Comparative Analysis of Maternal Care in the High-Yawning (HY) and Low-Yawning (LY) Sublines From Sprague–Dawley Rats

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ABSTRACT: High- and low-yawning rats (HY and LY) were selectively bred as a function of their spontaneous yawning frequency with the LY subline about 2 yawns/hr and the HY 20 yawns/hr. The HY rats have more grooming bouts and travel longer distances in an open field. HY dams spent less time in the nest, retrieved their pups faster, and show a longer latency to licking and mouthing the pups than the LY or outbred Sprague–Dawley (SD) animals. The percentage of HY dams that had atypical retrieving was higher, with a lower nest quality, and produced offspring whose weights were lower than those from the LY subline. We also showed that the pregnant HY dams have fewer pups and the percentage that had lost at least three pups during lactation was higher than the SD and LY dams. In conclusion, HY dams are motivated to take care of their pups, but the “fine tuning” of maternal care is disturbed. © 2010 Wiley Periodicals, Inc. Dev Psychobiol

Keywords: yawning; grooming; anxiety; retrieving; nest building; inbred rats; lactation; animal model; dopamine; stress

INTRODUCTION

Social interactions among members of a community are important for their organization and survival. These interactions need the display of affiliative behaviors such as maternal care, sexual behavior, and grooming, compared to the antagonistic ones such as aggression, isolation, and submissive behaviors. In altricial species, maternal behavior is the most important and most studied behavior not only because it allows the immature offspring to become independent over time, but it is also a good model for the offspring’s physiological, neuroendocrine, and cognitive display as adults (Beach & Jaynes, 1954; Hofer, 1994). Maternal behavior is the expression of a series of motor and somatosensory events by the mother at the end of pregnancy, parturition, and during the postnatal preweaning period (Rosenblatt, 1967; Rosenblatt & Lehrman, 1963). In rodents, once the pups are born, the mother retrieves them to the nest, licks their bodies and the anogenital region followed by a nursing posture over them in a highly stereotyped and defined organization (González-Mariscal & Poindron, 2002; Rosenblatt & Lehrman, 1963). Thus, the offspring receive warmth, nutrients, protection, and sensory and social stimulation in the form of social contact with the mother and their siblings (Beach & Jaynes, 1954; Hofer, 1994; Levine, Haltmeyer, Karas, & Denenberg, 1967). In rats and mice, differences in the timing and components of maternal behavior have been described over the course of the nurturing period, variations that mediate behavioral transmission of traits and thus transgenerational or nongenomic transmission to the offspring (Champagne,
Francis, Mar, & Meaney, 2003; Fleming et al., 2002). There are variations in mother–infant interactions within a same strain, that is, natural variations (Champagne, Curley, Keverne, & Bateson, 2007; Champagne et al., 2003; Meaney, 2001). For example, by choosing the extremes of the populations of the Long-Evans rats and comparing the frequency that the mothers lick their pup’s body and genitals, and their nursing posture, Meaney and his group had found that there are mothers that show high levels of licking and arched-back nursing (HG-ABN) and other mothers that show low levels of these behaviors (LG-ABN; Champagne et al., 2003). Interestingly, lactating rats from HG-ABN dams show a low reactivity of the hypothalamus–pituitary–adrenal (HPA) axis after exposure to stressful environment, with a small fear response, a good level of spatial learning, and mainly spent much time licking and nursing their pups compared to the LG-ABN dams. These differences are not caused by the genetic background because cross-fostering studies have shown that the offspring phenotypes depend on the mother that reared it (Champagne et al., 2003). Experimentally, it is possible to regulate how the mothers take care of their offspring. If lactating rats are exposed to a handling paradigm (pups are removed from the nest for 15 min), the dams spend more time licking the body and genitals of the pup’s when they are returned to the nest (Meaney et al., 1985; Pryce, Bettschen, & Feldon, 2001). Contrary to the handling paradigm, mothers who had been isolated from their mothers during infancy spent less time taking care of her pups (González, Lovic, Ward, Wainwright, & Fleming, 2001; Melo, Hernández-Curiel, & Hoffman, 2009; Melo et al., 2006). Different groups of mice showed remarkable variations in the expression of maternal behavior (Anisman, Zaharia, Meaney, & Merali, 1998; Brodia & Sware, 1982; Brown, Mathieson, Stapleton, & Neumann, 1999; Champagne et al., 2007; Ohta, Shiroma, Tohei, & Taya, 2002; Shoji & Kato, 2006). Thus, lactating female mice of C57BL/6, CBA/H, C3H/Ico, and CBA/J strains retrieved pups faster than BALB/c, NBZ, DBA/2, XLII, A/J, and AKR strains (Carlier, Roubertoux, & Cohen-Salmon, 1982). Furthermore, DBA/2J females built better nests and spent more time crouching over and nursing pups (Brown et al., 1999) than C57BL/6J dams.

A comparison between inbred and outbred mice has shown that the 129Sv strain had shorter latencies in nest building, built the nest less frequently, and spent less time engaged in licking the pups than outbred dams (Broida & Sware, 1983; Champagne et al., 2007; Meaney, 2001). In rats, psychogenetic selection has resulted in at least four different strains that, besides their own phenotype, have variations in postpartum maternal behaviors; (1) The Flinders Sensitive Line (FSL), considered a genetic animal model of depression, spent less time licking the pups and nursing them (Lavi-Avnon, Yadid, Overstreet, & Weller, 2005), a shorter latency to first pup retrieval, and more self-directed behavior than controls (Braw et al., 2009); (2) spontaneously hypertensive rats (SHR) more often had an arched and blanket-nursing posture and a lesser passive-nursing posture, spent less time licking their pups, and retrieved them more quickly than the Wistar strain (Myers, Brunelli, Squire, Shindeldecker, & Hofer, 1989); (3) Roman high (RHA-Verh)- and low (RLA-Verh)-avoidance sublines of rats were selected and bred for their rapid response compared to poor acquisition in a two-way active-avoidance response (Steimer, Escorihuela, Fernández-Teruel, & Driscoll, 1998). Female rats of RHA-Verh mothers had a high active avoidance, spent less time with their young, are more active, and also assumed the side-nursing position less often than the RLA-Verh mothers (Driscoll, Fümm, & Bättig, 1979); and (4) Hatano high- (HAA) and low- (LAA)-avoidance selective-breeding lines from the Sprague-Dawley strain (Ohta, Matsumoto, Nagao, & Mizutani, 1998) show a high variation in the expression of maternal behavior, with the low avoidance (LAA) females having longer latencies for retrieving the pups, spent less time with them, showed a decreased amount of milk ejection, a lesser increase in blood prolactin, and a greater increase of adrenocorticotropic hormone (ACTH) than the HAA mothers (Ohta et al., 2002).

Although the behavioral differences among inbred groups of mice or rats are attributed to genetic variations, it has been reported that the genetic–environment interactions early in life, mainly mediated by maternal care and their siblings, are the main cause of those variations (Francis, Szegda, Campbell, Martin, & Insel, 2003; Myers et al., 1989; Ohta et al., 1998; Shoji & Kato, 2009; Steimer & Driscoll, 2005). These data show that, besides the phenotype used, during inbreeding the process can generate other changes of the behavioral display that could be caused by maternal care.

Yawning is a phylogenetically old behavior and stereotypically shown by reptiles, fish, birds, and mammals (Walusinski & Deputte, 2004). It consists of a wide opening of the mouth with a long inspiration, followed by a short expiration. Yawning can be modulated by several peptides such as adrenocorticotropic hormone, alpha-melanocyte stimulating hormone, and oxytocin, and also by several neurotransmitters as GABAergic, dopaminergic, and muscarinic cholinergic systems in several strains of rats, as well as HY and LY sublines (for review, see Collins & Eguiabar, 2010; Doger, Urbá-Holmgren, Eguiabar, & Holmgren, 1989; Eguiabar, Barajas, & Moyaho, 2004; Eguiabar, Romero-Carbente, & Moyaho, 2003; Urbá-Holmgren, Santos, Holmgren, & Eguiabar, 1993). The HY males yawned more and also had more grooming bouts after exposure to a novel environment.
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than LY rats (Eguibar & Moyaho, 1997). The HY subline is also more active in an open-field arena (Moyaho, Eguibar, & Diaz, 1995). In addition, after wetting the HY showed a disorganized grooming-chain sequence compared to the LY animals with a clear cephalocaudal organization, similar to that obtained in other strains of rats (Moyaho et al., 1995). These observations suggest that the early life experience, such as maternal care and lactation, could be the cause of the differences among the sublines. In our experiments, we analyzed maternal care toward their own offspring of HY and LY dams and compared them with outbred Sprague–Dawley dams during the early-to-middle lactation period. In a second experiment, we compared the number of pups at parturition and weaning and the fertility index of the females of all groups.

METHODS

Subjects to Study

The subjects were outbred Sprague–Dawley (SD) HY and LY females of 90–100 days old obtained and bred in our animal room facilities with a control temperature (21 ± 2°C) and relative humidity (30–45%) with a 12:12 light/dark schedule, with lights on at 0700. Balanced rodent pellets (Zeigler, Gardners, PA) and tap water were provided ad libitum.

At the Institute of Physiology of the Benemérita Autonomous University of Puebla, México, we selectively inbred two sublines from Sprague–Dawley rats with a high- and low-spontaneous yawning frequency (Urbá-Holmgren et al., 1990). The high-yawning rats (HY) had a mean frequency of 20 yawns/hr and were obtained by an inbreeding process of more than 70 generations. The low-yawning rats (LY) were inbred for more than 60 generations. The HY subline had a mean frequency of around 2 yawns/hr (Urbá-Holmgren et al., 1990), with the males yawning more frequently than the females (Moyaho, Barajas, Ugarte, & Eguibar, 2009).

All procedures described in this study were in accordance with the Mexican guidelines NOM-062-200-ZOO-1999 for the care and use of laboratory animals, which are in accordance with the NIH Guide for the Care and Use of Laboratory Animals (HHS 85-23; Clark, 2002), and were approved by the University Animal Care and Use Committee.

Mating

At 90–100 days old, the nulliparous SD, LY, and HY female rats were placed in reproduction units with a sexually experienced male of the same group. Every day all females are visually inspected and the presence of a vaginal plug was taken as the beginning of pregnancy. The male was then removed from the cage.

Experimental Procedure

Experiment 1. On pregnancy days 16–18, each female was placed in a transparent Plexiglas cage (32 cm × 47 cm × 20 cm) which allowed us to observe all the behavioral repertoire of the female. The females were provided with paper towel strips for building a nest, and then were checked daily for parturition. The nest quality was rated on a 5-point scale ranging from 0 to 4, modified from Lisk et al. (Lisk, Pertlow, & Friedman, 1969). A score of 0 was given when no nest was built by the female. A score of 1 was given when nesting material was present in a corner of the cage, but no organized nest was built. A score of 2 was given when some kind of organization of nesting material, such as semicircular organization, was made in a corner of a cage, but no walls or more complex structure were made. A score of 3 was given when a complete circular or semicircular nest with walls was built and a score of 4 was given to a full nest with tall walls. Newborn litters found up to 1200 each day were designated as born on that day (Day 0). Only the females that mated successfully and had at least 7–8 pups per litter at parturition were used. The litters were culled to eight pups, with the gender distribution kept as equal as possible in each litter. All testing was done between 1000 and 1300 inside the same animal room to minimize the stress response. On postpartum day 1, pups were removed from the nest for 2–3 min, weighed, and returned to the opposite corner to where the nest had been built. Maternal behavior was immediately videotaped for 15 min. The same procedure was done at postpartum days 3, 5, 7, and 9 using the Observer video Pro software v. 5.0 (Noldus Information Technology, Amsterdam, the Netherlands). We measured the time to retrieve the first pup and each of the siblings to the nest, the time to pup licking, nest-building, nursing, and pup mouthing. Additionally, we recorded the nest quality and also the frequency and duration of each maternal behavior, which were (A) retrieval of pups, (B) licking of pup bodies, (C) licking of pup anogenital region, (D) crouching (the female adopting a high or low nursing posture), (E) nest building, (F) being close to the pups (the time the female spent in close proximity within 5 cm), (G) nest-height, (H) mouthing and sniffing the pups (rearrangement of the pups inside the nest by the dam), and (I) sniffing the pups. In addition, other nonmaternal behaviors, such as running, walking, jumping, eating, self-grooming, and rearings, were also recorded.

Experiment 2. To record the number of females that successfully mated (fertility), the size of the litter at birth, and the mortality at weaning, we used another cohort of females from each group. As for the first cohort, each female was placed in an individual Plexiglas cage at the end of pregnancy and checked daily for parturition. All the females that mated successfully were recorded and used independently of the number of pups per litter at birth. On the day of parturition (Day 0), the number of pups was recorded and returned to the nest and was not disturbed until weaning (about P22). The mothers were treated for cleaning, food, and water as for every rat in the vivarium. At weaning, the number of pups was again determined, and the number of dams that lost at least three pups.

Statistical Analysis

Because the data did not always show homogeneity of variance, the latencies to retrieve the first, the second, the third, and the last pup of the litter to the nest and the average latency to
began each behavior during the first 3 days of testing (postpartum days 1 and 3 (1 + 3)) and during the last 2 days of testing (postpartum days 7 and 9 (7 + 9)) were compared using nonparametric statistics, the Kruskall–Wallis test, an ANOVA, and a 2 test for three-group analyses, and the Mann–Whitney U-test, and a 2 test for two-group analyses. The same protocol of analysis was used to compare the frequency and duration of the different components of maternal behavior (nursing, body and genital licking, and nest-building), the nonmaternal behaviors, and the number of pups at birth and at weaning. The 2 test was used to compare the proportion of females that displayed atypical retrievings, re-retrievings, nest building of high and low quality, and those that lost at least three pups at weaning. 2-values of <.05 were accepted as statistically significant. Data were analyzed using SPSS software (Version 11.0 for Windows Vista).

RESULTS

Experiment 1

Latency for Retrieving Pups and the Total Time for Retrieving All the Litter. Figure 1 showed that the latency to retrieve to the nest the first pup (P < .02), second (P < .04), third (P < .03), and the last pup (P < .01) were different among the groups. The post hoc comparisons showed that the HY dams retrieved the first, second, third, and the last pup more quickly than the SD dams (P < .05, P < .008, P < .006, and P < .005, respectively). Furthermore, to analyze with detail the latency to retrieve the first pup, we compared them during the first 3 days of lactation (1 + 3) compared to middle lactation period (7 + 9). There were marginal group differences during the last 2 days of testing (P = .07; Fig. 2A), but not in the first 2 days of testing. The post hoc comparisons showed that the HY dams retrieved the first pups more rapidly than the SD dams (P < .05) and the LY dams (P < .05).

Figures 1. Latency for retrieving the first, second, third, and last pup of the litter by each group of dams. In all tested days, the HY dams (dark bars) were significantly faster than the LY (gray bars) and Sprague–Dawley (open bars) rats. *P < .05 and **P < .01. Data are the mean ± SE of 10 rats in each group.

Latency to Begin Maternal Behavior. To compare the differences among HY, LY, and SD dams at the beginning of each of the maternal characteristics in the early (1 + 3) compared to middle lactation (7 + 9), we averaged the latency of each behavior from the first days of lactation compared to middle lactation. There were no significant differences in the latency to begin nursing, body and genital licking, nest building, or mouthing in the first 2 days among groups. However, there were group differences in the last 2 days in the latency of body licking (P < .01, Fig. 2C), genital licking (P < .05, Fig. 2D), and mouthing the pups (P < .003, Fig. 2F). Figure 2C and D shows the latency to lick the pup’s body and genitals were longer in the HY dams than in the LY dams (P < .02 and <.03, respectively) and the SD dams (P < .03 and <.03, for both comparisons). In contrast, the HY mothers began mouthing the pups faster than the LY and SD mothers (P < .04 and <.001; Fig. 2E).

Maternal Behavior. There were group differences in the total time to retrieve (P < .03; Fig. 3A), with the HY quicker than the SD dams (P < .007). This is also true with the time that the mothers spent inside the nest, which is greater in the HY subline (P < .03; Fig. 3E). There were no significant differences in the total time of nursing, body and genital licking, or nest building among groups. Subsequent analyses showed that the HY mothers spent less time retrieving all pups and inside the nest compared to the SD dams (P < .03 and <.03, respectively). The LY dams spent less time inside the nest than the SD dams (P < .02, Fig. 3E). Furthermore, as shown in Table 1, only 10% of the HY mothers built a maternal nest of high quality, that is, a compact nest at least 5-cm high; score: (3–4), which was significantly less compared to that of 90% of the SD and 50% of the LY dams (P < .0001 and <.05 in the other two comparisons). In addition, the percentage of LY dams that built high-quality nests was significantly lower than the SD dams (P < .05; see also Table 1).

Atypical Maternal Behavior

Reretrievings. Under our conditions and without any selection of dams, some rats from all groups made the reretrieving behavior that is characterized by taking the pups outside the nest (once they were retrieved) and after a variable time the dams returned them to the nest. Because almost all females showed at least one reretrieving during the period of observation, we computed as reretrieving if the dam showed this behavior three or more times during 3 or more days. Eighty percent of the HY dams reretrieve pups, which was higher than the SD dams of just 40% (P < .05; Fig. 4A) and 50% in the LY subline (P < .05; Fig. 4A).
Atypical retrieving behavior. Mothers were considered to show atypical retrieving when they retrieved at least three pups by holding them by their leg, mouth, or belly during testing, and during 3 or more days. The 80% of the HY dams that had atypical retrieving was significantly higher than the 30% of the SD and LY mothers \((P < .0001\) and \(< .02; \text{Fig. 4B})\). A statistical tendency in the percentage of the HY dams that showed atypical retrieval was higher than for the LY mothers \((P = .06)\).

Nonmaternal Behaviors. Most of the mothers showed nonmaternal behaviors, such as exploring, digging the cage, hanging on the top of the cage, and self-grooming. We did find group differences for the exploration time of the maternal cage \((P < .007; \text{Fig. 5A})\), with a post hoc comparison showing that the SD dams spent less time exploring their cage than the HY dams \((P < .002)\) and LY dams \((P < .03)\). In addition, the SD mothers spent less time engaging in self-grooming than the LY mothers \((P < .05)\), but not the HY dams (Fig. 5B).
Experiment 2

Fertility. Although not significant, only 68% of the HY females became pregnant and these data were lower than in the SD and LY females, who had 82% pregnancy.

Weight of pups. As shown in Table 2, there were group differences for individual body weight on postnatal day (PND) 1, 5, 9, 14, and 18 ($P < .001$ in each age), with post hoc comparisons showing that the body weights of the offspring in PNDs 1, 5, 9, 14, and 18 from the HY dams were lower than the SD dams ($P < .0001$ in each age). Similarly, body weights of the offspring of PNDs 1, 5, 9, 14 ($P < .0001$), and 18 ($P < .02$) from the HY dams were lower than those from the LY dams. The body weight of

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Table 1. Percentage of Females That Built Nests of Low and High Quality

<table>
<thead>
<tr>
<th>Nest Quality Score</th>
<th>SD</th>
<th>LY</th>
<th>HY</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (1–2)</td>
<td>10</td>
<td>50</td>
<td>90</td>
<td>$&lt;.0001$ vs. HY</td>
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<td></td>
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<td>$&lt;.05$ vs. LY</td>
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<td>$&lt;.0001$ vs. HY</td>
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<td>$&lt;.05$ vs. HY</td>
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<tr>
<td>High (3–4)</td>
<td>90</td>
<td>50</td>
<td>10</td>
<td>$&lt;.0001$ vs. HY</td>
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<td>$&lt;.05$ vs. LY</td>
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<td>$&lt;.05$ vs. HY</td>
</tr>
</tbody>
</table>

SD, Sprague–Dawley; HY, high-yawning; LY, low-yawning.

Note. All comparisons are done using the $\chi^2$ test, followed by a Tukey test.
the offspring of PNDs 14 and 18 from the LY dams was lower than the offspring from the SD dams ($P < .009$ and $< .002$). When we compared the difference in the percentage of the pup’s body weight from the HY and SD mothers, we also showed that the HY offspring had 16% (PND 1), 19% (PND 5), 21% (PND 9), 17% (PND 14), and 26% (PND 18) lower weights than those obtained from the Sprague–Dawley rats. To determine whether the low weight of the HY pups at weaning remained until adulthood, the body weight of females from the LY and HY sublines of 75 and 90 days old were recorded. The body weight of the HY female rats was lower than the LY animals at both ages ($P < .05$, data not shown).

**Number of Pups Per Litter at Birth and Weaning.**

There were group differences among the groups of the number of pups per litter at birth ($P < .03$) and number of pups weaned ($P < .0001$). When we compared the number of pups per litter for the HY mothers it was significantly lower than those of the LY dams ($P < .01$) and compared to the SD dams ($P = .06$; see Fig. 6). Furthermore, the number of pups at weaning time from the HY mothers was significantly lower than from the SD and LY mothers ($P < .002$ and $< .0001$). In addition, the percentage of HY mothers that lost at least three pups during lactation was higher than that of the SD and LY mothers ($P < .03$ and $< .006$).

**DISCUSSION**

In this study we compared the maternal and nonmaternal behaviors of primiparous female rats of the HY and LY inbred sublines compared to outbred Sprague–Dawley rats during early-to-middle lactation. Our results showed that HY mothers express different patterns of maternal and nonmaternal behaviors compared to the Sprague–Dawley and LY dams. In addition, the number of pups per
litter at birth and weaning from the HY mothers was lower compared to the other groups of rats.

The results showed that the time engaged in nursing and licking the pups did not significantly differ among the groups, but the HY and LY mothers spent less time inside the nest than the Sprague–Dawley dams. In contrast, the HY dams retrieve all pups to the nest more quickly than the Sprague–Dawley mothers. These results are similar to that obtained in mice from the C57BL/6, CBA/H, C3H/Ico, and CBA/J strains, which retrieved pups more quickly than the BALB/c, NBZ, DBA/2, XLII, A/J, and AKR strains (Carlier et al., 1982). The HY dams not only retrieve the pups more quickly, but most of them made atypical retrievings and also displayed reretrieving and built the nest more rapidly, but with lower quality. These results match with those found in the 129Sv inbred strain that had shorter latencies in nest building, built the nest less frequently, and spent less time in licking the pups (Champagne et al., 2007). The above data suggest that mothers are maternally motivated but the “fine tuning” of the expression of all maternal characteristics are disturbed, similar to that already reported in the organization of grooming bouts in HY rats (Eguibar & Moyaho, 1997; Moyaho et al., 1995). Interestingly, when we compared the latency of each maternal component in the early compared to the middle lactation, we showed that mothers from all groups showed similar latencies during the early lactation. During the middle lactation the latency to retrieve the pups, mouthing them, and building the nest by the HY dams was shorter, but the latencies to begin licking the body and genital areas of the pups were longer than that of the LY and Sprague–Dawley dams.

The HY, the LY, and the Sprague–Dawley dams showed normal maternal motivation because once they get cues from the pups, they established contact with them (appetitive component), then they walk around the maternal cage with the pups in their mouth and put them in a different place and later on reretrieve them to the nest. This shows that they have enough motivation to engage in a behavioral interaction with a specific goal object, and they retrieve them to the nest (consummatory component), but their ability to show a specific behavior, that is, properly retrieve the pups is not adequate after putting them outside the nest, reretrieve them and also with atypical retrieving suggesting a disorganized pattern in the global organization of maternal care in HY rats (Everitt, 1990; Numan, Fleming, & Levy, 2006; Numan & Insel, 2003; Timberlake & Silva, 1995). The HY males also have a disorganized sequence of their grooming bouts because they showed caudocephalic or lateralcaudal sequences, instead of the cephalocaudal sequences shown by the LY rats and other rodent species (Berridge, 1990;

<table>
<thead>
<tr>
<th>LactationDay</th>
<th>SD</th>
<th>LY</th>
<th>HY</th>
<th>P-Value</th>
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<tr>
<td>1</td>
<td>7.1±.5</td>
<td>7.3±.3</td>
<td>6.0±.1</td>
<td>&lt;.005 vs. HY</td>
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<td>&lt;.000 vs. HY</td>
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<tr>
<td>5</td>
<td>12.9±.6</td>
<td>12.4±.4</td>
<td>10.4±.2</td>
<td>&lt;.001 vs. HY</td>
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<td></td>
<td>&lt;.002 vs. HY</td>
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<tr>
<td>9</td>
<td>21.1±.5</td>
<td>20.6±.8</td>
<td>16.5±.5</td>
<td>&lt;.001 vs. HY</td>
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<tr>
<td>14</td>
<td>26.7±.5</td>
<td>23.9±.7</td>
<td>21.8±.6</td>
<td>&lt;.05 vs. LY</td>
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<td>18</td>
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<td>30.2±1.1</td>
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<td>&lt;.0001 vs. HY</td>
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<td></td>
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<td></td>
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<td>&lt;.02 vs. HY</td>
</tr>
</tbody>
</table>

SD, Sprague–Dawley; HY, high-yawning; LY, low-yawning.
Note. Data are the mean ± SE. The data is in grams.

FIGURE 6  Fertility index and numbers of pups per litter at birth in the three groups of rats. The Sprague–Dawley (open bars) and LY (gray bars) rats have more pups at parturition and after the weaning period than the HY rats (filled bars, *P < .05). Data from the LY and HY rats are the mean ± SE of 15 rats and for the Sprague–Dawley rats are the mean ± SE of 10 rats.
Moyaho et al., 1995). These alterations could be caused, at least in part, to a greater number of D1 dopaminergic receptors in the ventral striatum in the HY compared to the LY animals (Diaz-Romero, Arias-Montaño, Eguíbar, & Flores, 2005). Matell, Berridge, and Wayne-Aldridge (2006) showed that the grooming syntactic chains can be altered after a lesion of the striatum or changing the dopaminergic transmission in the nigrostriatal pathway. It is well known that the basal ganglia play a crucial role in the organization, timing, and coordination of motor sequences including grooming (Cromwell & Berridge, 1996). Furthermore, systemic administration of SCH-23390, a specific dopaminergic D1 antagonist, produced a disruption of maternal care causing the mother to leave the pups outside the nest, so re-retrieving them (Byrnes, Rigero, & Bridges, 2002). This also happens with intra-accumbens injection of cis-flupenthixol, which inhibits maternal retrieving and licking the pups but enhances nursing behavior in lactating Long-Evans rats (Keer & Stern, 1999). The maternal-care deficits caused by haloperidol can be restored by demanding pups (12-hr deprived), showing that pups can reverse the effects produced by the dopaminergic antagonist and by bromocriptine, a dopaminergic agonist that produced an opposite effect (Pereira & Ferreira, 2006). Because the HY rats showed an increase of D1 receptors in the ventral striatum (Diaz-Romero et al., 2005) and a decrease in the dopamine levels in the nucleus accumbens (unpublished data), we suggest that dopamine changes could be responsible for the alterations in maternal care in the HY dams.

Recently, it has been reported that the mother not only gives somatosensory stimulation but also gives growth factors such as prolactin and growth hormone through the milk that could act in concert to aid growth, weight gain, and glucose homeostasis in the perinatal period (Fleenor et al., 2005). Prolactin plays a fundamental role not only to support milk production but also in the developmental and maturation of the pups (Melo et al., 2009). The above data suggest that because that HY offspring never gain normal weight during lactation it could be that these dams produce milk of lower quantity or quality. It is also possible that humoral factors such as growth, oxytocin, and thyroid hormones could be responsible for the lower rate of body-weight gain in the HY offspring (Bautista, Boeck, Larrea, Nathanielsz, & Zambrano, 2008; Glinoer, 1997; Hapon, Simoncini, Via, & Jahn, 2003; Valdez, Penissi, Deis, & Jahn, 2007).

A relationship between high emotionality and a deficit in the expression of maternal licking and grooming as well as arched-back nursing posture has been demonstrated (Francis, Diorio, Liu, & Meaney, 1999; González, Lovic, Ward, Wainwright, & Fleming, 2001; for review, see Numan & Insel, 2003). Female rats that are isolated early in life had a high stress response and show a deficit in the expression of maternal behavior, and these characteristics are transmitted to the next generation (González et al., 2001).

Because the HY offspring have lower weights during lactation that persists until adulthood, it is possible that behavioral disturbances found in these rats could be caused by differences in the maternal care, including grooming and the arched-back posture. There are some reports that partially support this hypothesis, that is, mothers that were undernourished during early life had an abnormal maternal care, as we found in the HY mothers. Thus, these dams show a decrease in nest quality and nursing time, an increase in the latency to retrieve pups, and atypical retrievals that can even produce sonic distress in the pups (Regalado, Torrero, & Salas, 1999; Salas, Torrero, Regalado, & Perez, 2002; Salas, Torrero, & Pulido, 1984; Smart, 1976). The HY mothers also had a lower nest-building rating and showed atypical retrievals. Rosenblatt and Lehrman (1963) reported that when a female cannot maintain a stable nest, she retrieves the pups too many times and deposits them anywhere in the cage, similar to that made by HY and LY dams, suggesting that the sublines had a disorganized pattern of maternal care.

It is important to emphasize that body weights of undernourished pups from 4 to 20 days old were about 20–50% lower than well-nourished rats (Bautista et al., 2008; Salas et al., 1984, 2002; Wiener, Fitzpatrick, Levin, Smotherman, & Levine, 1977; Zambrano et al., 2005). We found that body weights in the HY offspring were 16–25% lower during lactation compared to the Sprague–Dawley and LY animals. Because of the ad libitum disposition of rodent food pellets the disturbed expression of maternal behavior in HY dams are not because they are being underfed during neonatal period, but it is probably generated by maternal care. These deficits in birth weight can be corrected by improving postnatal nutrition, as reported in Wistar Kyoto rats, a good model of anxiety responses (Romano, Wark, Owens, & Wlodek, 2009). In our experiments the animals have free access to food (Zeigler) with 22% protein, but they did not reach weights similar to Sprague–Dawley pups surely because of their genetic background (Moyaho et al., 2009). The “fetal origin hypothesis” proposes that prenatal environmental exposures, including maternal stress, could have sustained effects across the lifespan (Kinsella & Monk, 2009). A positive correlation of food ingestion during pregnancy and low body weight in their offspring has been demonstrated (Massaro, Levitsky, & Barnes, 1974; Passos, Ramos, & Moura, 2000), including women with a poor diet before and during pregnancy who had babies with a low birth weight (Lechtig et al., 1975), and where the frequency of infant mortality is four times higher than
normal birth weight babies (Habicht, Yarbrough, Lechtig, & Klein, 1973).

Comparisons among inbred strains of rodents are important to determine the effect of environmental factors over behavioral traits made under laboratory conditions. Thus, environmental manipulations such as maternal separation early in life, handling, and enriched environments clearly affect subsequent juvenile or adult performances (Fleming et al., 2002). As in many other inbred strains of rodents, work has focused on the participation of genetic and epigenetic factors involved in the development of specific behaviors (Francis et al., 2003). The HY offspring had fewer contacts with their mothers, receive less grooming, and were retrieved carelessly (atypical retrievings and reretrievings), similar to that reported in stressed mothers (Salas et al., 2002). The HY mothers have fewer pups per litter at parturition, most of them lost pups during lactation, and the mean weight of their pups is lower from birth to weaning compared to the LY and Dawley offspring. These behavioral differences could be caused by some metabolic, hormonal, or emotional issues during pregnancy or lactation (Fleenor et al., 2005; Glinser, 1997; Hapon et al., 2003; Ozzane & Hales, 1999; Shono, Imagima, Zakaria, & Suita, 1999).

Pups, exposed to dexamethasone by its injection into their mothers during pregnancy, produced an offspring with lower weight and chronic hyperactivity of the HPA gland axis. These pups had higher plasma-corticosterone levels with an upregulation of hepatic gluconeogenesis and insulin resistance suggesting that glucocorticoids levels are a key factor for metabolic activity as adults (Buhl et al., 2007; Burlet et al., 2005).

The release of oxytocin in the paraventricular nucleus (PVN) of the hypothalamus at parturition probably facilitates a positive feedback in both parvocellular and magnocellular neurons to coordinate the high levels of oxytocin release that are important for the generation of maternal behavior, infant recognition, and bonding (Carter & Keverne, 2002). This is also true for yawning expression because the release of oxytocin in the PVN is a key factor for the generation of yawning, not only by this peptide, but also in this part of the brain the dopaminergic, excitatory amino acids, nitric oxide, GABA, and opioid receptors converge to increase yawning frequency, suggesting that neural mechanisms in the hypothalamus are important regulators for yawning and pair bonding (for review, see Collins & Eguíbar, 2010). Preliminary results showed that HY rats yawned more after the i.c.v. injection of oxytocin than the LY animals, but with similar grooming scores (unpublished data), suggesting different sensitivities in the neural pathways that mediate these behaviors. In future experiments, we will address oxytocin levels during parturition and lactation in both sublines.

In conclusion, our results were that the HY dams showed a different organization of maternal care with a reduced litter size and lower weights of pups at parturition and weaning. These changes can be caused by hormonal or neural mechanisms, which are able to alter somatosensory stimulation of the pups and also can produce hormonal and metabolic changes that ultimately are responsible for different behavioral characteristics of HY rats, such as yawning and grooming sequences in the adults.

NOTES

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