# Natural variations in postpartum maternal care in inbred and outbred mice

Frances A. Champagne [a,b,*], James P. Curley [a], Eric B. Keverne [a], Patrick P.G. Bateson [a]

[a] Sub-Department of Animal Behaviour, University of Cambridge, High Street, Madingley, CB3 8AA Cambridge United Kingdom
[b] Department of Psychology, Columbia University, 406 Schermerhorn, 1190 Amsterdam Avenue New York, NY 10027 USA

## Abstract

The role of maternal care in mediating variation in offspring phenotype has been examined in the rat and demonstrates that mother–infant interactions are critical for inducing long-term changes in behavior. Though phenotypic differences between mice strains are often attributed to genetic factors, the influence of early maternal environment has not been extensively explored. To understand maternal influence on phenotype in mice, we must first explore the nature of differences in behavior. In the present study, we examine aspects of maternal care differentiating mice strains and explore the relationship between postpartum behavior and measures obtained by a standard test of maternal responsivity (Retrieval Test). We compared inbred 129Sv ($n=25$), C57BL/6J ($n=23$), and outbred Swiss ($n=23$) lactating female mice. Swiss females had shorter latencies to retrieve and crouch over pups ($P<.01$), whereas 129Sv females had shorter latencies to nestbuild ($P<.05$). Conversely, observations of homecage behavior indicate that 129Sv females nestbuild less frequently. 129Sv females also engaged in very low levels of pup licking/grooming ($P<.001$) and long periods of nursing/contact ($P<.05$) with pups compared to C57BL/6J and Swiss females. Temporal analysis suggests that the magnitude of these differences varies both within and between days. No significant correlations were found between any aspect of maternal responsivity and postpartum behavior. These results illustrate that through detailed analysis of maternal behavior in mice, variations between strains can be observed. These variations represent strain specific strategies for promoting growth and survival of offspring during infancy that may also mediate "epigenetic" differences in phenotype in adulthood.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Maternal behavior; Mice; Strain; Natural variations; Licking/grooming

## 1. Introduction

Studies of the developmental origins of primate and rodent behavior illustrate how natural variations in maternal care can be associated with indices of stress-responsivity, social behavior, and cognition of offspring. In vervet monkeys, frequency of contact between mother and infant during the first 6 months of age predicts the maternal and exploratory behavior of adult offspring [1,2]. In rats, there are also naturally occurring variations in postpartum maternal care, notably licking/grooming of pups during the first week postpartum, which are stable across litters and associated with long-term changes in gene expression and behavior of offspring [3–8]. These variations in mother–infant interaction represent differences in maternal style which mediate the behavioral transmission of traits and thus transgenerational effects [8,9]. The evidence for the occurrence of these effects across species is striking and it is evident that within each species, distinctive behaviors confer this non-genomic transmission.

Though studies of rat maternal care and its impact on offspring development may be generalized to apply to all rodents, there are certainly species differences that need to be explored between rats and mice before these effects can be established. In mice, as indeed in rats, our knowledge of maternal behavior has typically been limited to that obtained during discrete tests of pup retrieval behavior [10–12]. Here, the mother is briefly separated from her litter while a few pups (typically 3–4) are removed from the nest and scattered in the homecage. Under this stressful and disturbed situation the dam is then activated to retrieve pups to the nest, nestbuild and crouch over pups. The latency to perform these behaviors and the amount of time engaged in each aspect of the response are taken as indices of

* Corresponding author. Department of Psychology, Columbia University, 406 Schermerhorn, 1190 Amsterdam Avenue, New York, NY 10027 USA. Tel.: +1 212 854 2589; fax: +1 212 854 3609.
E-mail address: fac2105@columbia.edu (F.A. Champagne).

"maternal responsivity". Though lactating female mice and rats certainly display higher levels of maternal responsivity compared to virgin and non-lactating females [13,14], it is evident that even amongst lactating females there are significant differences between strains, particularly in mice, in the latency to display maternal care. Three of the most commonly studied inbred strains used in these studies were the C57BL/6J, A/J and DBA strains, with the DBA strain generally being the slowest to retrieve pups [15–18]. Similar strain differences in maternal behavior of mice have been assessed in paradigms focused on nest-building and lactational aggression [19–22]. These strain dependent variations in maternal responsivity have been attributed to "genetic" differences [10–12,15,18,23] which has limited the exploration of maternal behavior as a mediating influence on offspring phenotype across strains.

To understand the role of maternal care in shaping offspring development in mice, we must first determine, as in the rat and primate, the characteristics of natural variations in maternal behavior, particularly between strains. The importance of understanding these natural variations is highlighted by recent studies that describe how pre- and postnatal maternal effects epigenetically alter pup development and adult behavioral phenotypes [24–29]. The present study provides a detailed analysis of the variability of postpartum maternal behavior of three commonly used strains of primiparous female mice (C57BL/6J, 129Sv, NIH Swiss). We also explore the relationship between traditional tests of maternal responsivity and these postpartum homecage mother–infant interactions.

## 2. Methods

### 2.1. Animal housing and care

All subjects were laboratory mice (*Mus musculus*) and all procedures were undertaken with the relevant ethical approvals. Animals were housed at the Sub-Department of Animal Behaviour (Madingley, Cambridge UK) on a reverse 12D, 12L light cycle, under a constant temperature of 21 °C and 55% humidity. All animals were given ad libitum access to water and the RM1(E) chow diet (Lillico, Surrey UK) and fresh bedding, wood-shavings (Lillico), placed into cages weekly. Following birth, mothers and pups were left undisturbed for the duration of observation (one week). Behavioral observations took place during the dark period of the light cycle, this being the period when mice are most active.

### 2.2. Subject animals and methodology

Subject animals ($n=71$) were inbred 129Sv ($n=25$), C57BL/6J ($n=23$) and outbred NIH Swiss female mice ($n=23$). The inbred 129Sv and C57BL/6J (hereafter B6) mice and outbred NIH Swiss (hereafter Swiss) were all taken from the colony at the Sub-Department of Animal Behaviour, Madingley. All animals were weaned from primiparous mothers at 28 days postnatal and housed in groups of four until mating aged 3–5 months. All females were virgins and were mated with males of the same strain. At late gestation (day 15–16) females were placed in transparent single cages (35 cm × 15 cm × 17 cm) to facilitate behavioral observations. Females were checked every 12 h to determine the day of birth. Pregnant females housed under reverse cycle lighting typically gave birth during the light cycle (8pm–8am). On the day of birth (Day 0pn), females were tested in a Retrieval Test and litter size and weights were recorded. For the following six days, (Days 1–6 pn), the behavior of the female in the homecage was observed. Only females that gave birth to litters of at least 3 pups were included in the study.

### 2.3. Retrieval Test

On the day of birth, the lactating female and pups were removed from the home cage briefly (approximately 10 s) and bedding was disturbed throughout the cage. Three pups from their own litter were then randomly placed away from the nest end of the home cage, and the mother was then reintroduced to the cage. The latency (in seconds) to sniff a pup, retrieve each of three pups, nestbuild and crouch over pups was recorded. If a female had not completed this response within 15 min the test was terminated, resulting in a latency of 900 s for any behaviors not yet observed. Following testing, all pups were returned to the home cage. All testing occurred within the colony room.

### 2.4. Maternal observations

The procedure for assessing maternal behavior was adapted from previous work examining natural variations in maternal care in rats [7,8,30]. Maternal behavior was scored from Day 1 through Day 6 postpartum. Observers were trained to a high level of interrater reliability (i.e., >0.90). Dams were observed in their home cage during the dark-phase of the light cycle under dim red light (<5 lx) and not disturbed for the duration of the 6-day observation period. Each day consisted of 4 observation periods, 2 within the first 5 h following the onset of the dark cycle (0800 to 1300; classified as EARLY) and 2 within 7 h of the end of the dark cycle (1300 to 2000; classified as LATE). Each observation was 60 min in duration and no observation sessions took place within the 1 h period before or after the transition from the light to dark cycle. Within each observation period, the behavior of each mother was scored every 3 min (20 observations/period × 4 periods per day = 80 observations/mother/day = 480 observations per mother over the 6 days). The following behaviors were scored [also described in [7,30]]: mother off pups, mother licking and grooming any pup (both body and anogenital licking were included), mother in nursing posture over pups, nest-building (whilst in contact with pups, nursing pups, or not in contact with pups), self-grooming, eating and drinking. Variations in nursing posture previously observed in lactating rat dams [7,30] were not evident in the 6 days postpartum and nursing thus describes a crouched arch posture over pups.

## 3. Results

28 females of each strain were mated, resulting in 25 129Sv litters, and 23 litters each for the B6 and Swiss strains. Litter

Table 1
Litter sizes and pup weights

|  | Litter size | Litter weight (g) | Mean pup weight (g) |
|---|---|---|---|
| 129Sv | 5.88±.39 | 8.23±.46 | 1.43±.02 |
| B6 | 6.26±.40 | 8.45±.47 | 1.37±.03 |
| Swiss | 9.56±.30*** | 14.36±.42*** | 1.51±.03** |

**P<.01, ***P<.001.

size and weight were recorded on the first day postpartum (Table 1). Significant differences between strains were found for litter size ($F_{2,70}=30.2$, $P<0.001$), litter weight ($F_{2,70}=58.1$, $P<0.001$) and mean pup weight ($F_{2,70}=6.9$, $P<0.01$). Tukey post-hoc analyses revealed that both litter size and weight were elevated in Swiss compared to both inbred strains ($P<0.001$), though there were no significant differences in either litter size or weight between the 129Sv and B6 strains. Likewise, the range of litter sizes was equivalent for the inbred strains (3–10 pups) whereas outbred Swiss litters ranged from 7 to 12 pups. Mean pup weight was also higher in the Swiss compared to B6 ($P<0.01$), but there were no significant differences in mean pup weight between 129Sv litters and either the B6 or Swiss litters.

## 3.1. Strain differences in maternal responsivity

On the day of parturition, the latency to maternal behavior in the Retrieval Test was measured in B6, 129Sv, and Swiss females. There were no significant strain differences in the percentage of females who exhibited all aspects of maternal behavior during the 15 minute task (129Sv=48.0%, B6=43.5%, Swiss=56.4%). No significant differences in the latency to sniff a pup or retrieve the first pup were observed (Fig. 1). However, significant strain differences were observed in the latency to retrieve the second ($F_{2,70}=4.8$, $P<0.05$) and third pups ($F_{2,70}=6.1$, $P<0.01$), to crouch over pups ($F_{2,70}=11.2$,



Fig. 2. Pie graph illustrating the frequency of observed behaviors during Day 1 to Day 6 postpartum exhibited by primiparous lactating female A) 129Sv, B) B6, and C) Swiss mice.
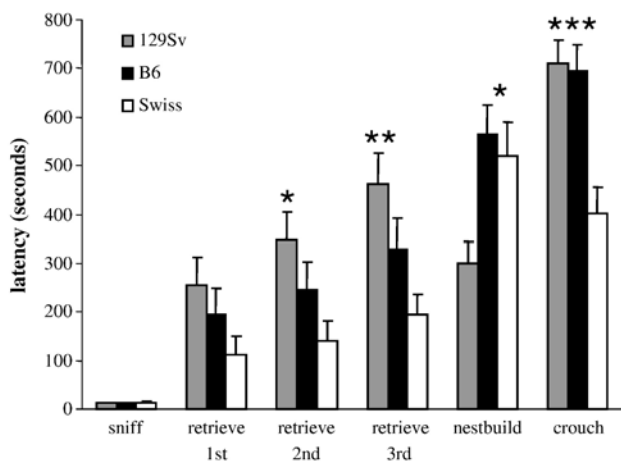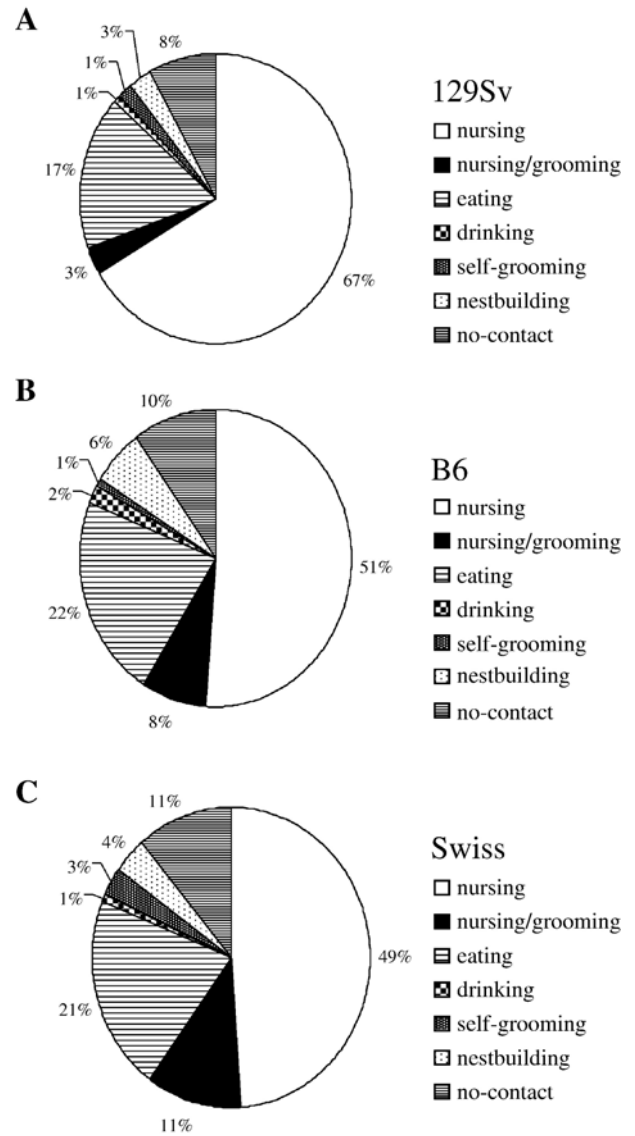


Fig. 1. Strain differences in latency (seconds) to perform maternal behavior on day 0pn in a 15 minute Retrieval Test (Mean±SE). 129Sv females were slower to retrieve 2nd and 3rd pups whereas they had shorter latencies to nestbuild compared to Swiss and B6 females. Swiss females had significantly shorter latencies to crouch over pups compared to B6 and 129Sv females. * $P<.05$, ** $P<.01$, *** $P<.001$.

$P<0.001$) and to nestbuild ($F_{2,70}=6.1$, $P<0.01$). Swiss females had shorter latencies to retrieve their second ($P<0.01$) and third pup ($P<0.01$) compared to 129Sv females. Swiss females also had significantly shorter latencies to crouch over pups than both inbred strains ($P<0.001$). B6 and 129Sv females did not significantly differ in their latencies to retrieve or crouch over pups. In contrast, 129Sv females had significantly shorter latencies to nestbuild than both B6 ($P<0.01$) and Swiss ($P<0.05$) females. There were also strain differences in the overall occurrence of behaviors during the Retrieval Test. Over 95% of all Swiss females retrieved all 3 pups whereas less that 83% of 129Sv and B6 showed this behavior. 88% of 129Sv females engaged in nest-building during the 15 minute task whereas less than 66% of B6 and Swiss females were observed to nest-build. Amongst Swiss females, greater than 86% crouched over pups during the test whereas less than 52% of 129Sv and B6
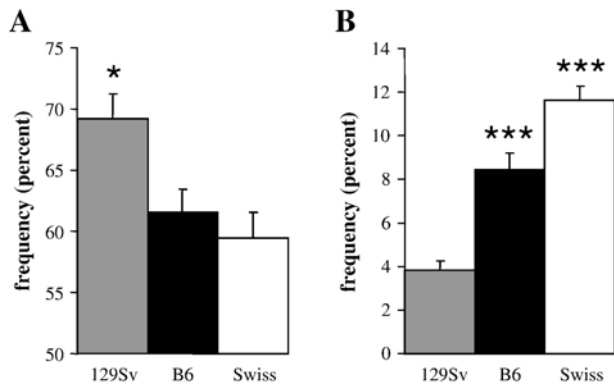
Fig. 3. The frequency of observed A) nursing and B) licking/grooming in 129Sv, B6, and Swiss lactating females averaged over days 1–6 postpartum (Means±SE). 129Sv females engaged in significantly higher levels of nursing and lower levels of licking/grooming compared to B6 and Swiss females. * $P<.01$, *** $P<.001$.

displayed this behavior. Interestingly, the significant differences in retrieval, nest-building and crouch latency emerge even when females who failed to display the behavior, thus receiving a latency of 900 s, were excluded from the analysis.

### 3.2. Strain differences in frequency of postpartum maternal care

From Day 1 though Day 6 postpartum, frequency of nursing, licking/grooming of pups, nest-building, self-grooming, eating, drinking, and overall contact with pups was rated in the lactating females (Fig. 2). Significant strain differences were observed in all aspects of postpartum maternal care averaged over these first six days postpartum (contact: $F_{2,70}=5.6$, $P<.01$; nursing: $F_{2,70}=8.2$, $P<.001$; nest-building: $F_{2,70}=6.6$, $P<.01$; licking/grooming: $F_{2,70}=47.6$, $P<.001$; eating: $F_{2,70}=4.1$, $P<.05$; drinking: $F_{2,70}=8.9$, $P<.001$; self-grooming: $F_{2,70}=16.5$, $P<.001$). 129Sv females were observed to be in contact with pups and nursing more frequently than B6 ($P<0.05$) and Swiss ($P<0.05$) females (Fig. 3A). B6 and Swiss females did not differ in their levels of nursing. Despite the longer latencies to engage in nest-building on the day of parturition, B6 females displayed significantly higher levels of nest-building during the first week postpartum compared to 129Sv females ($P<0.01$). Frequency of licking/grooming behavior differed significantly between all three strains, with B6 grooming pups twice as frequently as 129Sv females ($P<.001$) and Swiss exhibiting a three-fold greater level of pup grooming than 129Sv females ($P<.001$; Fig. 3B). B6 females spent more time eating and drinking during the postpartum period than 129Sv females ($P<.05$) and a significantly higher frequency of drinking than Swiss ($P<0.001$) females. Though no significant differences were found in self-grooming between the two inbred strains, Swiss females spent significantly more time self-grooming than both 129Sv ($P<0.001$) and B6 ($P<0.001$) females. Within each strain there was also considerable natural variation in postpartum maternal behavior as illustrated in Table 2. Thus, despite overall differences, within each strain there are individuals whose behavioral patterns overlap with those of another strain.

Table 2
Within strain variation (minimum and maximum levels) in frequency of postpartum maternal behavior

|       | Nursing | Licking/grooming | Nest-building | Eating | Drinking |
|-------|---------|------------------|---------------|--------|----------|
| 129Sv | 56–85%  | 1–8%             | 1–7%          | 8–29%  | 1–3%     |
| B6    | 37–73%  | 3–14%            | 1–21%         | 12–36% | 1–5%     |
| Swiss | 46–87%  | 6–19%            | 1–9%          | 10–36% | 0–2%     |

### 3.3. Frequency vs. duration of postpartum maternal behavior

Observations of postpartum care in rodents suggests that maternal behavior occurs in bouts that vary in both frequency and duration [7]. Analysis of the number of nursing bouts (defined as consecutive observations of nursing behavior) and the frequency of those bouts during the observation session was thus compared between strains. Significant strain differences were found in the number ($F_{2,70}=11.14$, $P<.001$; Fig. 4A) and duration ($F_{2,70}=13.07$, $P<.001$; Fig. 4B) of bouts of nursing over pups. B6 females were found to initiate bouts of nursing with pups more frequently than 129Sv ($P<.001$) or Swiss females ($P<.001$), however, the duration of these bouts was shorter than those exhibited by 129Sv females ($P<.001$).

### 3.4. Variation in maternal behavior across days

Previous work examining the temporal pattern of maternal behavior in rodents indicates that maternal behavior declines significantly over the postpartum period [6,7]. Analysis of the frequency of maternal care during the first week postpartum confirms the occurrence of this decline in both inbred and outbred mice. Two-way repeated measures ANOVA indicated a main effect of both day ($F_{5,70}=8.6$, $P<0.001$) and strain ($F_{2,70}=9.8$, $P<0.001$) on the frequency of nest-building, with females of all three strains reducing the frequency of nest-building with successive days postpartum (Fig. 5A). Analysis of licking/grooming indicated a main effect of both day ($F_{5,70}=16.0$, $P<0.001$), strain ($F_{2,70}=94.1$, $P<0.001$), and a significant interaction between day and strain ($F_{10,70}=3.4$, $P<0.001$). Post-hoc analyses revealed that 129Sv females do not alter their
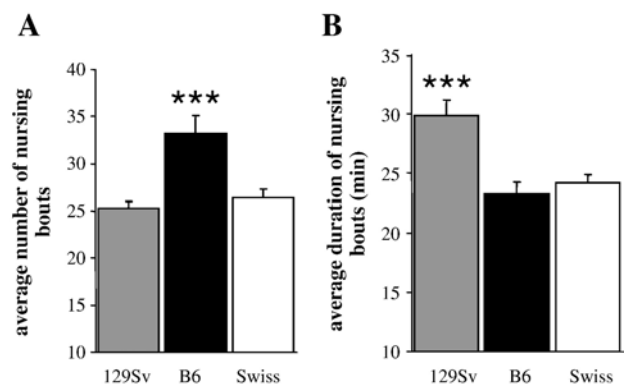


Fig. 4. A) Average number of nursing bouts, and B) average duration of nursing bouts (Mean±SE). B6 females engaged in nursing more frequently than 129Sv and Swiss females, however, the duration of nursing bouts in both B6 and Swiss was significantly lower than those observed in 129Sv females. ** $P<.01$, *** $P<.001$.

levels of licking/grooming over the first 6 days postpartum, whereas both B6 and Swiss females decline over this period (Fig. 5B). Analysis of nursing/contact indicated a main effect of both day ($F_{5,70}=6.0$, $P<0.001$) and strain ($F_{2,70}=13$, $P<0.001$), with females of all strains decreasing their levels of nursing over successive days. There was no significant strain by day interaction, with 129Sv females having significantly higher levels of nursing over all days (Fig. 5C). Coinciding with decreases in nest-building, nursing, and licking/grooming over successive days there are increases in eating and drinking in lactating females. Two-way repeated measures ANOVA of frequency of eating indicated a main effect of both day ($F_{5,70}=12.1$, $P<0.001$) and strain ($F_{2,70}=6.9$, $P<0.01$), with females of all strains increasing their levels of eating over successive days and with 129Sv females having significantly lower levels of eating over all days (Fig. 5D). Analysis indicated a main effect of both day ($F_{5,70}=4.6$, $P<0.001$) and strain ($F_{2,70}=12.5$, $P<0.001$), on frequency of drinking with females of all strains increasing their levels of drinking over successive days and with B6 females having significantly higher levels of drinking over all days. Finally, though self-grooming differed as a function of strain ($F_{2,70}=30$, $P<0.001$) with Swiss mice showing the highest levels of this behavior, no increases or decreases in this behavior are exhibited across days.

### 3.5. Variation in maternal behavior within the dark photophase

There is considerable variation during the course of the day in levels of activity in rodents which may have subsequent



Fig. 6. Frequency of postpartum A) nest-building, B) licking/grooming, C) nursing, and D) eating (Mean±SE) which occurred during observations conducted within the first 5 h after the onset of the dark cycle (EARLY) compared to observations conducted within the remaining 7 hours prior to the onset of the light cycle (LATE). Strain differences between 129Sv, B6, and Swiss lactating females in nursing and eating were only observed during the EARLY phase. * $P<.05$, ** $P<.01$.

effects on frequency of maternal care [31]. We compared the frequency of postpartum behavior in B6, 129Sv, and Swiss lactating females observed during the first five hours of the dark/active phase of the light cycle (EARLY) and the last seven hours of this phase (LATE). Equal numbers of observations were conducted between these two phases. Nest-building (Fig. 6A), licking/grooming (Fig. 6B), and self-grooming did not vary as a function of time of day. However, strain differences on other aspects of postpartum behavior did vary significantly between the EARLY and LATE phases, with differences being much more pronounced during the EARLY phase (Fig. 6).

Analysis of frequency of nursing behavior indicated a main effect of strain ($F_{2,70}=10.1$, $P<0.001$; Fig. 6C), and a significant interaction between time-of-day and strain ($F_{2,70}=6.6$, $P<0.01$). Strain differences in the frequency of nursing are apparent immediately after the onset of the EARLY phase, with 129Sv females exhibiting significantly higher levels of nursing than either B6 ($P<0.01$) or Swiss ($P<0.001$) females. No such significant strain differences in nursing levels are found in the LATE phase of the day. As was the case with frequency of nursing, we found a main effect of strain on the frequency of contact ($F_{2,70}=7.8$, $P<0.001$), as well as a main effect of time-of-day ($F_{1,70}=4.4$, $P<0.05$) and a significant interaction between time-of-day and strain ($F_{2,70}=8.6$, $P<0.001$). In the EARLY phase, 129Sv females have elevated levels of contact compared to both B6 ($P<0.001$) and Swiss ($P<0.001$) females.
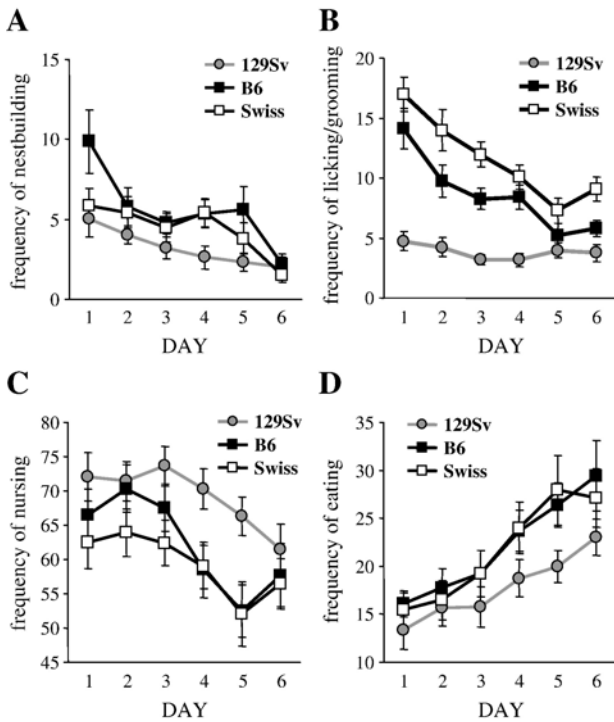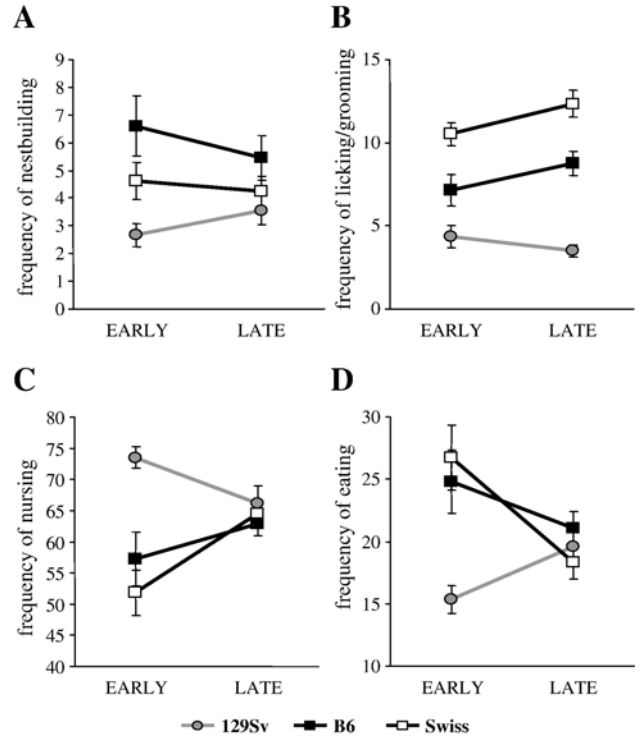


Fig. 5. Frequency of observed A) nest-building, B) licking/grooming, C) nursing, and D) eating behavior in lactating 129Sv, B6, and Swiss females over days 1–6 postpartum (Means±SE). There is an overall decrease in maternal behavior across days, with corresponding increases with frequency of eating.

In the LATE phase, no significant strain differences were found in the amount of contact with pups.

Repeated measures ANOVA of frequency of eating indicated a main effect of strain ($F_{2,70}=6.1$, $P<0.01$; Fig. 6D), and a significant interaction between time-of-day and strain ($F_{2,70}=6.1$, $P<0.01=6.6$, $P<0.01$). Strain differences in the frequency of eating are apparent immediately after the onset of the EARLY phase, with 129Sv females exhibiting significantly lower levels of eating than either B6 ($P<0.01$) or Swiss ($P<0.001$) females. No such significant strain differences in nursing levels are found in the LATE phase of the day. Moreover, analysis indicated a main effect of strain ($F_{2,70}=14.2$, $P<0.001$), and a significant interaction between time-of-day and strain ($F_{2,70}=4.1$, $P<0.05$) on the frequency of drinking. Strain differences in the frequency of eating are apparent immediately after the onset of the EARLY phase, with B6 females drinking significantly more than either 129Sv ($P<0.001$) or Swiss ($P<0.05$) females. No such significant strain differences in drinking are found in the LATE phase of the day between B6 and 129Sv females, however B6 females still drink more frequently than Swiss females ($P<0.01$).

### 3.6. Relationship between aspects of maternal responsivity in a Retrieval Test

We then examined the relationship amongst the several behavioral components of maternal responsivity (sniffing pups, retrieval, nest-building, and crouching over pups). Across strains, the latency of a female to sniff a pup was not significantly correlated with the latency to perform any of the other aspect of the maternal response. The latency to retrieve one, two and three pups were all inter-correlated with each other in all strains ($r \geq 0.7$; $P<0.001$). Strain differences in the correlations between other variables were observed. The latency to nestbuild was highly correlated in 129Sv females with the latency to retrieve ($r \geq 0.46$, $P<0.05$) and crouch ($r=0.38$, $P<0.05$) over pups. Similarly, the latency to nestbuild was significantly correlated with the latency to crouch ($r=0.38$, $P<0.05$). Amongst B6 females, latency to crouch was significantly correlated with nest-building ($r=0.69$, $P<0.001$) and retrieving pups ($r \geq 0.44$, $P<0.05$). However, the latency to retrieve pups and nestbuild were not significantly correlated. Amongst Swiss females, the latency to retrieve pups was significantly correlated with the latency to crouch ($r=0.49$, $P<0.05$), but no significant correlation was observed between the latency to nestbuild and the latency to any other behavior.

### 3.7. Relationship between relative frequencies of postpartum maternal behavior

Frequency of both licking/grooming and self-grooming were not significantly correlated with any other postpartum behavior. Nursing and contact were highly correlated with each other across all strains ($r \geq 0.96$, $P<0.001$), as were eating and drinking ($r \geq 0.45$, $P<0.05$). Moreover, both eating and drinking frequency are significantly negatively correlated with both contact and nursing frequency ($r \geq -0.42$, $P<0.05$). Strain differences in inter-correlations were only observed for the frequency

of nest-building. In B6 and Swiss females, the amount of time nest-building was not significantly correlated with any other postpartum behavior. However, in 129Sv females, nest-building was significantly negatively correlated with nursing and contact time ($r \geq -0.63$, $P<0.001$), and significantly positively correlated with eating ($r=0.49$, $P<0.01$).

### 3.8. Relationship between litter sizes, pup weights, and maternal behavior

Across all three strains, litter size and mean pup weight were not significantly correlated with the latency to any behavior in the Retrieval Test. In the Swiss strain, it was also observed that litter size and mean pup weight was not significantly related to the frequency of any maternal postpartum maternal behavior. However, in B6 females, both litter size and mean pup weight were significantly negatively correlated with nursing and contact ($r \geq -0.60$, $P<0.001$; Fig. 7A) and positively correlated with the frequency of eating ($r \geq 0.78$, $P<0.001$; Fig. 7B). In the 129Sv strain, females with larger litter sizes were observed to nestbuild more frequently ($r=0.40$, $P<0.05$). Frequency of licking/grooming was not correlated with litter size or average pup weight in any of these mice strains.
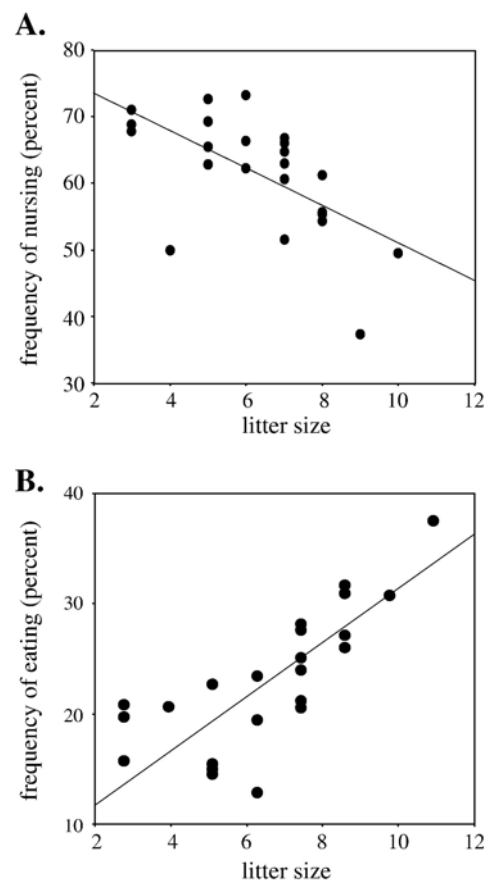


Fig. 7. Correlations between litter variables and frequency of postpartum behavior in B6 females. A) Frequency of nursing averaged over days 1–6 postpartum was negatively correlated with litter size ($r=0.60$, $P<.001$). B) Frequency of observed eating was highly positively correlated with litter size ($r=0.78$, $P<.001$).

### 3.9. Relationship between maternal responsivity and post-partum maternal care

The relationship between the performance of females during the Retrieval Test and the relative frequency of each postpartum maternal behavior was examined, however, no significant correlations were observed in the three strains tested.

## 4. Discussion

Semi-naturalistic observation of maternal care during the first week postpartum reveals highly significant differences in the mother–infant interactions between mice strains. Perhaps one of the most notable differences was found between 129Sv females compared to B6 and Swiss females. The frequency of licking/grooming behavior exhibited by females of this strain is extremely low (3.5%) and would likely be missed entirely in a more abbreviated observation schedule. However, amongst 129Sv females the frequency of time spent in a nursing posture over pups is very high (70%). Thus we observed two distinct maternal strategies, one in which females exhibit high levels of mother–infant contact yet low levels of infant stimulation (licking/grooming) as observed in 129Sv females and a second strategy involving low levels of contact and high levels of stimulation as exhibited by B6 and Swiss females (Fig. 3). Even between the Swiss and B6, this low nursing/high licking maternal style can be delineated by the short duration/high frequency nursing bouts exhibited by B6 females, and short duration/low frequency bouts exhibited by Swiss females (Fig. 4). Indeed, we also observed strain differences in the correlation between postpartum care and litter characteristics. For instance, amongst B6 females, litter size is highly negatively correlated with frequency of nursing (Fig. 7A), however, this same relationship is not observed in 129Sv and Swiss females.

It is important to note that despite the magnitude of these strain differences in maternal behavior, there were no strain differences in offspring mortality. Thus adequate levels of care were provided to promote growth and survival, hence this variation in behavior can be considered to be within the normal range of care. Postpartum behavior in lactating females reflects levels of hormones and hypothalamic receptor densities which are induced by prenatal hormonal priming [32–34]. During the gestational period, increases in levels of estrogen and prolactin mediate increases in central and peripheral levels of oxytocin receptors [32,35], induce anatomical changes in the paraventricular nucleus of the hypothalamus to facilitate lactation, and promote increases in feeding behavior [36,37]. These changes are necessary for both the physiological and behavioral aspects of maternal care. The changes in hormone levels that alter maternal physiology during gestation are induced by the developing fetus through the interactions between the placenta and maternal circulatory system [38,39]. Thus, even before birth, mother–infant interactions are critical for survival.

During the postpartum period the dependence of altricial young on these mother–infant interactions continues at a behavioral level. Thus the maternal behaviors we have characterized can be thought to promote survival through provision of adequate thermoregulation and nutrients to pups. When dams are crouched over pups in a nursing posture they provide ventral heat to offspring as well as access to nipples to allow suckling [40–42]. Licking/grooming of pups has been demonstrated to alter body temperature of pups, allows urination and defecation, and serves to stimulate movement of pups allowing them to gain access to nipples, promoting more efficient suckling [43–45]. The quality of the nest in which this occurs will contribute to the overall success of the interaction. A well formed nest that clusters the pups together will prevent loss of body heat and position pups to gain access to the mother's ventrum [46]. Finally, in order for dams to provide sufficient levels of nutrients to offspring, their own consumption must be increased requiring 2–3 fold increases in food and water intake. The time required for foraging also allows the temperature of the pups to decline and previous studies have illustrated that it is the temperature of the pups which stimulates the initiation and termination of contact between mother and infants [41]. This pattern may explain the correlation between larger litter sizes and reduced frequency of nursing observed in B6 females (Fig. 7A), with larger litters being better able to sustain high temperature levels in the absence of the dam. Thus, even time away from the nest serves a physiological function.

In the context of the strain differences in postpartum maternal behavior, 129Sv, B6 and Swiss females can be thought to ensure survival of offspring using different behavioral strategies to ensure adequate thermoregulation and nursing of young. While 129Sv females provide long periods of access to nipples and warmth from the mother's ventrum, they do not stimulate pups to allow them to improve their ability to suckle and they do not frequently replenish their own resources, likely reducing the efficiency of lactation. Conversely, B6 and Swiss would appear to be very efficient in the transfer of resources from mother to offspring, providing less access to nipples but high levels of stimulation to pups when they are in the nest, which may increase the likelihood of sucking. Strain differences between 129Sv and B6 females in frequency of nest-building may simply reflect the increased disruption of the bedding material occurring when B6 females leave the nest but may also reflect the need by these females to ensure pups are covered and huddled together to reduce loss of heat during frequent periods of mother–infant separation. These strategies appear to be highly adaptive from a biological perspective, ensuring survival and reproductive capability of young.

### 4.1. What is 'maternal'?: The relationship between maternal responsivity and postpartum care

Study of the neuroendcrine mechanisms which regulate postpartum increases in maternal care has made extensive use of the Retrieval Test paradigm [32–34]. This indice of maternal responsivity is particularly useful when comparing non-lactating females receiving artificial hormonal priming through either implants or peripheral injection. However, when determining variations in this behavior amongst lactating females, we find that approximately 50% of females fail to complete all of the behavioral components of the Retrieval Test. Moreover, despite

this apparent lack of maternal responsivity, almost all females are fully capable of rearing pups to weaning, with no correlation found between latency to sniff, retrieve, nestbuild, and crouch over pups in the Retrieval Test and frequency of postpartum licking/grooming and nursing/contact. These results are congruent with previous studies in the hamster [47] and rat [45,47,48] that have also demonstrated a lack of intercorrelation between postpartum care and Retrieval Test performance. These findings do not lessen the validity of maternal responsivity as a measure of maternal behavior but they do indicate that this measure will fail to predict the behavior that mothers will exhibit toward pups during the postpartum period. It is likely that these latency measures reflect response to novelty and stress reactivity which are triggered by the disruptive nature of this task [49,50]. The ability of females to orient themselves toward pups even under stressful conditions is certainly an important feature of maternal care but even those females who fail to respond to pups under these conditions are capable of being maternal.

## 4.2. Methodological considerations in studying maternal behavior in mice

Despite overall reliance on the Retrieval Test in mice, there has been previous work in mice exploring the frequency of undisturbed postpartum care. Laviola and Terranova [51] observed mother–offspring interactions for 10 min/day during the pre-weaning period to illustrate that litter composition can alter patterns of nest-building, nursing, and licking. Similar observational techniques were employed to examine the maternal care of Balb/c and B6 mice [27] implicating maternal phenotype as a mediator of strain differences in behavior. These and other studies [52] support our finding that B6 lactating mice engage in higher frequencies of pup licking/grooming compared to other mice strains. Previous observational studies also suggest strain differences in nest-building with B6 females building much higher quality nests [53]. Though we do not report on the quality of postpartum nests, these findings are consistent with our finding of higher frequency of nest-building observed in B6 females.

The use of observational data as an indice of maternal care in these studies clearly illustrates the utility of these techniques. The limited number of observations that are typical of these approaches may be suitable for simply detecting overall strain differences but may be insufficient for using maternal behavior as a predictor of shifts in offspring neurobiology and behaviour or for examining within strain variation in behaviour as has been accomplished in the rat [5]. The study of natural variations in maternal behavior requires detailed and lengthy behavioral observation, particularly when the behavior of interest occurs at a very low frequency [54]. We observed dramatic fluctuations in the frequency of eating, contact, nursing, nest-building and licking/grooming, during each observation period which is unsurprising given that individual bouts of these behavior patterns can last between 0–60 min. In addition to the number of observations required to generate reliable data for the frequency of these activities, timing of those observations is critical. The frequency of nursing/contact, nest-building and licking/groom-

ing are maximal immediately after birth and decline over subsequent days (Fig. 5). This is congruent with the within strain variation in licking/grooming reported in Long-Evans rats [6,7]. Group differences can only be detected during the first few days postpartum, when the levels of licking/grooming (LG) are relatively high. Attempting to differentiate behavior between strains or "High" and "Low" LG females beyond this point may not yield significant results for all aspects of care. Likewise, the timing of observations during the day is critical. Within the dark/light cycle, strain differences in nursing/contact were only apparent in observations conducted during the first 5 h after the onset of the dark-phase (Fig. 6). This directly corresponds to the period where inbred mice exhibit the greatest levels of home cage activity [31] and where strain differences in activity patterns are highest. Finally, it is also important that study of natural variations in maternal behavior should be undertaken on undisturbed females with their offspring. Disruption through even brief periods of handling may result in highly variable behavioral patterns that would not otherwise be observed during the postpartum period.

## 4.3. Epigenetic regulation of offspring behavior by maternal care

Maternal behavior is known to be a mediating influence for long-term changes in adult behavior across a number of species. Recent work has shown that variability in the quality of maternal care in the rat, specifically licking/grooming of pups, shapes the behavioral development of offspring [7,9,55]. This is epigenetically mediated via changes in the methylation status of the promoter regions of glucocorticoid receptor and the estrogen receptor $\alpha$ leading to expression differences in the brain [25,55,56]. Further evidence for epigenetic effects on offspring development come from the cross-fostering studies [26] and reciprocal breeding of inbred strains of mice [27,28]. These studies have demonstrated parent-of-origin effects on offspring development, and within cross-fostering paradigms, comparisons of the influence of biological compared to adoptive maternal strain on offspring development suggest a mediating role for maternal care. Francis et al. illustrated that the behavioral phenotype of Balb/c males could be shifted toward that of a B6 male through embryo transfer at the single cell pronucleus stage combined with cross-fostering at birth [26]. This finding may, however, be strain dependent, as several other studies have demonstrated that postnatal cross-fostering is sufficient to produce significant differences in offspring phenotype [57–59]. Differences in the frequency of licking/grooming behavior between the Balb/c and B6 inbred strains has been implicated as a mediating influence on offspring behavior during the postnatal period [26]. Our findings that 129Sv females exhibit relatively low levels of licking/grooming compared to B6 and Swiss females is consistent with the hypothesis that levels of maternal licking/grooming behavior can modify offspring anxiety responses in mice. Previous work has demonstrated strain differences in novelty seeking, open-field activity, and elevated plus-maze exploration of these particular strains with 129Sv consistently being found to be more anxious and

less active than B6 and Swiss [31,60,61]. To establish these effects, one approach would be to use a cross-fostering design, in which the influence of the low licking/grooming-high nursing style exhibited by 129Sv dams on B6 or Swiss off-spring can be assessed. However, consistent with the results of Francis et al. [26], preliminary findings from our lab suggest that this type of manipulation may be insufficient to completely shift the phenotype of inbred mice. Possible explanations for this finding could be that differences in the prenatal environ-ment between inbred mice strains may exert a particularly strong influence on offspring phenotype [26] or that due to gene–environment interactions, pups of a particular genotype may be relatively insensitive to the maternal care provided by other strains. However, there may also be some constraints of using postnatal cross-fostering as a tool in determining epige-netic effects of maternal behavior. For instance, as our data illustrate, there is considerable variation in maternal behavior within each strain and thus despite differences of genotype individual females may display similarity of maternal pheno-type, particularly when small samples are used. Consequently, for cross-fostering to be effective in revealing epigenetic effects on offspring development, females must be selected based on maximal differences in maternal style as has been show in previous studies of epigenetic programming [3–8].

## 5. Conclusions

The study of natural variation in maternal behavior provides an elegant model for understanding the developmental origins of behavior. This has certainly been appreciated in primates, in which field studies and observation in semi-naturalistic environ-ments has provided a valuable source of information regarding maternal styles and the transmission of behavior across gen-erations. Importantly, this same strategy can be implemented in rodents, facilitating the examination of multiple generations of offspring within a short period of time and allowing for the investigation of the neurobiological correlates of this transmis-sion. In rats, natural variations in maternal licking/grooming observed during the first week postpartum has a profound impact on gene expression and behavioral phenotype of offspring and is associated with epigenetic modification of genes involved in stress-responsivity and reproductive behavior. The transgenera-tional nature of these effects thus mimics a genetic inheritance to which strain differences in both mice and rats has been at-tributed. The exploration of strain differences in maternal care in mice described in the present study provides useful insights into the variation in care that exists in mice and provides a meth-odology for studying the potential role of mother–infant inter-actions for promoting offspring growth and development. The well characterized mouse genome combined with the detailed level of behavioral analysis we have described also provides an opportunity to use strain differences to explore gene–environ-ment interactions. From an evolutionary perspective, the ability to shift phenotype epigenetically in response to early environ-mental cues provides a dynamic mechanism for responding to fluctuations in environmental condition that would confer an adaptive advantage to genetically divergent strains.

## References

[1] Fairbanks LA, McGuire MT. Long-term effects of early mothering behavior on responsiveness to the environment in vervet monkeys. Dev Psychobiol 1988;21:711–24.

[2] Fairbanks LA. Early experience and cross-generational continuity of mother–infant contact in vervet monkeys. Dev Psychobiol 1989;22:669–81.

[3] 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.

[4] 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.

[5] Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 2001;24:1161–92.

[6] Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, 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.

[7] Champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav 2003;79:359–71.

[8] Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science 1999;286:1155–8.

[9] Champagne FA, Curley JP. How social experiences influence the brain. Curr Opin Neurobiol 2005;15:704–9.

[10] Ressler RH. Parental handling in two strains of mice reared by foster parents. Science 1962;137:129–30.

[11] Ressler RH. Genotype-correlated parental influences in two strains of mice. J Comp Physiol Psychol 1963;56:882–6.

[12] Ward R. Some effects of strain differences in the maternal behavior of inbred mice. Dev Psychobiol 1980;13:181–90.

[13] Rosenblatt JS. Psychobiology of maternal behavior: contribution to the clinical understanding of maternal behavior among humans. Acta Paediatr Suppl 1994;397:3–8.

[14] Cohen-Salmon C. Differences in patterns of pup care in *Mus musculus domesticus*. VIII. Effects of previous experience and parity in XLII inbred mice. Physiol Behav 1987;40:177–80.

[15] Wainwright P. Maternal performance of inbred and hybrid laboratory mice (*Mus musculus*). J Comp Physiol Psychol 1982;95:694–707.

[16] Cohen-Salmon C, Carlier M, Roubertoux P, Jouhaneau J, Semal C, Paillette M. Differences in patterns of pup care in mice. V-Pup ultrasonic emissions and pup care behavior. Physiol Behav 1985;35:167–74.

[17] Hennessy MB, Li J, Lowe EL, Levine S. Maternal behavior, pup vocalizations, and pup temperature changes following handling in mice of 2 inbred strains. Dev Psychobiol 1980;13:573–84.

[18] Carlier M, Roubertoux P, Cohen-Salmon C. Differences in patterns of pup care in *Mus musculus domesticus* l—comparisons between eleven inbred strains. Behav Neural Biol 1982;35:205–10.

[19] Schneider JE, Lynch CB, Possidente B, Hegmann JP. Genetic association between progesterone-induced and maternal nesting in mice. Physiol Behav 1982;29:97–105.

[20] Broida J, Svare B. Mice: progesterone and the regulation of strain differences in pregnancy-induced nest building. Behav Neurosci 1983;97:994–1004.

[21] Broida J, Svare B. Strain-typical patterns of pregnancy-induced nestbuild-ing in mice: maternal and experiential influences. Physiol Behav 1982;29:153–7.

[22] Lee CT. Genetic analyses of nest-building behavior in laboratory mice (*Mus musculus*). Behav Genet 1973;3:247–56.

[23] Carlier M, Roubertoux P, Cohen-Salmon C. Early development in mice: I. genotype and post-natal maternal effects. Physiol Behav 1983;30:837–44.

[24] Roemer I, Reik W, Dean W, Klose J. Epigenetic inheritance in the mouse. Curr Biol 1997;7:277–80.

[25] Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. Nat Neurosci 2004;7:847–54.

[26] Francis DD, Szegda K, Campbell G, Martin WD, Insel TR. Epigenetic sources of behavioral differences in mice. Nat Neurosci 2003;6:445–6.

[27] Calatayud F, Coubard S, Belzung C. Emotional reactivity in mice may not be inherited but influenced by parents. Physiol Behav 2004;80:465–74.

[28] Calatayud F, Belzung C. Emotional reactivity in mice, a case of nongenetic heredity? Physiol Behav 2001;74:355–62.

[29] Caldji C, Diorio J, Anisman H, Meaney MJ. Maternal behavior regulates benzodiazepine/GABAA receptor subunit expression in brain regions associated with fear in BALB/c and C57BL/6 mice. Neuropsychopharmacol 2004;29:1344–52.

[30] Myers MM, Brunelli SA, Squire JM, Shindeldecker RD, Hofer MA. Maternal behaviour of SHR rats and its relationship to offspring blood pressures. Dev Psychobiol 1989;22:29–53.

[31] Tang X, Orchard S, Sanford L. Home cage activity and behavioral performance in inbred and hybrid mice. Behav Brain Res 2002;136: 555–69.

[32] Bridges RS. Biochemical basis of parental behavior in the rat. Adv Study Behav 1996;25:215–42.

[33] Rosenblatt JS, Mayer AD, Giordano AL. Hormonal basis during pregnancy for the onset of maternal behaviour in the rat. Psychoneuroendocrinol 1988;13:29–46.

[34] Rosenblatt JS, Olufowobi A, Siegel HI. Effects of pregnancy hormones on maternal responsiveness, responsiveness to estrogen stimulation of maternal behaviour, and the lordosis response to estrogen stimulation. Horm Behav 1998;33:104–14.

[35] Bale TL, Pedersen CA, Dorsa DM. CNS oxytocin receptor mRNA expression and regulation by gonadal steroids. Adv Exp Med Biol 1995;395:269–80.

[36] Theodosis DT, Poulain DA. Neuronal-glial and synaptic plasticity of the adult oxytocinergic system. Factors and consequences. Ann N Y Acad Sci 1992;652:303–25.

[37] Denis RG, Williams G, Vernon RG. Regulation of serum leptin and its role in the hyperphagia of lactation in the rat. J Endocrinol 2003;176:193–203.

[38] Evain-Brion D. Maternal endocrine adaptations to placental hormones in humans. Acta Paediatr Suppl 1999;88:12–6.

[39] Haig D. Genetic conflicts in human pregnancy. Q Rev Biol 1993;68: 495–532.

[40] Croskerry PG, Smith GK, Leon M. Thermoregulation and the maternal behaviour of the rat. Nature 1978;273:299–300.

[41] Leon M, Croskerry PG, Smith GK. Thermal control of mother–young contact in rats. Physiol Behav 1978;21:79O–811.

[42] Stern JM, Johnson SK. Ventral somatosensory determinants of nursing behaviour in Norway rats. I. Effects of variations in the quality and quantity of pup stimuli. Physiol Behav 1990;47:993–1011.

[43] Sullivan RM, Shokrai N, Leon M. Physical stimulation reduces the body temperature of infant rats. Dev Psychobiol 1988;21:225–35.

[44] Sullivan RM, Wilson DA, Leon M. Physical stimulation reduces the brain temperature of infant rats. Dev Psychobiol 1988;21:237–50.

[45] Rosenblatt JS, Lehrman DS. Maternal behaviour in the laboratory rat. In: Rheingold Harriet L (Harriet Lange), editor. Maternal behaviour in mammals. New York: Wiley; 1963.

[46] Bult A, Lynch CB. Nesting and fitness: lifetime reproductive success in house mice bidirectionally selected for thermoregulatory nest-building behavior. Behav Genet 1997;27:231–40.

[47] Powell T. On the retrieving of young and other behavior in lactating golden hamsters. Proc Zool Soc Lond 1960;135:265–82.

[48] Slotnick B. Intercorrelations of maternal activities in the rat. Anim Behav 1967;15:267–9.

[49] Fleming A. Psychobiology of rat maternal behaviour: how and where hormones act to promote maternal behaviour at parturition. Ann N Y Acad Sci 1986;474:234–51.

[50] Fleming AS, Cheung U, Myhal N, Kessler Z. Effects of maternal hormones on 'timidity' and attraction to pup-related odors in female rats. Physiol Behav 1989;46:449–53.

[51] Laviola G, Terranova ML. The developmental psychobiology of behavioural plasticity in mice: the role of social experiences in the family unit. Neurosci Biobehav Rev 1998;23:197–213.

[52] Anisman H, Zaharia MD, Meaney MJ, Merali Z. Do early-life events permanently alter behavioral and hormonal responses to stressors? Int J Dev Neurosci 1998;16:149–64.

[53] Bond TL, Neumann PE, Mathieson WB, Brown RE. Nest building in nulligravid, primigravid and primiparous C57BL/6J and DBA/2Jmice (*Mus musculus*). Physiol Behav 2002;75:551–5.

[54] Martin P, Bateson P. Measuring behavior: an introductory guide. 2nd Edition. Cambridge: CUP; 1993.

[55] Champagne F, Meaney MJ. Like mother, like daughter: evidence for non-genomic transmission of parental behavior and stress responsivity. Prog Brain Res 2001;133:287–302.

[56] Champagne FA, Weaver IC, Diorio J, Sharma S, Meaney MJ. Natural variations in maternal care are associated with estrogen receptor alpha expression and estrogen sensitivity in the medial preoptic area. Endocrinology 2003;144:4720–4.

[57] Haug M, Pallaud B. Effect of reciprocal cross-fostering on aggression of female mice toward lactating strangers. Dev Psychol 1981;14:177–80.

[58] Bartolomucci A, Gioiosa L, Chirieleison A, Ceresini G, Parmigiani S, Palanza P. Cross fostering in mice: behavioural and physiological carry-over effects in adulthood. Genes Brain Behav 2004;3:115–22.

[59] Brake WG, Priebe K, Romeo RD, Sisti HM, Mueller A, McEwen BS, et al. Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/CJ mice: a cross-fostering study. Dev Psychobiol 2006;48: 95–6.

[60] van Gaalen MM, Steckler T. Behavioural analysis of four mouse strains in an anxiety test battery. Behav Brain Res 2000;115:95–106.

[61] Griebel G, Belzung C, Perrault G, Sanger DJ. Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. Psychopharmacology (Berl) 2000;148:164–70.