

Comparison of the “Nursing” and Other Parental Behaviors of Nulliparous and Lactating Female Rats

Joseph S. Lonstein,¹ Christine K. Wagner,
and Geert J. De Vries

*Center for Neuroendocrine Studies, Tobin Hall, Box 37720, University of Massachusetts,
Amherst, Massachusetts 01003*

Received March 24, 1999; revised May 14, 1999; accepted July 2, 1999

Virgin female rats display maternal behaviors after continuous exposure to pups (sensitization) that are in some respects similar to those of postpartum females. We herein provide a detailed comparison of the “nursing” and other parental behaviors of maternally sensitized virgin females and postpartum lactating dams. Ovariectomized and intact virgin females were exposed to pups until displaying maternal behavior. On the females’ fourth day of maternal responsiveness, the pups were removed for 3 h and then returned, and subject–litter interactions were observed for 45 min. Behavior of maternal virgins was compared with that of lactating dams observed on day 4 postpartum interacting with either suckling pups or pups unable to suckle due to perioral anesthesia. Ovariectomy had no effect on behavior of virgins. Retrieval and licking of pups were deficient in virgins compared with lactating dams. Suckled dams showed prolonged kyphosis (upright crouched nursing), whereas nonsuckled dams displayed little kyphosis but rather were often in a hunched position over pups. Some aspects of quiescent “nursing” behaviors of virgins were surprisingly similar to those of suckled dams, including the latency to and duration of quiescence. Nonsuckling pup stimulation elicited more kyphosis in virgins than in lactating dams, which was still much less than in suckled dams. Virgins also “nursed” pups in hunched and prone postures. Differences between sensitized and postpartum females in their maternal behaviors likely reflect differences in motivation as well as sensory inputs they receive from pups. In particular, sensory regulation of “nursing” behaviors is influenced by reproductive state because nonsuckling pups elicit different postural responses in sensitized and lactating mothers. © 1999 Academic Press

Key Words: lactation; maternal behavior; milk ejection; nipples; somatosensation; suckling.

The appearance of robust maternal behavior in rats during the periparturitional period ensures appropriate care of the young at birth and normally requires fluctuations in pituitary and ovarian hormones during pregnancy and parturition for its onset (Bridges, 1996; Numan, 1994; Stern, 1989). Maternal-like behaviors can be observed, however, in nonparturient animals. For example, juvenile rats of both sexes readily contact and carry young pups (Bridges, Zarrow, Goldman, and Denenberg, 1974; Brunelli and Hofer, 1990; Mayer and Rosenblatt, 1979) and adult virgin male and female rats can be induced to display maternal-like behaviors after repeated or continuous exposure to pups (termed sensitization) (Weisner and Sheard, 1933; Rosenblatt, 1967).

Early comparisons between the parental behaviors of sensitized adult virgin female rats and postpartum dams suggested that few differences existed, and those that did were only observable soon after the onset of parenting but not after numerous days of experience with young pups (Fleming and Rosenblatt, 1974; Reisbick, Rosenblatt, and Mayer, 1975). In contrast, subsequent comparisons focusing on retrieval of pups from a runway (Quadagno, DeBold, Gorzalka, and Whalen, 1974) or T-maze (Bridges, Zarrow, Gandelman, and Denenberg (1972); Stern and MacKinnon, 1976) extension of the home cage as a more sensitive measure of maternal motivation revealed both qualitative and quantitative differences between virgins and postpartum mothers. The parental responses of sensitized virgins in these cases were inferior to those of lactating females. Similar differences in retrieval have been observed between maternally sensitized and postpartum mice (Gandelman, Zarrow, and Denenberg, 1970). There has not been a detailed com-

¹ To whom correspondence should be addressed.

parison, though, of the types of quiescent huddling or “nursing” behaviors displayed by parental virgins and postpartum mothers.

It is very likely that differences in the details of their “nursing” behaviors exist and have in fact been anecdotally noted (Fleming and Rosenblatt, 1974). Often prior to nursing, lactating rats actively hover over their litter while repeatedly changing their position until a sufficient number of pups attach to nipples and begin suckling. After ample suckling the dam becomes quiescent and assumes one of numerous distinctive nursing postures (Stern and Johnson, 1990). Kyphosis (Stern, 1996), a nursing posture characterized by rigid limb support and pronounced upward arching of the back (Stern and Johnson, 1990), is one of the most frequently observed nursing postures during the first week of lactation (Stern and Levine, 1972) and requires suckling for its display. Lactating rats that have had their nipples removed or that interact with pups rendered unable to suckle display very little or no kyphosis and less quiescence over pups in any posture. In the absence of suckling-induced quiescence, nonsuckled dams actively hover over pups more and display increased pup licking, self-grooming, and exploration (Lonstein and Stern, 1997a,b; Lonstein, Simmons, Swann, and Stern, 1998; Stern and Johnson, 1990; Stern and Lonstein, 1996; Stern, Dix, Bellomo, and Thramann, 1992). Kyphosis can also be disrupted by destruction of neural sites that transmit (dorsolateral funiculus of spinal cord; Stern, Yu, and Crockett, 1993) or receive (lateral and ventrolateral regions of the caudal midbrain periaqueductal gray (cPAG_{l,vi}); Lonstein and Stern, 1997a; 1998; Lonstein, Simmons, and Stern, 1998) suckling inputs necessary for the mediation of this posture. In the absence of kyphosis, lesioned dams remain highly motivated to remain with the pups and nurse them, but do so in more atypical postures.

Maternally sensitized virgin females are not likely to be as susceptible to the behavioral consequences of ventral stimulation as lactating dams primarily because they do not have developed nipples onto which pups can attach and suckle; without such stimulation, neural sites such as the cPAG_{l,vi} that normally mediate quiescence and kyphosis in response to suckling (Lonstein and Stern, 1997a, 1998; Lonstein *et al.*, 1998a) are probably not highly activated. In the present study, we test the hypothesis that the details of the parental behaviors of maternal virgins differ from those of suckled lactating dams. The regulation of active maternal behaviors such as retrieval and licking could be influenced by differences in the somatosensory inputs

that these mothers receive from pups, as well as reflect differences in their maternal motivation. We also expected that their inactive “nursing” behaviors would differ dramatically because of differences in the ventral somatosensory inputs they receive, particularly suckling. In fact, we hypothesized that the maternal behaviors of sensitized virgins would be more similar to those of lactating dams interacting with pups rendered unable to suckle.

MATERIALS AND METHODS

Subjects

Subjects were thirty-one 90- to 120-day-old virgin female Sprague–Dawley rats born and raised in our colony from rats originally purchased from Taconic (Germantown, NY). Lights were on 12 h/day, beginning at 0700 h, food and water were freely available, and the room temperature was $22 \pm 1^\circ\text{C}$. Parental behavior was compared between sensitized virgin females on their fourth day of maternal responsiveness (virgin group; $n = 15$) and lactating rats on day 4 postpartum exposed to pups either capable of suckling (suckled group; $n = 8$) or not capable of suckling due to anesthetization of their mystacial pads (non-suckled group; $n = 8$). Females used for lactating groups were housed with stud males until a copulatory plug was found. Females were then housed in hanging wire cages in groups of two to three animals/cage until either 3 days before the beginning of sensitization or 3 days prior to the expected day of parturition, after which they were individually housed in polypropylene pan cages ($48 \times 28 \times 16$ cm) with wood shavings for bedding. Litters were each culled to eight pups on the day of birth (day of birth = day 0 postpartum) and remained with their biological mothers until 3 h before behavioral testing.

Ovarian cycling is suspended in postpartum females (e.g., van der Shoot, Lankhorst, de Roo, and de Greef, 1978), but may or may not be suspended in virgin females continuously exposed to pups (Jakubowski and Terkel, 1980; Marinari and Moltz, 1978; Fleming and Rosenblatt, 1974). To determine any effects of ovarian cyclicity on sensitization or subsequent maternal behavior in the virgin females, we observed the behavior of both ovariectomized and gonadally intact maternal virgins. At 60–70 days of age, females to be used for the virgin group were anesthetized with 90 mg/kg ketamine (ip) and 5 mg/kg xylazine (im) and received small bilateral dor-

solateral incisions of the skin and underlying muscle. Seven virgin subjects were ovariectomized while the remaining eight were sham ovariectomized. Muscle incisions were sutured closed and skin was closed with surgical staples. To eliminate the possibility that prior surgical manipulation of virgin females created differences between them and lactating females in their behavior with pups, females in the suckled and nonsuckled lactating groups received sham ovariectomy (incisions and suturing of skin and underlying muscle) 7 days after mating.

Sensitization Procedure

The parental responsiveness of the virgin females after continuous exposure to pups (sensitization) was evaluated as described previously (Mayer and Rosenblatt, 1977). Ten days after surgery, three 1- to 8 day-old freshly fed pups were placed in the subjects' home cage diagonal to where the subject was positioned. Subjects were then observed for 15 min and the incidence of sniffing, retrieving, or licking the pups and hovering over them was recorded. Each morning, the pups were removed from the virgin female's home cage and given to lactating surrogate mothers from our colony that were nursing pups of the same age. Approximately 15 min later, three freshly fed pups that had remained with a lactating dam for at least 24 h were removed from their dam and placed in the subject's home cage for another 15-min behavioral observation. Testing continued for 14 days. Subjects that performed all four behaviors within a single 15-min observation were considered to be fully parental. After 2 consecutive days of performing all four parental behaviors within the test period, subjects were assigned a sensitization latency corresponding to the first day of the two consecutive parental displays. After 2 days of full maternal behavior, virgins received five more freshly-fed pups (total of 8) for the next 2 days until behavioral testing as described below.

Behavioral Testing

Pups were removed from virgin females or lactating dams between 0900 and 1100 h. Pups used during testing were placed in a glass, paper-lined bowl placed under a warm lamp, and covered with a damp paper towel for 3 h. Lactating dams received their own litters of 4-day-old pups for behavioral testing; virgin females received litters of 3- to 5-day-old pups obtained from surrogate lactating dams from our colony. To

compare mother-litter interactions in the presence and in the absence of suckling, pups interacting with lactating dams received either bilateral mystacial pad injections of 0.02 ml of 2.5% lidocaine (Sigma, St. Louis, MO) dissolved in 0.9% saline or control injections of saline 10 min prior to testing. Mystacial pad anesthesia prevents the pups' ability to locate, adhere to, and suckle on a nipple, which greatly reduces the elicitation of kyphosis in their dam (Lonstein *et al.*, 1998b; Lonstein and Stern, 1997a, b; Stern and Johnson, 1990). Perioral lidocaine injections do not, however, alter the activity and rooting behavior of the pups (Lonstein *et al.*, 1998b) and dams display active maternal behaviors such as retrieval and licking toward the active but periorally anesthetized pups (Lonstein and Stern, 1997a, b; Stern and Johnson, 1990). To control for the perioral injections of the litters interacting with lactating dams, litters interacting with maternal virgins also received perioral injections of either lidocaine or saline ($ns = 7$ and 8 litters, respectively) 10 min before testing. Just prior to testing, pups were expressed of feces and urine and weighed.

Pups were scattered in the home cage opposite the nest and subject-litter interactions were continuously recorded for 60 min by an observer unaware of the subject's condition with the aid of a computerized data-acquisition system, similar to that described in detail previously (Stern and Lonstein, 1996). Active behaviors recorded included retrieval of the pups into the nest, sniffing the pups, full body and anogenital licking of the pups (both types combined), mouthing (short distanced oral repositioning of the pups), self-grooming, exploration, and nesting/burrowing in the wood shaving bedding. Contact and nursing behaviors recorded included *hovering over* the pups in the nest (a nonnursing position) while actively performing other behaviors such as licking of pups or self-grooming, and three mutually exclusive quiescent "nursing" postures that females were observed to display over pups: *kyphosis*, or upright crouching over the litter, in a high- or low-arched posture (Stern and Johnson, 1990), laying *prone* on top of the litter mass with little or no limb support, and sitting *hunched* over the litter with the body weight primarily resting on the hind limbs and hind flanks with the forelimbs often passively resting on the litter mass. Without developed nipples and the production of milk, maternal virgins cannot display true nursing behavior and we have used the term "nursing" within quotations to encompass the behavior of both virgin and lactating females. Pup stretch responses to milk receipt (Drewett, Statham, and Wakerley, 1974) also were re-

corded. After the observation period, pups were immediately removed from the dam's cage and weighed. The presence or absence of suckling was determined after behavioral testing by examining the appearance of the nipples, which were wet and protracted if suckling had taken place.

Data Analyses

Latency to hovering over, quiescence in any posture, and kyphosis were calculated as the elapsed time to a continuous period of ≥ 2 min of these behaviors, not interrupted by > 5 s of continuous activity. Total duration of quiescence and kyphosis, however, included bouts of all durations; note that bouts of kyphosis and other quiescent posture ≤ 120 s in duration have been in other studies included as hovering over the pups (Stern and Johnson, 1990; Stern *et al.*, 1992). Mean duration of quiescence and kyphosis bouts was calculated as the mean duration of only bouts > 30 s in length and uninterrupted by another behavior for > 5 s. The subjects' total time spent in physical contact with pups (TTWP) was calculated as the summed duration of time spent hovering over, in kyphosis, hunched, and prone over pups. Total activity includes durations of all active behaviors (carrying pups during retrieval, mouthing, licking, or sniffing pups plus self-grooming, exploring, nesting, eating/drinking). Behavioral data in all cases were analyzed with one-way ANOVAs followed by Fisher's PLSD *post hoc* tests. If either the assumptions of normality or homogeneity of variance were violated, data were analyzed nonparametrically with Kruskal-Wallis *H* analysis of ranks. There was no effect of either ovariectomy or whether pups received perioral lidocaine or saline before testing on any behavioral measure for virgin females during the 45-min continuous observations (all $P_s \geq 0.05$); all maternal virgins were therefore combined into a single group for data analyses ($n = 11$). Statistical significance in all cases was indicated by $P_s \leq 0.05$.

RESULTS

Four of the 15 virgin subjects (2 gonadally intact, 2 ovariectomized) did not display parental behavior after 14 days of pup exposure and were removed from the study. Responding virgin females took a median of 8.5 days of continuous exposure to pups before displaying full maternal behavior during two consecutive daily observations. One lactating dam interact-

ing with pups whose mystacial pads were anesthetized before reunion displayed more than 12 min of kyphosis and was found to have 2 pups attached to nipples at the conclusion of testing (see Stern *et al.*, 1992); this dam was removed from the study.

Active Maternal Behaviors

All females included in the study were highly maternal during testing with pups and had similar latencies to first contact pups after reunion. Measures of the latencies to begin retrieving pups from either reunion or from the first contact with them violated the assumptions of normality and homogeneity of variance and were therefore analyzed nonparametrically. The nonparametric analysis revealed that maternal virgins had a longer latency than lactating dams to begin retrieving after first contacting pups but not from reunion (Table 1). Though most subjects retrieved all eight pups to the nest, maternal virgins were less efficient, taking almost four times as long to retrieve each pup to the nest as lactating dams; differences between groups were statistically significant ($F(2,23) = 6.25$, $P \leq 0.01$; Fig. 1A). Longer durations of retrieval in maternal virgins were due to prolonged time spent in the nest between pup retrievals, rather than an inability to pick up, carry, or deposit the pups in the nest (see Lonstein and Stern, 1998). Licking was the most frequently observed pup-directed active behavior, but was significantly shorter in total duration in maternal virgins than in lactating dams ($F(2,23) = 9.06$, $P \leq 0.01$; Fig. 1B). Nonsuckled dams mouthed pups more and tended to be more active than suckled dams or maternal virgins. There were no significant differences between groups for any other active behavior measured (Table 1).

Inactive Huddling and "Nursing" Behaviors

The latency to begin hovering over the litter after reunion was significantly longer in maternal virgins, though this was due to their longer duration of retrieval; when calculated from the conclusion of retrieval, all dams joined the pups in the nest with a similar latency (Table 2). Groups of lactating dams did not significantly differ in their total time spent with the pups in the nest, though there was a tendency for maternal virgins to be in contact with pups for less time than lactating dams ($F(2,23) = 3.1$, $P \leq 0.06$; Fig. 2). The types of huddling and "nursing" behaviors displayed within the nest, however, were strikingly different between groups. Nonsuckled lactating dams

TABLE 1
Active Behaviors (Mean \pm SEM) of Suckled or Nonsuckled Lactating Females and Maternal Virgin Females Interacting with Pups during a 45-min Observation on the Fourth Day after the Onset of Maternal Behavior

	Suckled	Nonsuckled	Virgin	$F_{(2,23)}/H_{(2)}$
Latency (s)				
Contact pups	3 \pm 1	6 \pm 4	4 \pm 1	0.75
Retrieve pups a \rightarrow				
From reunion	13 \pm 8	16 \pm 5	55 \pm 27	4.36
From first contact	10 \pm 8 ^a	10 \pm 3 ^a	51 \pm 27 ^b	5.97*
Duration (s)				
Mouth pups	4 \pm 1 ^a	12 \pm 2 ^b	3 \pm 1 ^a	10.86*
Nest/burrow	65 \pm 32	99 \pm 32	50 \pm 29	0.63
Self-groom	60 \pm 15	139 \pm 30	93 \pm 26	2.17
Explore	113 \pm 33	152 \pm 35	190 \pm 20	2.00
Total activity	636 \pm 89	963 \pm 73	713 \pm 109	2.54
No. pups retrieved	8.0 \pm 0.0	8.0 \pm 0.0	7.1 \pm 0.7	1.23

Note. Statistically significant *post hoc* differences indicated by different superscript letters ($P \leq 0.05$).

^a Violated assumptions of normality and homogeneity of variance so were analyzed nonparametrically with Kruskal-Wallis *H*.

* ANOVA $P \leq 0.001$.

actively hovered over the litter longer than either suckled lactating dams or maternal virgins ($F(2,23) = 8.3, P \leq 0.002$; Fig. 2). After hovering over the pups for at least 5 min, all dams eventually became quiescent and displayed a "nursing" posture. Nonsuckled dams tended to become quiescent with a longer latency and shorter total duration than either suckled dams or maternal virgins. Suckled dams and maternal virgins did not differ on these measures.

Kyphosis (for any duration) was displayed by all subjects, with the exception of one nonsuckled dam. Groups of females significantly differed in their total duration of kyphosis with suckled dams displaying the longest and nonsuckled dams displaying the shortest total durations ($F(2,23) = 91.6, P \leq 0.0001$; Fig. 2). The duration of kyphosis displayed by maternal virgins was intermediate between the two lactating groups. All responding subjects assumed the low-arched kyphotic posture, and 100% of suckled lactating dams, 71% of nonsuckled dams, and 73% of maternal virgins also showed the high-arched kyphotic posture. Comparison between the display of low- and high-arched kyphosis revealed that suckled lactating dams spent a similar duration of time in each. Nonsuckled lactating dams and maternal virgins displayed approximately three- to four-fold more time in low-arched kyphosis than in the high-arched posture (Table 2). Suckled lactating dams showed more bouts of kyphosis than nonsuckled lactating dams, showed bouts of kyphosis that were longer in duration than those of either nonsuckled dams or maternal virgins,

and were more capable of displaying relatively long uninterrupted bouts of kyphosis than the other groups.

In the absence of kyphosis, nonsuckled lactating dams spent most of their time sitting quiescently over the litter in a hunched posture that was longer in total duration than that in the other groups ($F(2, 23) = 13.6, P \leq 0.0001$; Fig. 2). Maternal virgins, on the other hand, spent as much time hunched over the litter and laying prone on top of them as they did in kyphosis. Laying prone on top of the litter mass was rare in both groups of lactating dams compared with virgins ($F(2, 23) = 5.3, P \leq 0.02$).

Litter weights at the beginning of behavioral observations did not differ between groups ($F(2, 23) = 0.03, P \geq 0.9$, data not shown). All pups interacting with maternal virgins or rendered unable to suckle a lactating dam were observed to be in the nest with the subject during most of the observation period. Nipples of all dams interacting with pups capable of suckling showed evidence of the recent suckling; no evidence of suckling was observed on dams interacting with nonsuckling pups or maternal virgins. Suckling litters with lactating dams displayed 4.3 ± 0.5 stretch responses to milk receipt with a latency of 19 ± 1 min from reunion. Whereas litters permitted to suckle a lactating dam gained weight, those rendered unable to suckle their lactating dam or interacting with maternal virgins lost a small amount of weight (Table 2).

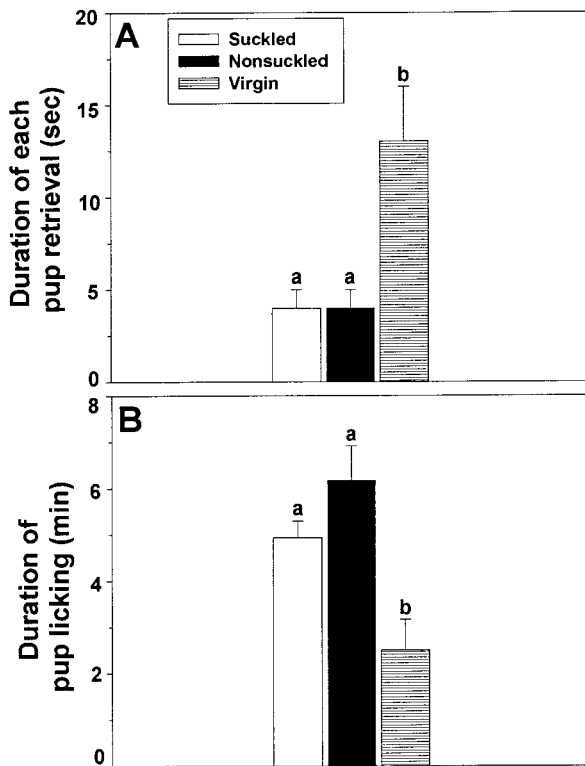


FIG. 1. Duration (mean \pm SEM) of time spent by suckled or nonsuckled lactating dams and sensitized maternal virgins (A) retrieving each pup to the nest and (B) licking the pups during a 45-min interaction with young. Differences between groups are indicated by different lowercase letters above bars, $P \leq 0.05$.

DISCUSSION

Distinct differences existed between maternally sensitized virgin females and postpartum lactating dams in their active maternal behaviors as well as in their quiescent huddling and "nursing" behaviors. Both retrieval and licking of pups were deficient for virgins compared with lactating dams and "nursing" behaviors were relatively unique in maternal virgins, being similar in some respects to suckled lactating dams and in other respects to nonsuckled lactating dams.

Active Maternal Behaviors

Our finding that the latency to begin retrieving pups was longer for maternal virgins compared with lactating female rats is consistent with many previous reports (Bridges *et al.*, 1972; Fleming and Rosenblatt, 1974; Quadagno *et al.*, 1974; Stern and MacKinnon, 1976). Their retrieval deficiencies also extended to the duration of time taken to successfully retrieve each

pup. Differences between virgin and postpartum mothers in their retrieval of pups have frequently been attributed to differences between their emotional state, such that less fearful lactating mothers (see Lonstein *et al.*, 1998a) may be more motivated to retrieve pups under relatively adverse conditions (Bridges *et al.*, 1972; Stern and MacKinnon, 1976). Sensitized virgin females are not less fearful than nonmaternal virgins (Hansen, 1990; Hard and Hansen, 1985) and their normal levels of fear and anxiety may contribute to their inferior retrieving. This cannot fully explain these behavioral differences in retrieval because retrieval within the home cage would not be expected to generate fear or anxiety. It is more likely that the differences between maternal virgins and postpartum dams in the endocrine history are responsible for their differences in retrieval. In support, exogenous steroid hormones elicit retrieval behavior in virgins similar to that seen in parturient mothers (e.g., Stern and MacKinnon, 1976). Furthermore, ovarian hormones increase the sensitivity of the dam's perioral region (Beiter and Barker, 1975), stimulation of which is necessary for retrieval and licking of pups (Stern and Johnson, 1989; Stern and Kolonie, 1991; Stern, 1997). The absence of elevated ovarian hormones and potentially heightened perioral sensitivity may result in prolonged retrieval in maternal virgins due to pauses between each pup that is carried back to the nest (Stern, 1997). The relationship between fear and anxiety and pup retrieval in sensitized virgin females is further complicated by the fact that the hormones that induce their maternal behavior either decrease (Fleming, Cheung, Myhal, and Kessler, 1989) or have no effect on (Hansen, 1990) anxiety. Additionally, we have recently found that neonatal treatment with the progesterone receptor antagonist RU486 decreases fearfulness in an elevated plus-maze but also tends to decrease sensitization in adult virgin rats (Lonstein, Quadros, and Wagner, submitted for publication).

Maternal virgins in the present study also displayed half the duration of licking the pups as lactating dams. This may be related to the latter group's increased physiological demands due to lactation and their need to recycle electrolytes and water from pup urine (Gubernick and Alberts, 1983). In the absence of increased metabolic requirements, maternal virgins may be less motivated to anogenitally lick the pups. In contrast, Fleming and Rosenblatt (1974) found that virgins tended to lick pups more than lactating dams, and Gubernick and Alberts (1987) found no difference between them. Methodological differences between the present and previous reports, such as the duration of

TABLE 2
Measures (Mean \pm SEM) of Quiescent Huddling and Nursing Behaviors for Suckled or Nonsuckled Lactating Females and Maternal Virgin Females Interacting with Pups during a 45-min Observation on the Fourth Day after the Onset of Maternal Behavior

	Suckled	Nonsuckled	Virgin	$F_{(2,23)}$
Latency (s)				
Hover over				
From reunion	137 \pm 38 ^a	111 \pm 16 ^a	239 \pm 31 ^b	4.89*
From end of retrieval	98 \pm 38	72 \pm 13	138 \pm 37	0.95
Quiescence				
From reunion	538 \pm 94 ^a	1103 \pm 170 ^b	426 \pm 67 ^a	10.89**
From hover over	401 \pm 84 ^a	992 \pm 156 ^b	188 \pm 44 ^a	19.90***
Duration (s)				
Total quiescence	1761 \pm 129	1235 \pm 96	1638 \pm 161	3.10 [†]
High kyphosis	808 \pm 80 ^a	32 \pm 14 ^b	113 \pm 35 ^b	67.35***
Low kyphosis	714 \pm 83 ^a	94 \pm 28 ^b	449 \pm 61 ^c	20.12***
Longest sustained kyphosis	946 \pm 168 ^a	40 \pm 11 ^b	159 \pm 23 ^b	27.42***
Longest sustained quiescence	1208 \pm 187	531 \pm 113	895 \pm 197	2.92 [†]
Mean kyphosis bout	422 \pm 90 ^a	36 \pm 12 ^b	81 \pm 11 ^b	17.61***
Mean quiescence bout	700 \pm 193	191 \pm 50	505 \pm 188	1.86
No. kyphosis bouts	4.5 \pm 0.7 ^a	1.3 \pm 0.5 ^b	5.5 \pm 0.8 ^a	8.47**
No. quiescence bouts	3.6 \pm 0.8 ^a	7.0 \pm 0.9 ^b	4.8 \pm 0.6 ^{ab}	4.48*
Relative litter weight change (g)	+3.8 \pm 0.9	-0.4 \pm 0.1	-0.5 \pm 0.1	24.58***

Note. Statistically significant *post hoc* differences between groups indicated by different superscript letters ($P \leq 0.05$). Total durations of quiescence and kyphosis include bouts of all durations; longest sustained durations, mean bout durations, and number of bouts include only bouts >30 s in length.

[†] ANOVA $P \leq 0.07$.

* ANOVA $P \leq 0.05$.

** ANOVA $P \leq 0.02$.

*** ANOVA $P \leq 0.001$.

mother-litter separation before behavioral testing and duration of the behavioral test itself, may explain the disparate results. The effects of ovariectomy on sensitized virgins' maternal behavior are also unclear because though we found no differences between intact and ovariectomized females, others have reported poor maternal behavior in the latter (LeRoy and Krehbiel, 1987). Finally, virgin females with more extensive maternal experience than the 4 days provided in the present study may show fewer deficits in their retrieval and licking behavior when compared with similarly experienced postpartum dams (Fleming and Rosenblatt, 1974).

Inactive Huddling and "Nursing" Behaviors

Females in all groups spent similar amounts of time with the pups, indicating that all were highly motivated to remain in physical contact with the young. The relative absence of kyphosis, and concomitant increase in hovering over the pups, in nonsuckled versus suckled lactating dams is consistent with pre-

vious reports (Lonstein and Stern, 1997a, b; Stern and Johnson, 1990; Stern *et al.*, 1992). The preferential display of a hunched posture by nonsuckled lactating dams observed in the present study, however, has not been previously described in similarly treated dams (Lonstein and Stern, 1997a, b; Stern and Johnson, 1990; Stern *et al.*, 1992). The assumption of the hunched posture in nonsuckled dams may have been previously overlooked due to the fact that in these previous reports bouts of quiescence (in any posture) ≤ 120 s in duration were recategorized for data analyses as hovering over the pups. Many of these short bouts of quiescence may actually have been while dams were hunched over pups and is supported by the fact that quiescence bouts were very short (~ 3 min) for our nonsuckled dams. As opposed to unmanipulated dams interacting with periorally anesthetized pups, when sensory inputs from the nipples and surrounding area are severely reduced via local anesthetic, dams lay in a prone posture over pups (Stern *et al.*, 1992). This would suggest that the hunched posture requires at least nonsuckling stimulation of the nip-

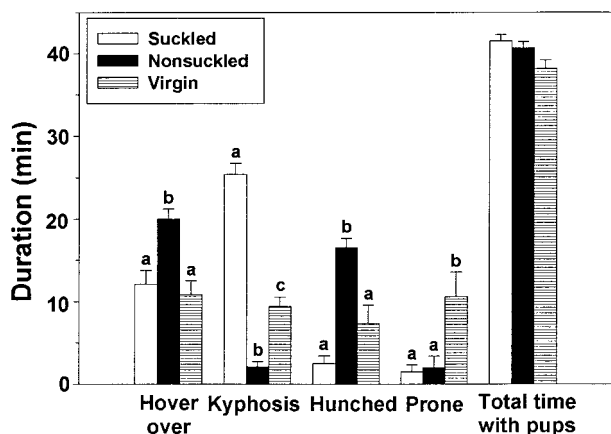


FIG. 2. Duration (mean \pm SEM) of time spent hovering over and in nursing postures by suckled lactating dams, nonsuckled dams, or sensitized maternal virgin females, during a 45-min interaction with pups. Differences between groups are indicated by different lower-case letters above bars, $P \leq 0.05$.

ples and ventrum. Dams with transections of the dorsolateral columns of the spinal cord also frequently display the hunched posture over pups (Stern *et al.*, 1993). Since spinal lesions may decrease nonnoxious sensory transmission from the entire ventrum, not just the nipples, the regulation of the hunched posture requires further investigation.

In the absence of suckling, one may have expected the quiescent behaviors of virgin females to be dissimilar to those of suckled lactating dams and more similar to those of nonsuckled dams. This surprisingly was not the case. Indeed, the latency of maternal virgins to become quiescent over pups, their number of bouts of kyphosis, duration of longest bout of quiescence, as well as durations of hovering over, quiescence in any posture, and sitting hunched over the pups were more similar to those of suckled dams than nonsuckled dams. The only apparent similarity between the behavior of virgin females and that of nonsuckled dams was their relatively low total durations of kyphosis, their inability to sustain it, and its appearance mostly in the low-arched posture. This detail of the "nursing" behavior in virgin females could have been predicted considering Fleming and Rosenblatt's (1974) anecdotal note that "a true lactating posture, with hind feet spread and back raised in an arch, was maintained for much longer periods by lactating animals than by virgins" and that maternal virgins "...would adopt an arched lactating position which would frequently degenerate into a partial flattening of the body over the pups." (pp. 964–965). They were presumably referring to the assumption of kyphosis

by virgin females that invariably deteriorated into a flattened prone posture, as observed in the present study.

Total durations of kyphosis were relatively low in both nonsuckled groups, but were almost fivefold greater in maternal virgins than in nonsuckled dams. This suggests differential sensory regulation of this posture in sensitized and lactating mothers. Under certain conditions, stimulation provided by active pups rooting in their dam's ventrum in search of a nipple produces cellular (Lonstein *et al.*, 1998b; Xerri, Stern, and Merzenich, 1994), endocrine (Jakubowski and Terkel, 1986; Stern and Siegel, 1978), and behavioral consequences in female rats (Stern, 1991). Rooting by pups is not adequate, though, for maximal *c-fos* transcription within the cPAG or the elicitation of kyphosis in normal lactating dams (Lonstein and Stern, 1997a, b), but is apparently somewhat effective for eliciting kyphosis in sensitized virgins. Female rats may have a basal level of responsiveness to rooting pups that promotes kyphosis which is lost soon after experience with suckling pups. After this experience, only suckling may be able to reliably elicit the posture. Though ovarian hormones influence peripheral somatosensory processing (e.g., Kow, Montgomery, and Pfaff, 1979; Rose and Beirber, 1984), the appearance of kyphosis even in ovariectomized maternal virgins suggests that these hormones are relatively unimportant for its display. Ventral stimulation provided by active pups produces kyphosis in nipple-less male prairie voles (*Microtus ochrogaster*) (Lonstein and De Vries, 1999), a member of a biparental species, and offers another example of how different hormonal and sensorimotor mechanisms can produce similar behaviors in parental mammals.

We do not know why nonsuckled lactating females spent more time in the hunched posture, whereas nonsuckled virgin females spent similar amounts of time in all quiescent postures. The greater propensity of virgin females to lay prone on the litter may indicate a relative insensitivity of their ventral skin since lactating dams with completely anesthetized ventrums also lay prone on top of pups (Stern *et al.*, 1992). The prone posture is the least effective nursing posture for milk letdown in lactating dams and probably offers the pups the least room to either move or breathe, as well as places the most weight of the female upon them (Lonstein *et al.*, 1998a). The high frequency of this posture in maternal virgins is another indicator of their inferiority in caring for the young.

It should be noted that though virgin and nonsuckled lactating females had similar durations of prior

experience with pups before behavioral testing, the nature of this experience differed. Whereas previous mother–litter interactions for the lactating dams included suckling, virgins experienced only nonsuckling interactions. Both received nonsuckling interactions during behavioral testing. One could argue that the behavioral alterations in the nonsuckled lactating dams were due not only to the lack of nipple stimulation, but also due to disruptions resulting from their inexperience with nonsuckling pups. This is probably not the case because when dams have their nipples removed prior to parturition (thelectomized), their active and inactive maternal behaviors are very similar to that of dams with nipples interacting with nonsuckling pups for the first time (Stern and Johnson, 1990; Stern *et al.*, 1992). These groups of dams have different types of experience with pups before behavioral testing (thelectomized dams have only nonsuckling interactions and nipples dams have only suckling interactions), but display similar behaviors during testing. This indicates that the absence of suckling is the important contributor to behavioral differences between suckled and nonsuckled dams and that experience with suckling or nonsuckling pups probably did not generate the behavioral differences between our nonsuckled lactating and virgin females.

Conclusions

It is unlikely that feral virgin female rats would have either no experience with young pups until adulthood (Calhoun, 1963; Davis and Hall, 1951; Gilbert, Burgoon, Sullivan, and Adler, 1983; Gray and Chesley, 1984; Stern and Rogers, 1988) or repeated access as adults to another female's litter (Calhoun, 1963). The sensitization paradigm is therefore an unnatural, but potentially useful, model to examine nonhormonal factors regulating maternal behavior. Since sensitized and postpartum females differ in both their endocrine histories and the somatosensory inputs they receive from pups, it may be difficult to ascribe differences in their behavior to only one of these factors. The active behaviors of maternally sensitized female rats differed in numerous respects from those of either suckled or nonsuckled lactating dams, which likely reflects both motivational and somatosensory differences between these groups. In addition, differences in the sensory regulation of "nursing" behaviors apparently exist that may be partly related to the female rat's reproductive state. These results emphasize the fact that maternally sensitized virgin female rats simply cannot be conceptualized as nonsuckled postpartum mothers.

ACKNOWLEDGMENTS

The authors thank Dr. Judith M. Stern and three anonymous reviewers for constructive comments that greatly improved this article and Mr. Ross Lonstein for creating the data acquisition software used in the present study. This research was supported by NIMH Grant 58006 and NICHD Grant 37244 to C. K. Wagner, NIMH Grant 47538 to Geert J. De Vries, and NICHD postdoctoral NRSA 08392 to J. S. Lonstein.

REFERENCES

- Bereiter, D. A., and Barker, D. J. (1975). Facial receptive fields of trigeminal neurons: Increased size following estrogen treatment in female rats. *Neuroendocrinology* **18**, 115–124.
- Bridges, R. (1996). Biochemical basis of parental behavior in the rat. In J. S. Rosenblatt and C. T. Snowden (Eds.), *Parental Care: Evolution, Mechanisms, and Adaptive Significance*, Advances in the Study of Behavior, Vol. 25, pp. 215–237. Academic Press, New York.
- Bridges, R., Zarrow, M. X., Gandelman, R., and Denenberg, V. H. (1972). Differences in maternal responsiveness between lactating and sensitized rats. *Dev. Psychobiol.* **5**, 123–127.
- Bridges, R. S., Zarrow, M. X., Goldman, B. D., and Denenberg, V. H. (1974). A developmental study of maternal responsiveness in the rat. *Physiol. Behav.* **12**, 149–151.
- Brunelli, S. A., and Hofer, M. A. (1990). Parental behavior in juvenile rats: Environmental and biological determinants. In N. A. Krasnegor and R. S. Bridges (Eds.), *Mammalian Parenting: Biochemical, Neurobiological and Behavioral Determinants*, pp. 372–399. Oxford Univ. Press, New York.
- Calhoun, J. B. (1963). *The Ecology and Sociology of the Norway Rat*, Public Health Service Publication 1008, Washington, DC.
- Davis, D. E., and Hall, O. (1951). The seasonal reproductive condition of female Norway (Brown) rats in Baltimore, Maryland. *Physiol. Zool.* **24**, 333–336.
- Drewett, R. F., Statham, C., and Wakerley, J. B. (1974). A quantitative analysis of the feeding behavior of suckling rats. *Anim. Behav.* **22**, 907–913.
- Fleming, A. S., and Rosenblatt, J. S. (1974). Maternal behavior in the virgin and lactating rat. *J. Comp. Physiol. Psychol.* **86**, 957–972.
- Fleming, A. S., Cheung, U., Myhal, N., and Kessler, Z. (1989). Effects of maternal hormones on "timidity" and attraction to pup-related odors in female rats. *Physiol. Behav.* **46**, 449–453.
- Gandelman, R., Zarrow, M. X., and Denenberg, V. H. (1970). Maternal behavior: Differences between mother and virgin mice as a function of the testing procedure. *Dev. Psychobiol.* **3**, 207–214.
- Gilbert, A. N., Burgoon, D. A., Sullivan, K. A., and Adler, N. T. (1983). Mother–weanling interactions in Norway rats in the presence of a successive litter produced by postpartum mating. *Physiol. Behav.* **30**, 267–271.
- Gray, P., and Chesley, S. (1984). Development of maternal behavior in nulliparous rats (*Rattus norvegicus*): Effects of sex and early maternal experience. *J. Comp. Psychol.* **98**, 91–99.
- Gubernick, D. J., and Alberts, J. R. (1983). Maternal licking of young: Resource exchange and proximate controls. *Physiol. Behav.* **31**, 593–601.
- Gubernick, D. J., and Alberts, J. R. (1985). Maternal licking by virgin and lactating rats: Water transfer from pups. *Physiol. Behav.* **34**, 501–506.

- Hansen, S. (1990). Mechanisms involved in the control of punished responding in mother rats. *Horm. Behav.* **24**, 186–197.
- Hard, E., and Hansen, S. (1985). Reduced fearfulness in the lactating rat. *Physiol. Behav.* **35**, 641–643.
- Jakubowski, M., and Terkel, J. (1980). Induction by young of prolonged dioestrus in virgin rats behaving maternally. *J. Reprod. Fertil.* **58**, 55–60.
- Jakubowski, M., and Terkel, J. (1986). Nocturnal surges and reflexive release of prolactin in parentally behaving virgin female and male rats. *Horm. Behav.* **20**, 270–286.
- Kow, L. M., Montgomery, M. D., and Pfaff, D. W. (1979). Triggering of lordosis reflex in female rats with somatosensory stimulation: Quantitative determination of stimulus parameters. *J. Neurophysiol.* **42**, 195–202.
- LeRoy, L. M., and Krehbiel, D. A. (1978). Variations in maternal behavior in the rat as a function of sex and gonadal state. *Horm. Behav.* **11**, 232–247.
- Lonstein, J. S., and De Vries, G. J. (1999). Comparison of the parental behavior of pairbonded male and female prairie voles (*Microtus ochrogaster*). *Physiol. Behav.* **66**, 33–40.
- Lonstein, J. S., Quadros, P. S., and Wagner, C. K. Effects of neonatal RU486 on adult sexual, parental, and fearful behaviors in rats. Submitted for publication.
- Lonstein, J. S., and Stern, J. M. (1997a). Role of the midbrain periaqueductal gray in maternal nurturance and aggression: *c-fos* and electrolytic lesion studies in lactating rats. *J. Neurosci.* **17**, 3364–3378.
- Lonstein, J. S., and Stern, J. M. (1997b). Somatosensory determinants of *c-fos* activity in the periaqueductal gray of lactating rats: Role of suckling, rooting and perioral stimulation from pups. *Horm. Behav.* **32**, 155–166.
- Lonstein, J. S., and Stern, J. M. (1998). Site and behavioral specificity of periaqueductal gray lesions on postpartum sexual, maternal, and aggressive behaviors in rats. *Brain Res.* **804**, 21–35.
- Lonstein, J. S., Simmons, D. A., and Stern, J. M. (1998a). Functions of the caudal periaqueductal gray in lactating rats: Kyphosis, lordosis, maternal aggression, and fearfulness. *Behav. Neurosci.* **112**, 1502–1518.
- Lonstein, J. S., Simmons, D. A., Swann, J. M., and Stern, J. M. (1998b). Forebrain expression of *c-fos* due to active maternal behaviour in lactating rats. *Neuroscience* **82**, 267–281.
- Marinari, K. T., and Moltz, H. (1978). Serum prolactin levels and vaginal cyclicity in concaveated and lactating female rats. *Physiol. Behav.* **21**, 525–528.
- Mayer, A. D., and Rosenblatt, J. S. (1977). Effects of intranasal zinc sulfate on open field and maternal behavior in female rats. *Physiol. Behav.* **18**, 101–109.
- Mayer, A. D., and Rosenblatt, J. S. (1979). Ontogeny of maternal behavior in the laboratory rat: Early origins in 18- to 27-day-old young. *Dev. Psychobiol.* **12**, 407–424.
- Numan, M. (1994). Maternal behavior. In E. Knobil and J.D. Neill (Eds.), *The Physiology of Reproduction*, Vol. 2, pp. 221–302. Raven Press, New York.
- Quadagno, D. M., DeBold, J. F., Gorzalka, B. B., and Whalen, R. E. (1974). Maternal behavior in the rat: Aspects of concaveation and neonatal androgen treatment. *Physiol. Behav.* **12**, 171–1074.
- Reisbick, S., Rosenblatt, J. S., and Mayer, A. D. (1975). Decline of maternal behavior in the virgin and lactating rat. *J. Comp. Physiol. Psych.* **89**, 722–732.
- Rose, J. D., and Bieber, S. L. (1984). Joint and separate effects of estrogen and progesterone on responses of midbrain neurons to lordosis-controlling somatic stimulation in the female golden Syrian hamster. *J. Neurophysiol.* **51**, 1040–1054.
- Rosenblatt, J. S. (1967). Nonhormonal basis of maternal behavior in the rat. *Science* **156**, 1512–1514.
- Stern, J. M. (1989). Maternal behavior: Sensory, hormonal, and neural determinants. In F. R. Brush and S. Levine (Eds.), *Psychoneuroendocrinology*, pp. 103–226. Academic Press, San Diego.
- Stern, J. M. (1991). Nursing posture is elicited rapidly in maternally naive, haloperidol-treated female and male rats in response to ventral trunk stimulation from active pups. *Horm. Behav.* **25**, 504–517.
- Stern, J. M. (1996). Somatosensation and maternal care in Norway rats. In J. S. Rosenblatt and C. T. Snowden (Eds.), *Parental Care: Evolution, Mechanisms, and Adaptive Significance*. Advances in the Study of Behavior, Vol. 25, pp. 243–294. Academic Press, New York.
- Stern, J. M. (1997). Trigeminal lesions and maternal behavior in Norway rats. III. Experience with pups affects retrieval. *Dev. Psychobiol.* **30**, 115–126.
- Stern, J. M., and Johnson, S. K. (1989). Perioral somatosensory determinants of nursing behavior in Norway rats (*Rattus norvegicus*). *J. Comp. Psychol.* **103**, 269–280.
- Stern, J. M., and Johnson, S. K. (1990). Ventral somatosensory determinants of nursing behavior in Norway rats. I. Effects on variations in the quality and quantity of pup stimuli. *Physiol. Behav.* **47**, 993–1011.
- Stern, J. M., and Kolunje, J. M. (1991). Trigeminal lesions and maternal behavior in Norway rats: I. Effects of cutaneous rostral snout denervation on maintenance of nurturance and maternal aggression. *Behav. Neurosci.* **105**, 984–997.
- Stern, J. M., and Levine, S. (1972). Pituitary–adrenal activity in the post-partum rat in the absence of suckling stimulation. *Horm. Behav.* **3**, 237–246.
- Stern, J. M., and Lonstein, J. S. (1996). Nursing behavior is impaired in small nestboxes and with hyperthermic pups. *Dev. Psychobiol.* **29**, 101–122.
- Stern, J. M., and MacKinnon, D. A. (1976). Postpartum, hormonal, and nonhormonal induction of maternal behavior in rats: Effects on T-maze retrieval of pups. *Horm. Behav.* **7**, 305–316.
- Stern, J. M., and Rogers, L. (1988). Experience with younger siblings facilitates maternal responsiveness in pubertal Norway rats. *Dev. Psychobiol.* **21**, 575–589.
- Stern, J. M., and Siegel, H. I. (1978). Prolactin release in lactating, primiparous, and multiparous thelectomized and maternal virgin rats exposed to pup stimuli. *Biol. Reprod.* **19**, 177–182.
- Stern, J. M., Yu, Y-L., and Crockett, D. P. (1993). Spinal pathway mediating suckling-induced nursing behavior and neuroendocrine reflexes. *Soc. Neurosci. Abstr.* **19**, 1610.
- Stern, J. M., Dix, L., Bellomo, C., and Thramann, C., (1992). Ventral trunk somatosensory determinants of nursing behavior in Norway rats. 2. Role of nipple and surrounding sensations. *Psychobiology* **20**, 71–80.
- Van der Shoot, P., Lankhorst, R. R., de Roo, J. A., and de Greef, W. J. (1978). Suckling stimulus, lactation, and suppression or ovulation in the rat. *Endocrinology* **103**, 949–956.
- Weisner, B. P., and Sheard, N. M. (1933). *Maternal Behavior in the Rat*. Oliver and Boyd, London.
- Xerri, C., Stern, J. M., and Merzenich, M. M. (1994). Alterations of the cortical representation of the rat ventrum induced by nursing behavior. *J. Neurosci.* **14**, 1710–1721.