

The effects of early rearing environment on the hormonal induction of maternal behavior in virgin rats

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Abstract

The present study investigated the effects of isolation rearing, through the artificial rearing paradigm (AR), on the hormonal induction of maternal behavior (MB) in female Sprague–Dawley rats. Between postnatal days (PND) 4 and 18, rat pups were raised either with their mothers (MR) or artificially, without their mothers (AR). As well, some of the AR pups were provided with additional maternal-like licking stimulation (AR-MAX) while the others were not given any additional stimulation (AR-MIN). At PND 60–100, AR ($n = 28$) and MR ($n = 25$) animals were ovariectomized (OVX). One week after the surgery, rats were either treated with a 2-week estrogen (E2) and progesterone (P) hormonal regimen (Bridges, R.S., 1984. *A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat. Endocrinology* 114, 930–940) or not treated with the hormone replacement. Maternal behavior testing with foster pups commenced 24 h following the removal of P treatment. Results demonstrated that MR animals showed increased pup licking and hover-crouching in comparison to AR animals and that hormonally primed groups became maternal more quickly than non-primed groups, regardless of the rearing history. There was also a significant interaction between the rearing condition (MR vs. AR) and hormonal treatment on the quality of maternal behavior exhibited. The highest level of licking and crouching was shown by the hormone-treated MR group. Mechanisms for these effects are discussed.

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Introduction

There is considerable research on adult maternal behavior and its neuroendocrinology (Fleming and Li, 2002; Numan, 1994). The mother rat begins to care for her offspring shortly following parturition (Wiesner and Sheard, 1933). The dam retrieves her newborn to the nest by carrying them in her mouth. She then exhibits nursing by crouching over the pups and licking them to elicit urination and defecation (Wiesner and Sheard, 1933; Fleming and Li, 2002).

In contrast, nulliparous female rats require continuous exposure to young pups to exhibit maternal responding, a process called ‘sensitization’. This process can require approximately 6–10 days of continuous exposure to pups

to elicit maternal responsivity and does not depend on hormonal changes (Rosenblatt, 1967).

The rapid appearance of maternal behavior at parturition is due to hormonal changes prior to and following parturition. In the rat, the ovarian steroid progesterone (P) is high during most of gestation, but then declines a few days prior to the end of pregnancy (Bridges, 1990). Conversely, estradiol (E2), also an ovarian hormone, and prolactin (PRL), a milk-producing peptide, both increase towards the end of pregnancy and peak at parturition, following the initially low levels associated with the start of pregnancy (Rosenblatt et al., 1987). The administration of a hormonal regimen that mimics the hormonal profile characteristic of pregnancy and parturition can reduce the latency of nulliparous rats to respond to young foster pups (Bridges, 1984; Moltz et al., 1970).

Bridges (1984) devised an endocrine model for the study of the hormonal regulation of maternal responsiveness. The

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model employs the administration of physiological amounts of the steroids estradiol (E2) and progesterone (P) via Silastic implants to OVX virgin rats and measurement of the effects of these implants on maternal behavior. One week following ovariectomy, a single E2 capsule is implanted in the dorsal region behind the neck, while three P capsules are implanted in the same region 2 days afterwards. Duration of exposure to hormones can range from 5 days to 13 days to 21 days with maternal testing commencing on the day after the removal of the progesterone capsules. Bridges (1984) found that the 13- and 21-day regimens stimulated the short latency maternal behavior to foster pups, whereas the 5-day treatment did not.

In addition to hormones, prior care provided by the mother influences the expression of adult maternal behavior (Gonzalez et al., 2001). Females that are reared without their mothers (AR) and that do not receive adequate licking stimulation during early development grow up to show reduced licking and crouching in comparison to mother-reared (MR) animals when tested with their own offspring (Francis et al., 1999; Gonzalez et al., 2001; Champagne and Meaney, 2001). Stroking infant rats with a paintbrush can reverse some of these separation effects (Gonzalez et al., 2001; Zhang et al., 2002; Akbari et al., submitted for publication).

The mechanisms through which early maternal deprivation influences later maternal behavior are numerous and include producing changes in brain activation patterns (Fleming et al., 2002; Gonzalez and Fleming, 2002; Gonzalez and Fleming, in preparation), in neurogenesis (Akbari et al., submitted for publication), in levels of different hormones and/or neuropeptides (Pryce and Feldon, 2003), and in hormone receptor distribution or number in brain sites where parturitional hormones act (Champagne et al., 2001, 2003a). Again, additional tactile stimulation with a paintbrush can reduce the severity of these deprivation-induced changes in the brain (Kuhn et al., 1990; Sucheki et al., 1993; Zhang et al., 2002).

The purpose of the present study was to assess whether administration of estrogen and progesterone for 2 weeks via silastic implants would stimulate the onset of maternal responsiveness in artificially reared virgin rats and how early life maternal separation would affect the initial expression and quality of adult maternal behavior. The process of simple sensitization of maternal behavior is based on direct continuous sensory stimulation from pups and does not depend on hormonal mechanisms. Hence, we predicted that the latency to become maternal in control virgins would not differ between AR and MR animals whereas under hormonal priming, MR animals would be expected to show shorter latencies than AR animals. In addition, once animals had become maternal, the MR animals were expected to show an increased frequency and duration of maternal behaviors in comparison to the AR animals. Hence, the prediction was that there would be an interaction between early rearing experience and hormonal

condition in maternal behaviors, once they are exhibited, and that the most responsive group would be hormonally stimulated MR animals.

The 13-day hormonal regimen was chosen because it was found to induce short-latency maternal behavior in virgin rats (Bridges, 1984) and hence was expected to initiate rapid responding to foster pups.

Materials and methods

Subjects

A total of 53 female Sprague–Dawley rats ranging in weight from 222 g to 410 g was used. All rats were born between October 2003 and February 2004. The experimental rats were the offspring of primiparous 60- to 90-day-old Sprague–Dawley rats. In addition, donor mothers and their offspring (1- to 5-day-old pups) were used during adult maternal testing. Animals were bred and raised at the University of Toronto in Erindale College animal facilities. The animals were singly housed under a 12:12 light/dark cycle (lights on from 0800 to 2000 h) with free access to food and water. Experimental rats were placed into AR and MR groups.

During behavioral testing, the animals were individually housed in clear, Plexiglas cages (22 × 44 × 30 cm). All behavioral measures were obtained using the subject's own cage. Behaviors were observed and recorded on computer using the Behavioral Evaluation Strategy and Taxonomies (BEST) program (S and K Computer Products, Toronto, Ontario).

Pup surgery and artificial rearing

Female virgin rats were mated by being placed with a proven stud male for a period of 5 days and left alone until parturition. On postnatal day one (PND 1), litters were culled to four males and six females. On PND 4, four female pups from each litter were randomly assigned to one of four experimental conditions: minimum group (AR-MIN), maximum (AR-MAX), a mother-reared (MR) surgical control (SHAM), and an MR control group (CTRL). All animals were weighed on PND 4. The AR-MIN and AR-MAX groups received a cheek cannulation. The pups were anesthetized using 1–2 ml of halothane (CDMV Inc.) in a bell jar. Once, anesthetized, a leader wire (stainless steel, 0.25 mm in diameter, VWR) sheathed with silastic tubing (0.3 mm, VWR) was slid over the cannula (polyethylene tubing, PE 10 tubing). The PE 10 tubing was flared on one end with a flat washer to hold the tubing in place. The leader wire was dipped into reagent grade mineral oil (Sigma) to lubricate tubing. The leader wire was led over the tongue and penetrated through the translucent cheek, muscle, and skin. Then, it was gently pulled until the flared end contacted the inside wall of the cheek. The leader wire

and silastic tubing were then removed. Polysporin antibacterial cream was applied to the site of penetration. A flat washer, followed by a T-washer (flared PE 50 tubing) was placed on the PE 10 tubing, which was placed firmly against the outside wall of the pups' cheek. The washers were held in place with Superglue (Gonzalez et al., 2001). The PE tubing was flushed with double-distilled water to prevent blocking. The SHAM group received a similar surgery. However, the tubing was removed, the tail marked, and the pups were returned to their mother and left undisturbed until weaning. The pups in the CTRL also had their tails marked and left undisturbed with their mothers until weaning.

Rearing and weaning

AR pups were individually placed in plastic cups (11 cm diameter $\times$ 20 cm deep) with corncob bedding (Renseed). These cups were then placed in a second weighted cup that was submerged 1 in. into a heated, temperature-controlled aquarium tank in a room that was maintained at 25°C, with a humidity level of 48%. Cup temperatures were controlled by 2 heaters in each tank. Cup temperature was maintained at nest temperature according to PND (decreasing temperature with increasing age). The top of the cups remained open to allow each pups' tubing (PE 10) to be attached to a PE 50 tubing (60 cm in length) that was in turn connected to a nearby 10-cc syringe with a 26G1 needle. Each syringe was filled with milk formula diet (Messer diet, University of Iowa) placed on a timer control infusion pump (Harvard Apparatus). The pumps were programmed to infuse the formula for 10 min every hour, 24 h daily. Every morning, pups were removed from the cups, weighed, and their lines were flushed with double-distilled water and a new infusion rate was calculated. The infusion rate was calculated based on a specified fraction of the mean pup weight. The amount of milk formula the pumps delivered was based on a specific fraction of the mean pup weight. We started by giving pups formula volume equal to 33% of their body weight, and then increased the volume by 1% each day.

To initiate and facilitate urination and defecation, pups in the MIN group received two anogenital stimulations (AGS) a day, once in the morning and once in the evening for 45 s each. AGS was administered using a wet paintbrush. The MAX group received the same AGS, along with five dorsal stimulations (DS) to mimic mothers licking, for 90 s each, administered with a dry paintbrush. These stimulation manipulations were carried out from the day the pups were placed on the pumps (PND 4) until the day of weaning (PND 18).

On PND 18, AR pups were removed from the pumps and placed in small (11 in. $\times$ 7 in. $\times$ 5 in.) cages and given free access to crushed cat chow mixed with formula and water. The cages were placed on heating pads that were set on the lowest setting. On PND 21, AR animals were paired with non-experimental MR social partners until the adult surgeries. MR animals weaned from their mothers were

paired together (MR-SHAM and MR-CON—from the same litter). Food and water were available ad lib.

Ovariectomy and hormone replacement

At 60–120 PND, all rats were anesthetized with isoflurane gas (Aerrane Brand) during surgery. Bilateral incisions were made to extract the ovaries and polysporin antibacterial cream was placed on the incision sites at the end of surgery. Seven days following OVX, animals were anesthetized and a single E2 (17 β -Estradiol 3-Benzoylate, E-8875; Sigma Chemical Co.) capsule was administered s.c. via a 20-mm Silastic implant (0.078 in. ID $\times$ 0.125 in. OD; Dow Corning, Midland, MI) in the dorsal region behind the neck. Two days following the E2 capsule implants, animals were again anesthetized and an additional 3 progesterone (Sigma Chemical Co.) capsules (40 mm) were also administered subcutaneously in the dorsal region as well.

Prior to implantation, the silastic capsules were fitted with 5-mm wood piece ends (Canlab) that were sealed with Silastic Medical Adhesive (Dow Corning) after being filled with either E2 or P. The capsules were left to dry for a 24-h period, after which they were washed in ethanol and incubated for 48 h in phosphate-buffered saline (pH 7.0). Control rats received empty (unfilled) silastic implants that resembled the hormone-filled capsules. Following each surgery, rats were wrapped in hand towels and placed into a new cage. Progesterone capsules were removed 10 days after implantation and maternal behavior testing commenced the following day. Testing was administered between 0900 and 1800 h.

Adult maternal behavior testing

Maternal behavior was assessed for 11 consecutive days. Testing stopped when an animal reached maternal criterion. The criterion for designating the animals as maternal was the retrieval of pups to the nest site within the 10-min test period on two consecutive days and the adoption of a crouching posture on both of these days. During testing, 6 (1–5 days old) pups provided by the donor mothers were placed in the diagonally opposite corner of the rat's nest. Recorded behaviors included pup retrieval to the nest (carrying the pups to the nest), pup body-licking, anogenital licking, and nursing posture over the pups (low and high crouch). Spot checks were performed 1 h following the initial time of testing, in which position of the rat and the pups was recorded. The next day, testing began immediately after the pups had been replaced with six new, recently fed pups.

Statistical analyses

For analyses, some behaviors were combined into single measures. Pup licking included body and anogenital

licking, while hover-crouching consisted of hovering, a low crouching posture, and a high crouching posture. Self-grooming and sniffing air were combined as non-pup behaviors. Other maternal behaviors, such as pup retrieval, mouthing, and nest building were not grouped with any other measures. In addition, because there were no differences between MR-SHAM and MR-CTL in any of the comparisons, the 2 MR groups were combined for all subsequent analyses. Given the nature of the latency data (non-normal in distribution), a between-group, non-parametric Kruskal–Wallis test was used to compare all 6 groups in their latencies to reach the maternal criterion. This was followed by comparisons among pairs of groups, using a series of Mann–Whitney U tests. In addition, a set of 3 (experience condition) $\times$ 2 (hormone condition) univariate ANOVAs was used to analyze the first 2 days and the last 2 days of testing to compare changes in behavior. A final univariate ANOVA was used to compare hormonally treated AR (AR-HORM) and MR (MR-HORM) animals that became maternal, i.e., 2 consecutive days of maternal responding. Tukey's post hoc test was used for post hoc comparisons. The level of statistical significance was $P < 0.05$. Data were analyzed using SPSS (SPSS 12.0 for Windows).

Results

Maternal latency

There was a significant difference among the six groups in latencies to become maternal ($\chi^2 = 43.1$, $df = 5$, $P < 0.001$). Post hoc Mann–Whitney U tests showed no differences in latencies among the 3 early experience groups that did not receive hormonal priming or among the 3 groups that received hormonal priming. However, when comparisons between hormone- and non-hormone-treated groups were made within each experience condition, the hormonally primed groups had significantly

shorter latencies than the non-primed groups (AR-MIN, HORM vs. CON, $U = 0$, $n_1 = n_2 = 7$, $P < 0.001$; AR-MAX, HORM vs. CON, $U = 0$, $n_1 = n_2 = 7$, $P < 0.001$; MR HORM vs. CON, $U = 0$, $n_1 = 10$, $n_2 = 15$, $P < 0.001$) (Fig. 1).

First two and last 2 days of testing

Duration of each of the maternal behaviors was averaged across the first 2 days and the last 2 days of testing. All animals were included in the analysis.

Licking

There was an effect of hormone condition on pup licking during the first 2 days [$F(1,47) = 6.0$, $P < 0.05$] and last 2 days [$F(1,47) = 80.5$, $P < 0.001$] of testing. The CON animals did not lick the pups during the first 2 days, and showed less licking during the last 2 days than the HORM animals (see Figs. 2a and b). An early rearing experience effect was also found during the first 2 days [$F(2,47) = 10.5$, $P < 0.001$] and last 2 days [$F(2,47) = 6.5$, $P < 0.05$] of testing. Among the HORM group, post hoc tests indicate that MR rats showed higher levels of licking than did both AR groups on first 2 days (Tukey's, P s < 0.05), and more licking than the AR-MIN rats on the last 2 days (Tukey's, $P < 0.05$). AR-MAX rats did not differ significantly from the MR rats on the last 2 days of testing. However, no significant difference in pup licking was found among the CON animals during the first or last 2 days of testing. In addition, there was also an interaction between early rearing experience and hormone condition. The MR-HORM animals engaged in more pup licking on both the first 2 days [$F(2,47) = 6.5$, $P < 0.05$] and the last 2 days [$F(2,47) = 6.5$, $P < 0.05$] of testing compared to all other remaining groups.

Hover-crouching

As can be seen in Figs. 2c and d, results for hover-crouching were similar to that of pup licking. There was an effect of hormone condition on the first 2 days [$F(1,47) = 7.1$, $P < 0.05$] and last 2 days [$F(1,47) = 97.1$, $P < .001$] of testing, in which the CON animals did not hover-crouch over the pups during the first 2 days and showed less hover-crouching on the last 2 days than the HORM animals. An early rearing experience effect was also found during the first 2 days [$F(2,47) = 5.7$, $P < 0.05$] and last 2 days [$F(2,47) = 9.6$, $P < 0.001$] of testing. Among the HORM animals, post hoc tests indicate that MR rats showed higher levels of hover-crouching than did both AR groups on the first 2 days of testing (Tukey's, P s < 0.05), and more hover-crouching than the AR-MIN rats on the last 2 days of testing (Tukey's, $P < 0.05$). AR-MAX rats did not differ from the MR rats on the last 2 days. Once again, there was no significant

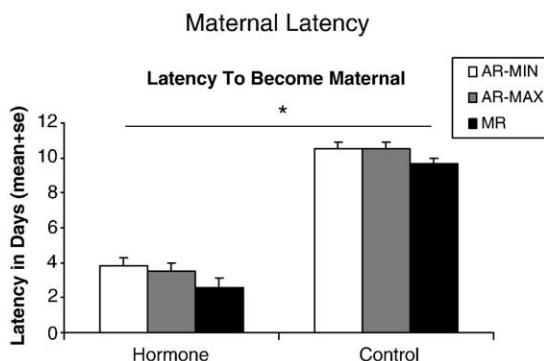
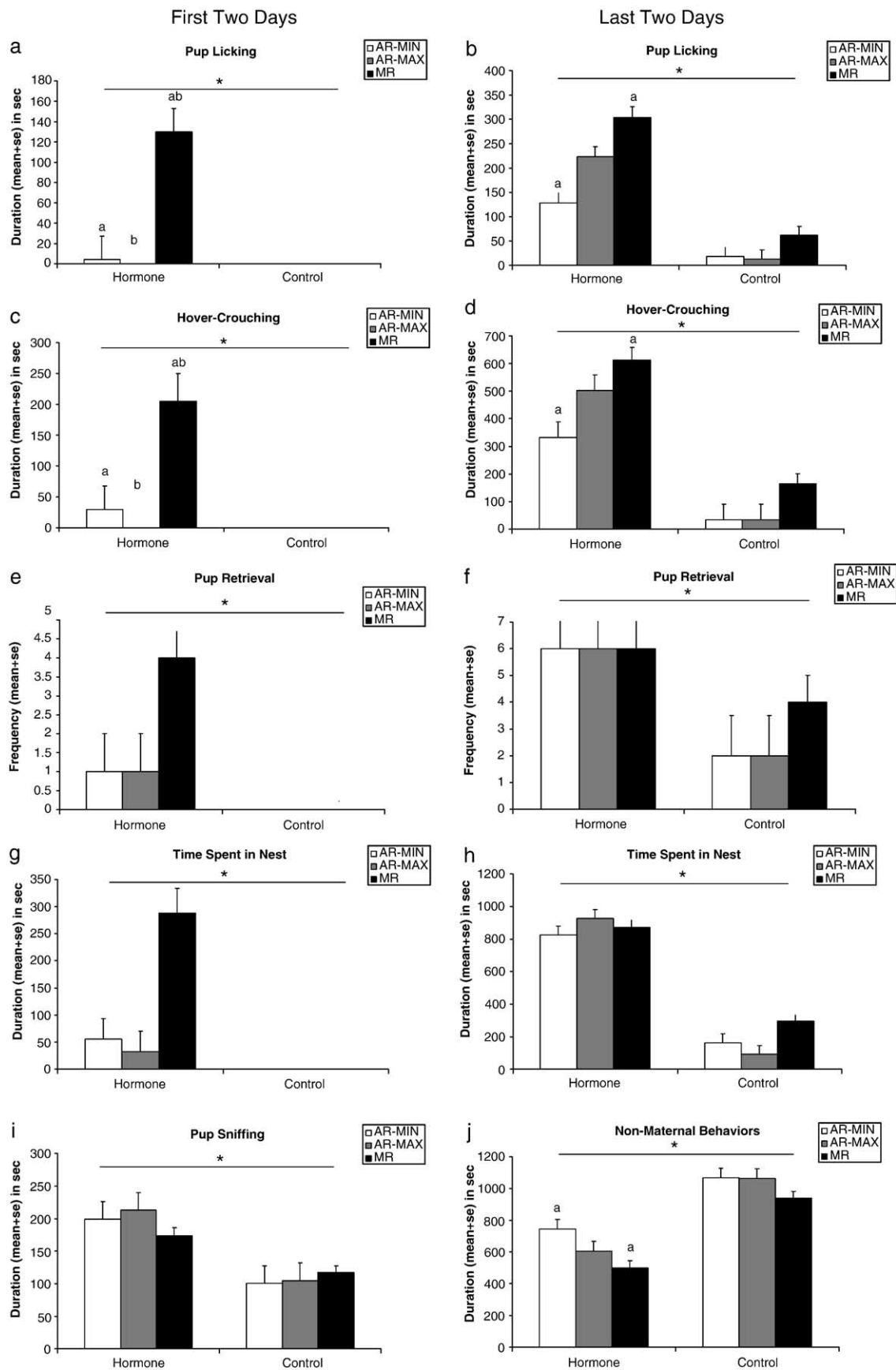


Fig. 1. Mean maternal latencies (days) for hormonally treated mother-reared (MR) ($n = 10$) and artificially reared (AR) MIN ($n = 7$) and MAX ($n = 7$) rats, and non-hormone control MR ($n = 15$) and AR MIN ($n = 7$) and MAX ($n = 7$) rats (mean $\pm$ SEM). An asterisk represents a significant difference between the hormone treated and control groups, $P < 0.05$.



difference in hover-crouching among the CON animals during the first or last 2 days. Furthermore, an interaction between early rearing experience and hormone condition was also found during the first 2 days of testing [$F(2,47) = 5.7, P < 0.05$], in which the MR-HORM animals engaged in more hover-crouching than all of the other animals.

Retrieval frequency

There was an effect of hormone condition in pup retrieval during the first 2 days [$F(1,47) = 6.0, P < 0.05$] and last 2 days [$F(1,47) = 59.6, P < 0.001$] of testing. The CON animals did not retrieve any pups during the first 2 days and retrieved less than the HORM animals during the last 2 days (see Figs. 2e and f). An early rearing experience effect was also found during the first 2 days [$F(2,47) = 4.3, P < 0.05$] of testing. Among the HORM animals, the MR rats retrieved more pups than did both AR groups, while no difference in retrieval frequency was found among the CON animals.

Time spent in nest

There was an effect of hormone treatment for time spent in nest during the first 2 days [$F(1,47) = 7.1, P < 0.05$] and last 2 days [$F(1,47) = 73.1, P < 0.001$] of testing. The CON animals did not spend any time in the nest with pups during the first 2 days and spent less time in the nest with pups on the last 2 days than did the HORM animals (see Figs. 2g and h). Early rearing experience was also found to be significant during the first 2 days [$F(2,47) = 3.6, P < 0.05$] of testing. Among the HORM animals, MR rats spent more time in the nest compared to both AR groups, while no difference in time spent in nest was found among the CON animals.

Pup sniffing

As can be seen in Fig. 2i, there was an effect of hormone condition in terms of the animals' initial response to the pups. HORM animals engaged in more pup sniffing on the first 2 days compared to the CON animals [$F(1,47) = 18.3, P < 0.001$]. No early rearing experience effect was found on the first two or last 2 days of testing.

Non-maternal behaviors

In addition to maternal behaviors, non-maternal behaviors were also analyzed. Results show an effect of hormone condition for the first 2 days [$F(1,47) = 26.7, P < 0.001$] and last 2 days [$F(1,47) = 79.5, P < 0.001$] of testing. CON animals engaged in more non-maternal activity during the

first and last 2 days than did the HORM animals. Early rearing experience was also found to be significant on the last 2 days [$F(2,47) = 6.6, P < 0.05$] (see Fig. 2j). Among the HORM animals, MR animals engaged in less non-maternal behaviors than both AR groups (Tukey's, $P_s < 0.05$). However, no significant difference in non-maternal activity was found among the CON animals.

Quality of maternal behavior

The 2 consecutive days of maternal responding were investigated to assess the effects of early rearing experience in hormonally primed animals on the quality of maternal behavior shown once it is exhibited. Only hormone-treated animals were compared because very few of the control animals became fully maternal within the testing period. Duration of each of the maternal behaviors was averaged across the first 2 days that animals exhibited maternal behavior. Results obtained from these analyses would be expected to be comparable to those of non-manipulated adult AR and MR postpartum animals that undergo natural hormonal changes at the time of parturition.

There were main effects for both pup licking [$F(2,21) = 8.6, P < 0.05$] and hover-crouching [$F(2,21) = 10.7, P < 0.001$] (see Figs. 3a and b). The MR rats showed higher levels of licking and hover-crouching than did the AR-MIN rats (Tukey's, $P_s < 0.05$). The AR-MAX rats, on the other hand, licked and hover-crouched at levels similar to that of the MR rats. Hence, among AR animals, maximal stimulation reduced some of the deficits in licking and crouching produced by artificial rearing.

For other maternal behaviors, there were main effects for mouthing [$F(2,21) = 4.7, P < 0.05$] and nest building [$F(2,21) = 5.7, P < 0.05$]. In each case, AR-MAX animals engaged in significantly more mouthing and nest building than the MR and AR-MIN animals (Tukey's, $P_s < 0.05$). AR-MIN and MR animals did not differ in either mouthing or nest building behaviors (see Figs. 3c and d). No significant differences were found in the amount of time animals spent in the nest.

Discussion

The present study assessed whether a hormone regimen of estrogen and progesterone that mimics the natural changes associated with late pregnancy and parturition would facilitate the induction of maternal behavior in ovariectomized virgin rats that had been reared without their mothers in a manner similar to that of mother-reared

Fig. 2. (a–i) Average duration of time exhibiting the different maternal behaviors (indicated on top of each figure) during the first 2 days (a, c, e, g, and i) or during the last 2 days (b, d, f, h, and j) of testing in hormonally treated mother-reared (MR) ($n = 10$) and artificially reared (AR) MIN ($n = 7$) and MAX ($n = 7$) rats, and non-hormone control MR ($n = 15$) and AR MIN ($n = 7$) and MAX ($n = 7$) rats (mean $\pm$ SEM). Histograms sharing letters indicate significant differences between the rearing groups and an asterisk represents a significant difference between the hormone-treated and control groups, $P < 0.05$.

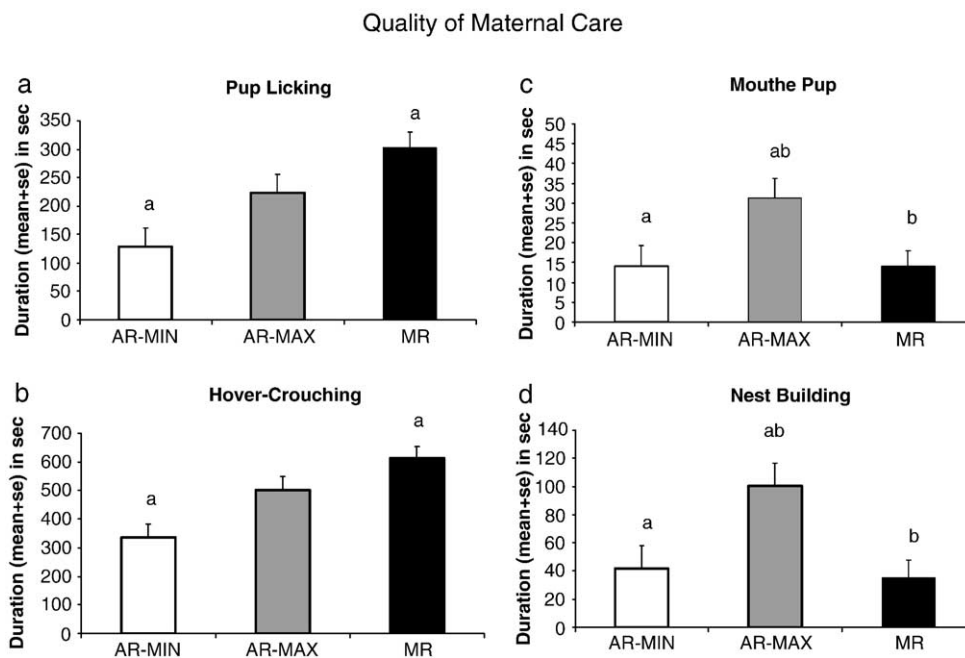


Fig. 3. (a–d) Average duration of 4 different maternal behaviors (indicated on top of each figure) in hormone-treated mother-reared (MR) and artificially reared (AR) MIN and MAX rats during 2 consecutive days of maternal behavior (mean $\pm$ SEM). Histograms sharing letters indicate significant differences between rearing groups, $P < 0.05$ (MR, $n = 10$; AR-MIN, $n = 7$; AR-MAX, $n = 7$).

animals (Bridges, 1984). Results show that hormonal priming served to facilitate rapid onset of maternal behavior in both AR and MR animals, regardless of early rearing experience. However, during the first 2 days of testing, MR animals as a group displayed more maternal behaviors than did AR groups, indicating a greater sensitivity to hormones at the initial stages of testing.

Another purpose of the study was to test the prediction that early experience would interact with hormone treatment on the quality of maternal behaviors shown. Our hypothesis that the most responsive group would be the hormone-treated MR animals was supported. As well, we explored the role of replacement tactile stimulation to AR animals (AR-MAX) and the effects of hormonal priming on maternal behavior in these animals. The hormone-treated MR animals spent significantly more time licking and hover-crouching than did the hormone-treated AR animals, with the AR-MIN rats exhibiting the shortest durations of these behaviors and with the AR-MAX rats displaying levels of responding more similar to the MR than the AR-MIN animals. Also, the hormone-primed groups were more responsive than the non-primed groups. Thus, additional tactile stimulation reduced deficits in pup licking and hover-crouching in hormone-treated AR animals.

These results, using exogenous hormone treatment, are striking in that they are similar to previous findings for postpartum animals tested with their own young (Gonzalez et al., 2001; Lovic et al., 2001; Lovic and Fleming, 2004). Females reared without their mothers that receive minimal licking-like stimulation (AR-MIN) grow up to show the full pattern of maternal behavior when they give birth (hence,

immediate maternal responding), but their behavior is disrupted and characterized by reduced licking and crouching (Gonzalez et al., 2001). Moreover, in both the present study and in the study by Gonzalez et al. (2001), replacement licking-like stimulation (AR-MAX) reversed some of the deprivation effects on licking and crouching. These results support the conclusion that additional tactile stimulation can reduce deficits in adult maternal behavior irrespective of whether the adult female is caring for her own young or foster young.

The dissociation of the hormone $\times$ experience interactions in terms of latency and licking/crouching behavior may reflect a difference in the mechanisms that underlie the initial motivation to respond to pups (measured by latency to respond) and the intensity or quality of the behavior once it is exhibited. It seems that the hormones, estrogen and progesterone, act primarily to facilitate the initial motivation to respond to pups, whereas the earlier experiences in relation to mother, nest, and litter affect the quality/intensity of maternal behavior. However, because the hormone-primed MR group showed the highest levels of maternal licking and crouching behaviors, this suggests that the latency–quality dissociation may not be complete. Early experience may only affect quality of behavior, but the hormones likely affect both motivation and quality of responsiveness. The elevated levels of licking and hover-crouching in the MR hormone groups suggest that either AR animals have reduced sensitivity to the hormones or the findings reflect a methodological constraint in that too few non-hormonally primed animals in either rearing condition exhibited maternal behavior, thus preventing us

from analyzing their maternal licking and crouching behaviors.

Non-hormonal influences also play a role in how the dam responds to her young (Fleming and Li, 2002; Orpen et al., 1986). Although pregnancy hormones are necessary to induce rapid maternal responding, maintenance of maternal behavior is sustained through learning and reinforcement (Orpen et al., 1986). The notion that maternal responding is sustained over time independently of hormones is important when taking into the account the fact that adult AR animals show deficits in attention (Lovic and Fleming, 2004).

Although evidence for the role of early maternal care on subsequent behavior is commonly obtained through experimental methods, such as separation studies, there are also natural variations in maternal care that occur under normal rearing conditions. Rats that receive less licking/grooming (LG) during the neonatal period lick their own offspring less, and in turn, these offspring persist in licking their own young (granddaughters) less (Champagne and Meaney, 2001). This observation provides evidence that variations in maternal care are transmitted across generations. Moreover, animals that lick more possess higher levels of estrogen-inducible oxytocin receptors as well as estrogen receptors in mPOA (Champagne et al., 2003b).

A similar transgenerational effect has been found with artificially reared animals (Gonzalez et al., 2001). In comparison to AR-MIN animals, AR-MAX females that licked their young more had daughters who also licked their own offspring more. Therefore, the level of somatosensory stimulation from the mother is transmitted to her daughters who will exhibit corresponding levels of licking in adulthood.

An additional factor that may have influenced the development of the artificially reared pups is the source of protein in the milk formula. In this experiment, we used the standard Messer diet (Messer et al., 1969), which includes soy-based protein in the formula. Soy contains some phytoestrogens, which are non-steroidal estrogens produced by plants (Han et al., 2002). Phytoestrogens have weaker estrogenic activity than estradiol, and are considered to be antiestrogens because they prevent estrogen from binding to the receptor (Jacob et al., 2001). Evidence from the literature has shown that phytoestrogens can influence not only the neuroendocrine development of neonatal rat pups, but also the integrity of the estrous cycle and lordosis behavior in female rats (Kouki et al., 2004; Whitten et al., 1995). In contrast to these findings, however, a study on the effects of lifelong and multigenerational exposure to dietary phytoestrogen found that mated female animals became pregnant, and showed no severe effects of diet in nursing behavior (Flynn et al., 2000). Results from the current study also suggest minimal effects of diet. Although the hormone-primed MR animals spent more time crouching over the pups than did the hormone-primed AR groups, AR animals given additional stimulation (AR-MAX) did not differ from

the MR animals in their maternal crouching and licking behavior, although they received the soy diet. It would, however, be very informative to investigate more closely the effects of diets used in artificial rearing paradigm on behavior to dissociate these from the effects of isolation rearing.

Findings from this study suggest an early rearing and hormone interaction for quality/intensity of maternal licking and crouching but no interactive effect on the initial motivation to be maternal. To preclude an interactive effect on motivational systems, it would be necessary to assess in a future study the effects of threshold levels of hormones administered to AR and MR animals on maternal latencies. Hence, a follow-up study will investigate the effects of a shorter hormone regimen on the induction of maternal behavior in mother-reared and artificially reared rats. Furthermore, estrogen receptors suggests that estrogen receptor activity should also be examined for AR and MR animals exposed to different durations of the hormone replacement regimen.

In conclusion, results from the present study provide evidence that AR and MR animals are equally responsive to the effects of estrogen and progesterone in relation to the rapid initiation of maternal responding, although the MR animals responded more quickly. However, evidence of the importance of early rearing experience on quality of adult maternal behavior under the influence of hormones is more profound, with lack of maternal care during early development producing deficits in pup licking and hover-crouching. Finally, deficits associated with motherless rearing can be reduced in hormone-treated animals through additional licking-like stimulation.

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