dyads were maintained under one of three foraging conditions: low foraging demand (LFD), where food was readily available, high foraging demand (HFD) where ample food was available, but required long periods of searching, and variable foraging demand (VFD), a mixture of the two conditions on a schedule that did not allow for predictability. At the time that these conditions were imposed, there were no differences in the nature of mother–infant interactions. However, following a number of months of these conditions there were highly significant differences in mother–infant interactions related to the “economic” demands of the condition. The VFD condition was clearly the most disruptive [37]. Mother–infant conflict increased in the VFD condition. Infants of mothers housed under these conditions were significantly more timid and fearful. These infants showed signs of depression common observed in maternally separated macaque infants, remarkably, even while the infants were in contact with their mothers. As adolescents, the infants reared in the VFD conditions were more fearful, submissive and showed less social play behavior. More recent studies have demonstrated the effects of these conditions on the development of neural systems that mediate behavioral and endocrine response to stress. As adults, monkeys reared under VFD conditions showed increased CSF levels of CRF [35,36]. Increased central CRF drive would suggest altered noradrenergic and

serotonergic responses to stress, and this is exactly what was seen in adolescent VFD-reared animals. It will be fascinating to see if these traits are then transmitted to the next generation.

The critical issue here is the effect of environmental adversity on maternal behavior. In the rat, females exposed to stress during pregnancy showed increased retrieval latencies [26,38,39] a finding that would seem to reflect an effect of stress on maternal responsiveness. In a recent study we examined the effect of such gestational stress on maternal behavior in High and Low LG-ABN mothers [40]. Females that had been previously defined as High or Low LG-ABN mothers with their first litter were exposed to restraint stress during the last half of gestation or to control conditions. Gestational stress decreased the frequency of maternal licking/grooming and arched-back nursing in the High, but not Low LG-ABN mothers. Thus, a stressful environmental signal during gestation was sufficient to reverse completely the pattern of maternal behavior in High LG-ABN mothers. These findings then led to us to question whether such effects of gestational stress would be apparent with a subsequent litter, even in the absence of any further stress. Indeed, the effects of gestational stress were fully evident with the third litter, reflecting a potent, transgenerational effect. Taken together, these findings suggest that environmental adversity alters the emotional well-being of the mother: chronic stress increases anxiety and fearfulness, and thus decreases maternal responsiveness which, in turn, influences the development of stress reactivity in the offspring. For humans, these are not isolated conditions: One in five teens and one in six adult women experience abuse during pregnancy [41,42]. Also, in humans Fleming and Corter [6] reported that many factors contribute to the quality of the mother's attitude towards her newborn, but none were correlated more highly than the women's level of anxiety. Mothers who felt depressed and anxious were, not surprisingly, less positive towards their baby [43]. Moreover, there is evidence for the behavioral transmission of anxiety. Highly anxious mothers are more likely to have children who are shy and timid, and the behavior of the mother predicts the level of such behavioral inhibition in the child [44,45].

These patterns of transmission likely reflect very adaptive patterns of development. Children inherit not only genes from their parents, but also an environment [46]: Englishmen inherit England, noted Francis Galton. We believe that the findings on intergenerational transmission via maternal behavior represent an adaptive approach to development [29,34]. Under conditions of increased environmental demand, it is commonly in the animal's interest to enhance its behavioral (e.g., vigilance, fearfulness) and endocrine (HPA and metabolic/cardiovascular) responsiveness to stress. These responses promote detection of potential threat, avoidance learning, and metabolic/cardiovascular responses that are essential under the increased demands of the stressor. Since the offspring usually inhabit a niche

that is similar to their parents, the transmission of these traits from parent to offspring could serve to be adaptive. A metaphor for this argument exists in the physiology of the thrifty phenotype in rodents [47,48]. In response to the deprivation of energy substrates in fetal life, rodents show a pattern of development that favors energy conservation and an increased capacity for both gluconeogenesis and lipolysis in adulthood. Both effects appear to reflect "anticipatory" patterns of development that would be adaptive under repeated periods of food shortages. Interestingly, these effects are mediated by sustained changes in the expression of genes in hepatic tissues that mediate glucose and fat metabolism [47,49]. We believe that the effects of maternal care of the expression of genes involved in the regulation of behavioral and endocrine responses to stress reflect a comparable effect.

The key issue here is that of the potential adaptive advantage of the increased level of stress reactivity apparent in the offspring of Low LG-ABN mothers. The research of Farrington et al. [50] and Haapasalo and Tremblay [51] on young males growing-up in a low SES and high crime in urban environments provides an excellent illustration of the potential advantages of increased stress reactivity. In this environment, the males that were most successful in avoiding the pitfalls associated with such a "criminogenic" environment were those that were shy and somewhat timid. Under such conditions a parental rearing style that favored the development of a greater level of stress reactivity to threat would be adaptive. It is thus perhaps understandable that parents occupying a highly demanding environment would transmit to their young an enhanced level of stress reactivity in "anticipation" of a high level of environmental adversity. Such a pessimistic developmental profile would be characterized by, for example, an increased level of hypothalamic and amygdaloid CRF gene expression. In contrast, more favorable environments would encourage an optimistic pattern of development, characterized by more modest levels of stress reactivity and increased hippocampal synaptogenesis. The quality of the environment influences the behavior of the parent, which in turn, is the critical factor in determining whether development proceeds along an optimistic vs. pessimistic pattern of development. The obvious conclusion is that there is no single ideal form of parenting: Various levels of environmental demand require different traits in the offspring. In essence, in instances where the threats experienced by the parents predict those likely to be encountered by the offspring, the parental behavior should function in the direction of endowing the offspring with an appropriate level of defensive responses [29,34]. As such, licking/grooming in the rat may serve to transduce information concerning the quality of the maternal environment to the offspring and to regulate the expression of defensive responses in a manner that provides a certain measure of "preparedness". This logic forms the basis of a hypothesized relationship between environmental conditions, maternal behavior and development. The find-

ings reported here suggest such ideas can be meaningfully tested in the Norway rat, a species with a remarkably rich behavioral repertoire.

References

- [1] Ammerman RT, Cassisi JE, Hersen M, van Hasselt VB. Consequences of physical abuse and neglect in children. *Clin Psychol Rev* 1986;6: 291–310.
- [2] Ladd CO, Huot RL, Thirivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM. Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Prog Brain Res* 2000;122:81–103.
- [3] Suomi SJ. Early determinants of behavior: evidence from primate studies. *Br Med Bull* 1997;53:170–84.
- [4] Trickett PK, McBride-Chang C. The developmental impact of different forms of child abuse and neglect. *Dev Rev* 1995;15:311–37.
- [5] Maccoby EE. Parenting and its effects on children: on reading and misreading behavior genetics. *Annu Rev Psychol* 2000;51:1–27.
- [6] Fleming AS, Corter C. Factors influencing maternal responsiveness in humans: usefulness of an animal model. *Psychoneuroendocrinology* 1988;13(1–2):189–212.
- [7] Fleming AS. Psychobiology of rat maternal behavior: how and where hormones act to promote maternal behavior at parturition. *Ann N Y Acad Sci* 1986;474:234–51.
- [8] Numan M. Neural basis of maternal behavior in the rat. *Psychoneuroendocrinology* 1988;13:47–62.
- [9] Pedersen CA. Oxytocin control of maternal behavior. Regulation by sex steroids and offspring stimuli. *Ann N Y Acad Sci* 1995;126–45.
- [10] Stern JM. Offspring induced nurturance: animal–human parallels. *Dev Psychobiol* 1997;31:19–37.
- [11] Berman CM. Intergenerational transmission of maternal rejection rates among free-ranging rhesus monkeys on Cayo Santiago. *Anim Behav* 1990;44:247–58.
- [12] Fairbanks LA. Early experience and cross-generational continuity of mother–infant contact in vervet monkeys. *Dev Psychobiol* 1989;22(7): 669–81.
- [13] Myers MM, Brunelli SA, Squire JM, Shindeldecker RD, Hofer MA. Maternal behavior of SHR rats and its relationship to offspring blood pressures. *Dev Psychobiol* 1989;22(1):29–53.
- [14] Levine S. The ontogeny of the hypothalamic–pituitary–adrenal axis. The influence of maternal factors. *Ann N Y Acad Sci* 1994;746: 275–88.
- [15] Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky P, Meaney MJ. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc Natl Acad Sci U S A* 1998;95:5335–40.
- [16] Fleming AS, O'Day DH, Kraemer GW. The neurobiology of mother–infant interactions: experience and central nervous system plasticity across development and generations. *Neurosci Biobehav Rev* 1999; 23(5):673–85.
- [17] Francis DD, Diorio J, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* 1999;286:1155–8.
- [18] Blasser-Hrdy S. Mother nature: a history of mothers infants, and natural selection. New York: Pantheon Books; 1999.
- [19] Fairbanks LA. Individual differences in maternal style of old world monkeys. *Adv Study Behav* 1996;25:579–611.
- [20] Maestripieri D, Tomaszycski M, Caroll KA. Consistency and change in the behavior of rhesus macaque abusive mothers with successive infants. *Dev Psychobiol* 1999;34(1):29–35.
- [21] Champagne F, Diorio J, Sharma S, Meaney MJ. Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proc Natl Acad Sci U S A* 2001;98:12736–41.
- [22] Francis DD, Champagne FC, Meaney MJ. Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat. *J Neuroendocrinol* 2000;12:1145–8.
- [23] Fahrbach SE, Morrell JI, Pfaff DW. Possible role for endogenous oxytocin in estrogen-facilitated maternal behavior in rats. *Neuroendocrinology* 1985;40(6):526–32.
- [24] Moore CL. Maternal contributions to mammalian reproductive development and the divergence of males and females. *Adv Study Behav* 1995;24:47–118.
- [25] Moore CL, Morelli GA. Mother rats interact differently with male and female offspring. *J Comp Physiol Psychol* 1979;93:677–84.
- [26] Moore CL, Power KL. Prenatal stress affects mother–infant interaction in Norway rats. *Dev Psychobiol* 1986;19:235–45.
- [27] Liu D, Diorio J, Day JC, Francis DD, Meaney MJ. Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat Neurosci* 2000;3:799–806.
- [28] Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. *Science* 1997; 277:1659–62.
- [29] Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu Rev Neurosci* 2001;24:1161–92.
- [30] Mousseau TA, Fox CW, editors. Maternal effects as adaptations. New York: Oxford Univ. Press; 1998.
- [31] Rossiter MC. The role of environmental variation in parental effects expression. In: Mousseau TA, Fox CW, editors. Maternal effects as adaptations. New York: Oxford Univ. Press; 1998. p. 112–34.
- [32] Tolliran R, Dodson SI. Inducible defenses in Cladocera: Constraints, costs and multipredator environments. In: Tolliran R, Harvell CD, editors. The ecology and evolution of inducible defenses. Princeton, NJ: Princeton Univ. Press; 1999. p. 177–202.
- [33] Tolliran R, Harvell CD, editors. The ecology and evolution of inducible defenses. Princeton, NJ: Princeton Univ. Press; 1999.
- [34] Agrawal AA, Laforsch C, Tollrian R. Transgenerational induction of defences in animals and plants. *Nature* 1999;401:60–3.
- [35] Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, et al. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci U S A* 1996;93(4): 1619–23.
- [36] Coplan JD, Trost RC, Owens MJ, Cooper TB, Gorman JM, Nemeroff CB, et al. Cerebrospinal fluid concentrations of somatostatin and biogenic amines in grown primates reared by mothers exposed to manipulated foraging conditions. *Arch Gen Psychiatry* 1998;55(5):473–7.
- [37] Rosenblum LA, Andrews MW. Influences of environmental demand on maternal behavior and infant development. *Acta Paediatr Suppl* 1994;397:57–63.
- [38] Fride E, Dan Y, Gavish M, Weinstock M. Prenatal stress impairs maternal behavior in a conflict situation and reduces hippocampal benzodiazepine receptors. *Life Sci* 1985;36:2103–9.
- [39] Kinsley CH, Bridges RS. Prenatal stress and maternal behavior in intact virgin rats: response latencies are decreased in males and increased in females. *Horm Behav* 1988;22(1):76–89.
- [40] Champagne F, Meaney MJ. Gestational stress effects on maternal behavior. *Abstr Soc Neurosci* 2000;26:2035.
- [41] Newberger EH, Barkan SE, Lieberman ES, McCormick MC, Yllo K, Gary LT, et al. Abuse of pregnant women and adverse birth outcome. Current knowledge and implications for practice. *JAMA* 1992;267: 2370–2.
- [42] Parker B, McFarlane J, Soeken K. Abuse during pregnancy: effects on maternal complications and birth weight in adult and teenage women. *Obstet Gynecol* 1994;84:323–8.
- [43] Field T. Maternal depression effects on infants and early interventions. *Prev Med* 1998;27(2):200–3.
- [44] Hirshfeld DR, Biederman J, Brody L, Faraone SV, Rosenbaum JF. Expressed emotion toward children with behavioral inhibition: asso-

- ciations with maternal anxiety disorder. *J Am Acad Child Adolesc Psychiatry* 1997;36(7):910–7.
- [45] Hirshfeld DR, Biederman J, Brody L, Faraone SV, Rosenbaum JF. Associations between expressed emotion and child behavioral inhibition and psychopathology: a pilot study. *J Am Acad Child Adolesc Psychiatry* 1997;36(2):205–13.
- [46] West MJ, King AP. Setting nature and nurture into an ontogenetic niche. *Dev Psychobiol* 1987;20:549–62.
- [47] Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992;35: 595–601.
- [48] Neel JV. Diabetes mellitus: a “thrifty” genotype rendered detrimental by “progress”? *Bull World Health Organ* 1999;77:694–703.
- [49] Bauer MK, Harding JE, Bassett NS, Breier BH, Oliver MH, Gallaher BH, et al. Fetal growth and placental function. *Mol Cell Endocrinol* 1998;140(1–2):115–20.
- [50] Farrington DP, Gallagher B, Morley L, St. Ledger RJ, West DJ. Are there any successful men from criminogenic backgrounds? *Psychiatry* 1988;51(2):116–30.
- [51] Haapasalo J, Tremblay RE. Physically aggressive boys from ages 6 to 12: family background, parenting behavior, and prediction of delinquency. *J Consult Clin Psychol* 1994;62(5):1044–52.