Chapter 13

Measuring Variations in Maternal Behavior: Relevance for Studies of Mood and Anxiety

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Abstract

The assessment of variations in maternal behavior in laboratory rodents is challenging yet may provide an essential tool for understanding the mechanisms linking early life experiences to individual differences in stress responsivity and behavioral indices of depression and anxiety. In this chapter, the methodology for characterizing the quality and quantity of mother–pup interactions in mice as well as the strategy for analyzing this observational data is described in detail. Successful use of this approach is dependent on careful consideration of the wide variety of environmental variables that may influence maternal behaviors, such as pup licking/grooming, which have been demonstrated to be associated with a wide range of behavioral phenotypes in offspring. Maternal behavior can moderate the effect of genetic and neurobiological manipulations that are being developed in mice to study the etiology of psychopathology. The protocol described in this chapter can be applied to these studies to examine the interplay between genes and the environment.

Key words: Maternal behavior, Licking/grooming, Home-cage, Time-sampling, Postnatal

1. Background and Historical Significance

Adverse early life experiences have been demonstrated to be a significant predictor of later life psychopathology in humans, including risk of depression and anxiety (1). This risk is particularly evident among individuals who have experienced reduced or disrupted mother–infant interactions, as in the case of childhood neglect or abuse (2). These epidemiological findings have motivated the development of laboratory models in rodents where manipulation of the degree of contact between the dam and pups is altered during the postnatal period and the behavioral and neurobiological outcomes in offspring are assessed (3). Two classic approaches to inducing postnatal changes in the experience of

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rodent pups are neonatal handling and maternal separation. These paradigms have been used extensively within the literature and lead to significant effects on brain development, stress physiology, and behavior in adulthood, though the exact nature of the effect of these manipulations is dependent on the methodological approach taken and the strain/species being studied (4, 5).

The handling paradigm involves brief daily removal of pups from the home-cage after birth and was developed as a form of early life stimulation (6). The duration of separation between mothers and pups in handling studies can vary from 3 to 20 min and the condition of the pups during the separation, particularly whether the pups are provided with an external source of heat (i.e., placed under a heat lamp), also varies from study to study. The period during which the handling occurs typically includes the week following parturition and may extend across the entire preweaning period. Effects of brief maternal separation on corticosterone response to stress in adult offspring can also be observed among pups that remain in the home-cage while the mother is briefly removed (7). Though much of the early work on neonatal handling was conducted in rats, the long-term consequences of handling on emotionality in mice are also evident (8, 9). For example, among C57Bl/6 (B6) mice, the experience of postnatal handling reduces the motivation to escape a novel environment and reduces the corticosterone response to stress (9). Handling can also attenuate increased stress responsivity associated with genetically induced upregulation of the stress response in transgenic mice and illustrates the potential of the handling paradigm in studies of gene–environment interactions (10). The effectiveness of handling to induce behavioral and neurobiological change is significantly related to the background strain, genotype, and sex of the mouse being used, and these factors are an important methodological consideration when implementing this paradigm (11).

In contrast to the effects of handling, more prolonged separations between mother and pups have been used to enhance emotionality and behavioral indices of depression and anxiety (4). The maternal separation paradigm typically involves removing pups from the home-cage for at least 1 h daily during the postnatal period. Similar to the handling manipulation, there are a wide range of variations in methodology used within the maternal separation paradigm, including the duration of separation (ranging from 1 to 24 h), the timing of the separation within the postnatal period (i.e., daily separations during the first week postnatal, throughout the preweaning period, or a single separation within the postnatal period), and the condition of mother and pups during separation (i.e., both placed in novel cages, pups kept warm vs. at ambient temperatures). Maternal separation in mice has been demonstrated to have acute effects on hypothalamic-pituitary-adrenal (HPA) function leading to increased stress-induced

glucocorticoids and reduced hippocampal glucocorticoid receptor gene expression (12). In B6 maternally separated male mice, increased risk assessment behaviors (stretch-attend postures) during open-field testing increased immobility during forced swim, and memory deficits have been reported (13). However, as in the case of neonatal handling, variation in methodological parameters as well as the strain, genotype, and sex of the mice being assessed will influence the effectiveness of this early-life manipulation to induce long-term neurobehavioral outcomes (8, 11).

Neonatal handling and maternal separation are both considered early life stressors as they both lead to increases in plasma corticosterone levels in pups when they are separated from the dam (14). However, the reduced stress response that is observed in adults who experienced brief maternal separation in the form of neonatal handling has led to the hypothesis that the effect of handling may be due to separation-induced increases in the maternal behavior of dams toward the pups following the handling procedure (5, 15). In laboratory rodents, there are increased levels of maternal care, particularly licking/grooming (LG), observed in dams toward pups among handled litters (16). If mouse dams are treated with anxiolytic drugs during the separation period, there are no increases in maternal care directed toward pups following reunion and no long-term reductions in emotionality observed in the handled offspring (17). Though it may not be necessary to induce changes in maternal care in order to observe handling effects in offspring (18, 19), these studies suggest that variation in the quality and quantity of maternal care may be a critical aspect of the developmental experience that shapes the emotionality of offspring.

Natural variations in maternal care observed in laboratory environments among inbred and outbred rodents can be used as a predictor of offspring neurobiology and behavior (20, 21). Though this approach has been more thoroughly explored in rats, there are emerging data from studies in mice illustrating the persistent effects on emotionality of variations in maternal care (22–24). The maternal behavior of rodents varies across the postnatal period and consists of crouching over pups, nursing, licking pups, and nest-building (NB) in addition to time off the nest spent eating, drinking, and self-grooming (20, 21). There are considerable between-strain and within-strain differences in the frequency of the various aspects of maternal behavior. Studies of the long-term impact of maternal care indicate that the frequency of LG may be a critical feature of the mother–infant interaction that shapes emotionality and HPA response to stress as well as multiple dimensions of behavior and physiology (cognition, social behavior, and reproduction) correlated to changes in region-specific gene expression within the brain (25). These findings are consistent with the handling literature, which has identified increased LG in handled dams. The frequency

of LG can be influenced by the environmental experiences of the dam prior to the postpartum period (26–30) and by the conditions of the postnatal rearing environment (23, 31) and thus exhibits a high degree of plasticity. The genotype of the dam can also influence the frequency of LG (32), which is an important consideration in studies where the phenotypic consequences of a transgenic manipulation are being assessed. In rodents, cross-fostering of offspring between strains that differ in the frequency of postnatal LG can lead to shifts in the development of offspring (33, 34), an effect that can also be observed in within-strain cross-fostering designs (20).

Assessment of variations in maternal behavior in mice is challenging but if successful can yield exciting new insights into the pathways linking early life experience to molecular, neurobiological, and behavioral outcomes relevant to studies on mood and anxiety. Traditionally, measures of maternal behavior in mice have focused on motivation to retrieve pups or the latency or degree of maternal aggression displayed toward an intruder (35–37). While informative regarding the mechanisms of maternal behavior (38), these approaches are likely not relevant for understanding the rearing experience of laboratory mice, since disruption to the nest or introduction of unfamiliar mice into the home-cage of a postparturient female is not typical in laboratory rearing environments. Thus, methodological approaches involving home-cage maternal observations and assessment of individual differences in maternal care toward offspring are essential in studies examining the direct impact of maternal care on offspring development as well as the moderating effect of these experiences on the influences of environment, genotype, and strain. In the subsequent sections, we provide a detailed description of this methodology, outline strategies for analyzing maternal data, and discuss tips for troubleshooting this protocol.

2. Equipment, Materials, and Setup

2.1. Animals

Both inbred and outbred strains of laboratory mice are suitable for measuring home-cage maternal behavior. However, as mentioned in the previous section, strains vary considerably in the frequency of mother–pup interactions, particularly in LG behavior. For example, Balb/c and 129Sv mice engage in significantly reduced levels of LG compared to B6 and Swiss. If the goal of the study is to assess offspring who have received low vs. high levels of LG in a within-strain model, then selecting a strain with a high average level of LG (i.e., B6) would be recommended. Alternatively, a cross-fostering design could be used between strains that exhibit low (i.e., Balb/c) vs. high (i.e., B6) levels of LG (33, 39). To generate

litters for these studies, female and male mice can be successfully mated at 8 weeks of age with mating success declining after 32 weeks. Within a cohort of mating females, it is best to use animals that are similar in age.

2.2. Animal Facility

The maternal behavior and rearing experiences of laboratory animals can be easily disturbed by the routine traffic and maintenance activities that occur in all animal facilities. Ideally, these studies should be conducted in smaller animal housing rooms separate from large vivariums. Though investigators often do not control the light cycle within animal housing rooms, studies should avoid transferring mice to and from rooms that differ in this cycle. For example, if offspring are going to be tested on measures that require reverse lighting (i.e., dark during the day, lights on at night), it would be preferable that they are reared under these same lighting conditions. There has been no systematic study of whether maternal data collected exclusively under light vs. dark conditions are better predictors of the overall pattern of maternal care, and both strategies have been successful approaches in studying mother–pup interactions in mice (22, 31, 34). If observations are being conducted during the dark phase of the cycle, lamps with red light bulbs can be arranged within the observation area to provide suitable illumination.

2.3. Housing and Husbandry

The observation of home-cage maternal behavior will require that mice are housed in Plexiglas cages that permit a clear view of the dam and litter. Though the shoe-box cages that are typically used for housing mice will be sufficient, larger cages permit the observation of more dynamic interactions between the dam and pups. The bedding material is also an important consideration. Corncob bedding does not provide sufficient nesting material for lactating females and if using this type of bedding, nestlets will need to be provided. However, the nestlets allow mice to build very elaborate nests which will prevent reliable observations of mother–pup interactions. An alternative is to use pine shavings as the bedding material. Mice can build nests from this bedding yet still be viewed from the exterior of the cage, making nestlets unnecessary.

2.4. Mating

It is recommended that if mice are being used from a commercial breeder, a 2-week period of acclimatization to the animal facility should be implemented to promote reduced stress at the time of mating. Multiple females (2–3) can be housed together with one male to promote the breeding of multiple litters. If the intention of the study is to examine the effects of within-strain variations in maternal care, 30–40 litters will be necessary to generate sufficient numbers of "low" vs. "high" litters. Since the determination of the relative level of maternal care exhibited by a female will be dependent on comparison to a cohort mean and standard deviation (SD)

(see Sect. 4.2), it will be ideal to breed the females as a cohort so that litters will be born at roughly the same time. Males can be removed after a 2-week period to avoid any contact with postnatal pups which will reduce the risk of infanticide and prevent the male from influencing the postnatal development of offspring.

2.5. Postpartum Monitoring and Husbandry

Mated females should be routinely monitored and singly housed a few days prior to parturition. Though it is often difficult to ascertain when a female will give birth (particularly if the litter is small), the weight gain of pregnant females will be evident 2 weeks after mating. Once singly housed, females should be monitored daily to establish the date of birth of the litter. The process of parturition may take over an hour to complete, particularly if the litter is large, and it is best to leave the female undisturbed until this process is completed. After birth, the dam will retrieve her pups to the nest and clean them to remove remnants of the placenta. Once this process is complete, pups can be weighed and counted and placed with the dam into a clean cage, mixing in some of the soiled bedding material from the old cage to provide olfactory continuity and reduce rehousing stress. During the postnatal observations of maternal care, cage cleaning is very disruptive. Cleaning the cage on the day of birth (day 0) will allow for undisturbed assessment of home-cage behavior from postnatal days 1–6 and cages should not be cleaned until after observations are completed on postnatal day 6. We recommend cleaning the cage every 7 days thereafter until weaning. Cleaned cages can then be positioned on the racks in the animal housing room to permit viewing of activity within the cage (see Fig. 1). Observations of maternal behavior can then commence the following day on postnatal day 1.

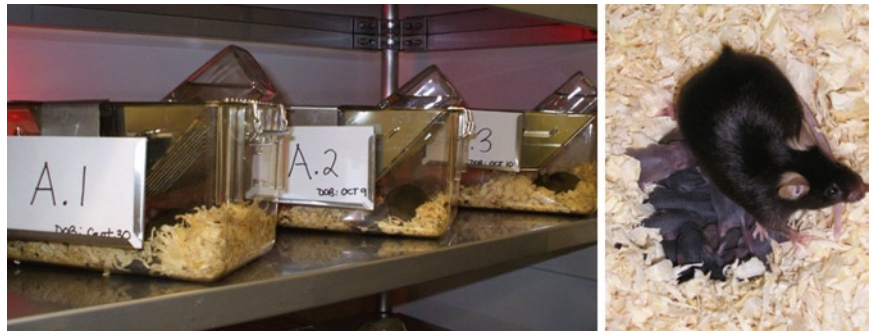

Fig. 1. Photo of home-cage observation set-up (*left*) with unique cage identifiers (*A.1*, *A.2*, *A.3*) and a close-up of a lactating female B6 mouse with a litter of pups (*right*).

3. Procedure

The acquisition of behavioral data from the home-cage can be achieved either through video recordings or by observers who document the behavior in the home-cage from within the animal housing room. The later approach will be preferable when there are numerous cages to be observed and when viewing the mother–pup interactions require dynamic changes in the position of the observer (as is often the case). In both cases, raters/observers will need to be trained to a high degree of inter-rater reliability. The description of maternal behaviors that can be observed is provided in Table 1 and includes nursing postures, LG pups, NB, self-grooming, eating, and drinking. These behaviors are not mutually exclusive and can occur in a variety of combinations. There may be a wide variety of nursing postures that can be observed, and a more detailed account of these postures has previously been described (22). However, for most studies, the behaviors outlined in Table 1 will capture the features of mother–pup interactions that are predictive of long-term outcomes in offspring. Each individual behavior should be assigned a unique alphanumeric identifier (e.g., nursing = N, nest-building = B) and raters/observers should be provided with

Table 1
Description of home-cage maternal behaviors in mice

Behavior	Description	Code
Nursing (crouch)	Dam is positioned over the pups to permit sucking or thermoregulation with a low to moderate arch in her back	N
Arched nursing	Dam is positioned over the pups with a high arch in her back to permit sucking and pup movement	A
Passive nursing	Dam is lying on her side with her ventrum exposed to the sucking pups	P
Licking/grooming	Dam is licking pups (anogenital or body region)	G
Nest-building	Dam is picking up pieces of bedding and retrieving these to the nest or moving bedding in the nest with her snout	B
Self-grooming	Dam is licking herself (often occurs during bouts of pup licking)	S
Eating	Dam is eating	E
Drinking	Dam is drinking	D
Contact with pups	Dam is in contact with the pups but not in a posture that promotes sucking (i.e., sitting next to pups)	C
No contact with pups	Dam is off the nest and not in contact with any pups (and not engaging in any of the other behaviors noted above)	X

a detailed legend outlining these identifiers. The schedule of observations during the day relative to the light cycle should be consistent across days and for each litter. Furthermore, it is important to avoid conducting observations within an hour before or after the light–dark transition in room lighting. It is recommended that a minimum of 4 h of observations (e.g., 11 am, 12 pm, 3 pm, 4 pm) be conducted each day for each litter. Across the postpartum period, there is a significant decline in maternal behavior (particularly LG (20, 21)) and it is generally accepted that the critical window for many long-term developmental effects will require assessment of maternal care from the time of birth to at least 6 consecutive days.

3.1. Time-Sampling Observation of Maternal Behavior

In rodent studies examining the long-term consequences of maternal behavior for offspring development, a typical data collection technique involves time-sampling the home-cage behavior of the dam during each observation session (20, 23). At the start of the observation session, observers should acquaint themselves with the location of each cage to be observed. Data collection sheets should be organized such that there is a row for each "time of sampling" and a column for each litter to be observed (for an example observation sheet, see Fig. 2a). For data collection and analysis, it will be necessary for each cage to have a unique alphanumeric identifier code (see Fig. 1). A stopwatch will also be necessary to time the intervals between observations. Once the observer is ready to commence the data collection, the stopwatch is started and the observer notes the behavior of the dam in the first cage. For example, if the dam is crouched over the pups in a nursing posture and licking the pups, the observer can write "NG." If the dam is eating and not in contact with the pups, the observer can write "E" on the observation sheet. It is important to take a visual "snap-shot" of behavior (lasting 1–2 s) within the cage since the behavior is likely to change if the cage is observed continuously. Once the behavior in the first cage is noted, the observer should move to the next cage and repeat the rating process. Once all of the cages have been observed and the maternal behavior recorded, the observer returns to the first cage and repeats the entire process. A good time-sampling interval is 3 min. When using a 3-min interval, series of observations are conducted at 0, 3, 6 min and so on until the 1-h session is completed. This will result in 20 observations of each cage within the session. With a schedule of four sessions per day across 6 consecutive days, a resulting 480 observations (20 observations × 4 sessions × 6 days) per litter will be obtained.

3.2. Focal Observations of Maternal Behavior

In cases where there are fewer cages to be observed, maternal behavior can also be measured through continuous observation of a single cage. In this case, the observer also needs a stopwatch to note the time at which behaviors emerge or change and data collection sheets can be arranged with multiple rows in which the

A

DATE	10-Oct	
TIME	11AM	
OBSERVER	Frances	
Litter ID	A.1	A.2
0	X	N
3	X	N
6	X	N
9	X	NG
12	X	NG
15	E	NG
18	E	NS
21	E	NS
24	D	NG
27	D	N
30	X	C
33	X	X
36	X	X
39	CB	X
42	C	X
45	C	E
48	N	E
51	NG	E
54	N	B
57	A	B

B

DATE	10-Oct-10
TIME	11AM
OBSERVER	Frances
Litter ID	A.1
TIME	BEHAVIOR
0	off nest
0:56	contact with pups
1:48	pup licking starts
2:38	licking pups stops
2:59	nursing starts
8:09	nursing & nest-building
9:35	nest-building stops
10:20	arched nursing starts
12:34	contact (not nursing)
15:22	crouch nursing
20.41	pup licking starts
28.34	pup licking stops
29.12	self-grooming starts
36.44	self-grooming stops
45.34	off nest
52.11	eating
56.17	eating stops
57.56	nest-building starts
59.33	nest-building stops

Fig. 2. Example observation sheets and maternal behavior ratings. (**a**) Time-sampling observation data of two litters (*A.1* and *A.2*). (**b**) A focal observation of litter *A.1* indicating the start and stop times of maternal behavior.

time and behaviors can be noted (e.g., observation sheet, see Fig. 2b). At the start of the session, the observer will start the stopwatch and note the behaviors that are evident. When there is a transition to another behavior or a current behavior stops, this should also be recorded. This continues for 1 h until the session is completed. This data collection strategy is ideal for looking at the duration and number of bouts of maternal behavior (20) and provides a rich source of information on the temporal patterns of mother–pup interactions.

3.3. Observing Communal Maternal Behavior

Communal rearing of pups is an early life experience that can have a significant impact on behavioral and neurobiological phenotypes related to anxiety and depression (40). Observation of mother–pup interactions in a communal nest can be conducted using a time-sampling procedure as outlined for a single dam and litter. However, this will require individual marking of each dam in the nest. For albino mice, black bands marked on the tail (i.e., 1-band, 2-bands, 3-bands) can clearly distinguish each female (31). For B6 mice, ear punches or tags might be necessary to distinguish between females.

3.4. Cross-Fostering, Handling, and Maternal Separation

Experimental approaches to studying environmental and/or genetic influences on phenotype in rodents often utilize a manipulation of the early rearing experiences. In this context, cross-fostering can be used to alter rearing experiences, and can lead to varying developmental trajectories among offspring (33). However, though most laboratory mice will readily accept foster pups and provide maternal care toward these pups, the genotype or treatment history of the pups may influence the frequency of maternal behavior (34). To maintain significant strain differences in maternal behavior when cross-fostering is being used may require an increased sample size to account for these pup-induced effects. When cross-fostering is done on the day of birth, the behavioral sampling protocol that has been outlined can be used to verify the frequency of maternal care. If the fostering is being done at some point later in the postnatal period, the timing of the manipulation relative to the observation session should be consistent over days and litters and be noted as a potential influence on the observational data collected immediately after the fostering procedure. This strategy should also be used if daily handling or maternal separation is being conducted.

4. Data Analysis

The observation protocols outlined in the previous section result in numerous individual data points that can be used in multiple ways depending on the overall experimental design of the study. There are multiple statistical approaches to using this data and the choice of approach will depend on the independent or dependent variables associated with maternal behavior.

4.1. Calculating Average or Daily Frequencies of Maternal Behavior

Time sampling observational data collected for each litter can be entered into a spreadsheet in which the litter ID, time, and date are noted. Frequencies of the occurrence of each combination of behaviors (e.g., nursing and licking (NG), nursing and eating (NE), nursing and NB) or of a single behavior of interest (e.g., licking – G) can then be calculated. The frequency

of a behavior will be determined by calculating the number of times a behavior occurs as a proportion of the total number of observations conducted. For example, if pup licking (G) is observed to occur 48 times during the 480 observations of a dam, the average frequency of pup licking for that dam would be 10%. A similar approach can be taken if calculating average daily maternal behavior, with the numerator and denominator reflecting the frequency of behavior observed during a single day and the total number of observations conducted during this particular day.

4.2. Determining Low vs. High Maternal Behavior

When observational data is available on numerous dams (i.e., 30–40), a normal distribution of maternal behavior is likely to be evident. As such, females can be selected as engaging in low vs. high levels of a particular aspect of maternal behavior and this grouping may be a useful strategy in subsequent analysis of offspring characteristics. For example, Fig. 3a illustrates a distribution of pup LG behavior collected during postnatal days 1–6 in observations of B6 dams ($n = 40$). The values are the average frequency of observed LG during this 6-day period. Low LG dams are defined as engaging in this behavior at a frequency that is less that 1 SD below the cohort average whereas high LG dams engage in this behavior at a frequency that is greater than 1 SD above the mean. In the sample cohort illustrated in Fig. 3a, the average frequency of LG is 9.46% with a SD of 3.16%. Using this information, low (<6.30%; $n = 7$) and high (>12.62%; $n = 6$) licking grooming dams can be identified, with remaining females classified as "mid" ($n = 27$), as illustrated in Fig. 3b. Offspring born to these dams can undergo subsequent testing to determine the relationship between maternal care and behavioral phenotypes. For example, in the sample cohort described, one male from each litter was assessed in the open-field test at 60 days of age and the duration of time spent in the inner area of the apparatus was measured as an indication of anxiety-like behavior (41). In this sample, using the average LG frequency for each dam yields a significant correlation with time (seconds) spent in the inner area ($r = 0.75$, $p < 0.001$; illustrated in Fig. 3c). One-way ANOVA using the group classifications (low, mid, high) of LG behavior also indicates a significant effect ($F(2,39) = 16.25$, $p < 0.001$), and Tukey's post hoc analysis indicates a significant difference between all three groups ($p < 0.01$). Offspring behavioral data can be presented as a function of the group classification of maternal LG (Fig. 3d).

4.3. Calculating Maternal Behavior Bout Lengths

Focal observational data can be used to assess the frequency and duration of bouts of maternal behavior. This assessment can also be done with time-sampling protocols; however, if the behavior is very short in duration (i.e., less than the interval between observations) then the duration estimates will not be accurate. Assessment of bout duration requires determining the start and stop times of

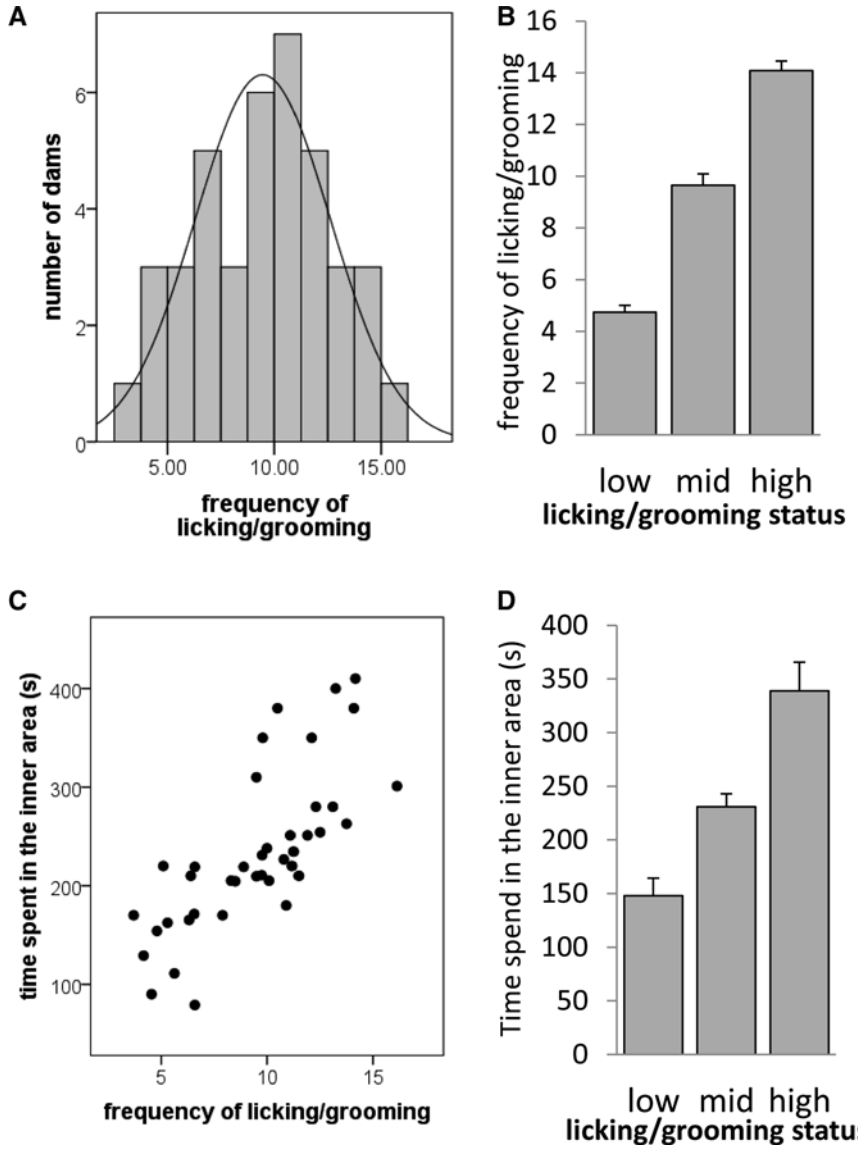

Fig. 3. Analysis of variations in licking/grooming (LG) in B6 mice and relationship to offspring open-field behavior. (**a**) In a cohort of 40 B6 females, average LG behavior observed from postnatal days 1–6 is normally distributed. (**b**) Based on the cohort mean, B6 females can be classified as low, mid, or high in LG behavior. (**c**) Average LG behavior can be used as a continuous variable to permit analysis of the linear correlation with offspring open-field performance (time (s) spent in the inner area of a 10-min open-field test). (**d**) Categorical maternal data (low, mid, high) can likewise be used to compare mean differences in open-field behavior.

the behavior (based on the noted times on the observation record – see Fig. 2b) and calculating the length of time of each start/stop occurrence. The average bout duration for a dam would be the sum of the individual bout durations divided by the total number of bouts. Mice strains differ significantly in the duration and frequency of bouts of nursing and LG, likely reflecting differences in activity levels between strains (21).

4.4. Using Multilevel Modeling in Studies of Maternal Behavior

To analyze change over time, to parse the relationship between two co-occurring variables, or to examine how early maternal care affects later litter neurobiology and behavior, a more sophisticated statistical methodology can be useful. For example, moment to moment, the pups' corticosterone may increase/decrease while interacting with dams, but this lower level effect can be distinct from the higher level effect, i.e., pups with high corticosterone in general tend to have mothers that provide more/less care. Traditional statistical methods would be ill equipped to differentiate between these two effects, but they are easily identifiable with multilevel modeling (42). Multilevel (hierarchical or random effects) modeling is now well established as a standard technique in the statistical toolkit and eminently suited to answer these questions (43, 44). By accounting for repeated observations within a single group (i.e., multiple observations of a single litter), multilevel modeling allows researchers to retain the bulk of their data in raw unaggregated form, thus gaining statistical power and permitting more refined experimental questions. To use this approach, data should be arranged in a spreadsheet so that all similar observations are stacked within a single column/one variable, while observation type (e.g., postnatal day, time) and litter ID are indicated with two other variables. The data are then analyzed as with GLM or regular regression but using a multilevel error structure. These techniques are easily applied in most current statistical packages (43, 45).

5. Experimental Variables and Troubleshooting

5.1. Low Levels of Maternal Behavior with Minimal Variation

Though this may be due to specific characteristics of the strain or genotype being used, a common cause of reduced maternal behavior in rodents are stressors within the physical or social environment. Fluctuations in temperature, humidity, and noise can disturb patterns of mother–pup interactions. Collaborating with animal care staff is essential to maintaining appropriate conditions for assessing home-cage behaviors and observations should be conducted when there are few if any ongoing activities in the housing room. Schedule observations during the day so as to avoid times when routine cage maintenance/cleaning are being conducted in the room.

5.2. Outliers in Maternal and Offspring Behavior

It is not uncommon for there to be dams or offspring that exhibit extremes of behavior. In some cases, there are predictors of when this will occur. Very small litter (i.e., fewer than four pups) or very large litter (i.e., greater than 14 pups) sizes can influence the pattern of maternal behavior, average pup weight, and affect the experience of individual pups within the litters. One approach to address this issue is to cull litters to a standard size (i.e., eight pups) and to

not use very small litters. However, litter size and average pup weight can also be used as covariates in data analyses and may yield interesting interactions between life-history variables and outcome measures. In general, it is best to record as much information as possible on each litter, particularly any adverse experiences that may alter the data.

5.3. High Levels of Mortality

There are often periods of time during the course of the year where breeding success is very low and the mortality of litters is very high. Reproduction in rodents is highly dependent on olfactory cues and any change in the olfactory environment may lead to a reduction in the number of litters produced that survive to weaning age. If the source of the reduced reproductive success cannot be identified, it may be recommended to use a different mouse strain, as there are likely to be strain difference in resilience to these environmental fluctuations.

5.4. Avoiding Observer Effects

Variability in behavioral data can often be linked to the particular characteristics of individual experimenters/observers. In addition to appropriate training to obtain an inter-rater reliability greater than 90%, observers should be provided with detailed instructions regarding the importance of not disturbing the litters while conducting the observations. Cell phones, loud noises, lighted computer screens, and heavy perfumes/aftershaves are definitely to be avoided.

5.5. Making Fine-Tuned Behavioral Distinctions

Training observers is a difficult task. In addition to using video recordings to generate inter-rater reliability, accompanying observers during sessions and providing feedback will improve the quality of the maternal data. This will be particularly important for difficult behavioral categories, such as the distinction between contact and nursing or between self-grooming and pup-grooming.

6. Concluding Remarks

Across species, the quality of the early life environment can shape development leading to increased risk or resilience to later-life disorder. Measures of variation in maternal behavior have proven to be valuable tools in understanding the mechanisms of these developmental effects, and the successful use of mice as a model for these studies is dependent on the appropriate assessment of home-cage maternal behavior. The plasticity of maternal behavior in response to the environment contributes to the challenge of such an approach but with detailed observational data, a meaningful characterization of mother–pup interactions is possible.

This methodology can be applied to studies of induced genetic and neurobiological modification that are being developed in mice and may be a critical methodological approach for examining the interplay between genes and the environment.

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