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The effects of adrenalectomy and corticosterone replacement on maternal memory in postpartum rats

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Abstract

Hormones associated with parturition prime rats to behave maternally, although hormonal changes are not necessary for these behaviors to occur. Experience with pups after birth enhances maternal responsiveness after a period of isolation, creating a maternal memory. The purpose of this study was to determine the role of corticosterone in the formation of maternal memory. Adrenalectomy or sham surgeries were performed in late gestation with corticosterone or vehicle pellets being given to adrenalectomized rats. Pups were removed immediately following parturition, and half of the rats received 4 h of pup experience, while the other half received only brief pup experience associated with parturition. Ten days following pup experience, foster pups were given to all rats. Latency to become maternal and maternal behaviors on the first 2 days of re-exposure and the first two maternal days were recorded. Among adrenalectomized rats given corticosterone, 4-h experience with pups decreased maternal latency when compared to brief experience with pups. This maternal experience effect was not found in comparisons between adrenalectomized rats not given corticosterone. In addition, corticosterone decreased latencies regardless of pup experience. Corticosterone also increased maternal behavior upon initial exposure to foster pups. In conclusion, corticosterone enhanced maternal memory and initial maternal behavior in postpartum rats.

Keywords: Corticosterone replacement; Maternal memory; Adrenalectomy; Maternal behavior; Postpartum; Rat

Introduction

New mother rats begin responding maternally as soon as their young are born (Rosenblatt and Lehrman, 1963; Weisner and Sheard, 1933). This is reflected in immediate retrieving, licking, and nursing of pups. Terkel and Rosenblatt (1972) found that the exhibition of these maternal behaviors is caused by humoral changes in the few days leading up to parturition. Subsequent studies indicated that a profile of hormones in the mother rat, including high levels of progesterone followed by its decline and rising levels of estrogen, prolactin, and possibly oxytocin, enhances maternal responsiveness when pups are born (Bridges, 1996; Insel, 1990; Moltz et al., 1970; Numan and Insel, 2003; Zarrow et al., 1971). However, maternal responsiveness in virgin rats shows that hormonal changes are not necessary for maternal behavior to be exhibited, although a more rapid responsiveness to pups is observed if hormonal priming is used to elicit these behaviors (Bridges, 1984; Rosenblatt, 1967). Behavioral modifications (such as reduced timidity and increased attractiveness to pup odors) accompany hormonal changes in mother rats and, in turn, facilitate the expression of maternal behavior (Fleming et al., 1989; also see Li and Fleming, 2003; Numan and Insel, 2003 for review).

In contrast to their role in the initial expression of maternal behavior, the hormones released at the time of parturition are not required to maintain maternal behavior over lactation (Bridges, 1975, 1977; Cohen and Bridges, 1981; Lee et al., 1999; Orpen and Fleming, 1987). Maternal behavior was not sustained during later postpartum days if the mother rats were separated from their pups during the first few hours after birth when parturitional hormones normally have the greatest effect on maternal behavior. However, if mother rats were allowed to interact with their pups during a brief postpartum period,

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then responsiveness was sustained for many days, and sometimes weeks, even in the absence of hormones (Bridges, 1975, 1977; Cohen and Bridges, 1981; Orpen and Fleming, 1987). Originally, Bridges (1975) found that an initial 1–1.5 h of interaction with pups was enough to sustain maternal responsiveness after a 25-day isolation from pups so long as the interaction occurred immediately following parturition. If tests were undertaken 10 days after birth, then as little as 30 min of interaction was necessary for responsiveness to be sustained (Orpen and Fleming, 1987).

The fact that Orpen and Fleming (1987) found that 15 min of pup exposure was insufficient to retain maternal responsiveness in the new mother rat suggests that there may be a threshold of experience required for retention of motivation to show maternal behavior. More evidence for this threshold comes from the finding that the length of pup interaction after birth, as well as the length of time between exposure and retention, affects the quality of maternal responsiveness observed in rats (Fleming and Sarker, 1990). Li and Fleming (2003) have termed this experience-based maternal responsiveness the maternal experience effect (MEE) or "maternal memory", which is defined as "the long-term retention of maternal responsiveness as a consequence of rats' prior experiences with pups". It has been suggested that, while the hormones necessary for the demonstration of postpartum maternal behaviors cause enduring changes in the rat central nervous system, additional changes also stem from the experience of interacting with the pups (Lee et al., 1999).

Lee et al. (1999) argue that the learning involved in retaining maternal responsiveness is comparable to other types of learning, in terms of both its temporal constraints and physiology. It has been shown, for instance, that the synthesis of proteins in the brain is involved in the formation of maternal memory, as is the case in other standard forms of memory (Malenfant et al., 1991a,b). Using pharmacological agonists and antagonists, it is clear that, as with other forms of memory, multiple neurochemical systems are involved in aspects of the formation, consolidation, and retention of maternal memory (Byrnes and Bridges, 2000; Byrnes et al., 2002; Lee et al., 1999; Li and Fleming, 2003; Moffat et al., 1993). For instance, the β-adrenergic blocker propranolol was shown to block the effect of pup experience on maternal memory, while the adrenergic agonist isoproterenol enhanced the memory formed during pup experience (Moffat et al., 1993). In addition, Byrnes and Bridges (2000) found that, if β-funaltexamine, a long-lasting μ opioid receptor antagonist, was administered before parturition, long-term retention of a maternal memory was disrupted. Similar effects were found for dopamine receptor blockers, which disrupted retention of a maternal experience when administered during the pup experience phase (Byrnes et al., 2002).

Where in the brain these neurochemical effects occur is not clear, although in a series of studies, Lee et al. (1999) found that lesions of the hippocampus, the amygdala, the dorsomedial thalamus, or other sites previously implicated in other kinds of memory were without effect on maternal memory. However, recently, a set of studies by Li and Fleming (2003) found that one particular part of the nucleus accumbens, the shell region, was essential for the consolidation of a maternal memory. Lesions or the administration of a protein synthesis blocker, cycloheximide, to this area before or directly after pup experience prevented a maternal memory from forming, whereas lesions of the core region of the nucleus accumbens had no effect (Li and Fleming, 2003).

The HPA axis of the rat is responsible for some control over the emotional and physiological responses towards new, potentially fearful or noxious stimuli (Tannenbaum et al., 1997), which could possibly include pups. In fact, the presence of pups following the cessation of lactation increases corticosterone levels in postpartum rats (Koranyi et al., 1977). In addition, after 5 h of isolation from pups, corticosterone increases were seen in postpartum rats (Koranyi et al., 1977). Corticosterone also plays a role in the expression of maternal behavior of the postpartum rat. The removal of the adrenal glands, the source of corticosterone, by way of adrenalectomy results in reduced maternal licking, crouching, and in nest behaviors exhibited by primiparous rats (Rees et al., 2004). This replicates the results found by Hennessy et al. (1977) who found that pup retrieval is deficient in mother rats that were adrenalectomized. When corticosterone is replaced in adrenalectomized mother rats, the maternal deficits are reversed (Rees et al., 2004).

Whether corticosterone is important in the onset or maintenance of maternal behavior is unclear. Potentially, corticosterone plays a role in the maintenance of maternal behavior, a form of learning about pup characteristics, as corticosterone has been implicated in the regulation of other more standard forms of learning and memory, including spatial memory (McCormick et al., 1997; Schaaf et al., 1999), non-spatial object recognition tasks (McCormick et al., 1997), and inhibitory avoidance (Borrell et al., 1983). The long-term consequences of adrenalectomy include impaired spatial memory, while corticosterone replacement reverses the deficits caused by adrenalectomy (McCormick et al., 1997). Adrenalectomy also impairs the retention of an inhibitory avoidance response, but corticosterone replacement does not reverse this deficit (Borrell et al., 1983), suggesting either that other disruptions caused by adrenalectomy cause this effect or that other components of the HPA axis play a more important role than corticosterone.

The present study was designed to: (1) determine whether corticosterone is involved in the maternal memory or maternal experience effect; that is, whether corticosterone exposure at the time of the maternal experience would lower the latency to display maternal behavior after a period of isolation from pups; (2) replicate prior studies in assessing effects of corticosterone on maternal behaviors exhibited by postpartum rats (Rees et al., 2004).

Methods

Subjects

A total of 36 female primiparous Sprague–Dawley rats, approximately 4 months in age, were subjects in this experiment. These rats were born at the University of Toronto at Mississauga from a stock originally obtained from

Charles River Farms in St. Constant, Quebec, Canada. Each was isolated in a Plexiglas cage (27 × 47 × 15 cm) with shaved woodchip bedding and a metal grid roof allowing ad libitum access to Purina rat chow and water. Light was provided in a 12:12 light/dark cycle, starting at 08:00 h. Room temperature was set at approximately 25°C, and humidity was approximately 40–50%. All procedures involving animals were approved by the University of Toronto Animal Care Committee. All animals were monitored daily after adrenalectomies for health status with a view to removing and providing additional care to animals if they showed signs of lethargy or sickness.

Treatment groups

The rats were divided into six groups, four of which were adrenalectomized and two of which received sham surgeries. Two of the four adrenalectomized groups received a corticosterone pellet, while the other two groups received empty silastic tubing. The two sham groups both received empty silastic tubing. Within each of these conditions, one group received 4 h of pup-exposure during the postpartum period and one group received only brief pup exposure at parturition (approximately 15 min); this is designated as "brief experience" (Brief Exp). The six groups were: (1) adrenalectomized, corticosterone replacement, brief experience (ADX/CORT/Brief Exp); (2) adrenalectomized, corticosterone replacement, 4-h experience (ADX/CORT/4HR Exp); (3) adrenalectomized, no corticosterone replacement, brief experience (ADX/No CORT/Brief Exp); (4) adrenalectomized, no corticosterone replacement, 4-h experience (ADX/No CORT/4HR Exp); (5) sham, brief experience (Sham/Brief Exp); and (6) sham, 4-h experience (Sham/4HR Exp).

Adrenalectomy and corticosterone replacement

Pregnant rats were anesthetized between gestation days 16 and 18 using a mixture of isoflurane, oxygen, and nitrous oxide in a 2:2:1 ratio for inhalation. Adrenal glands were removed through bilateral dorsal mid-flank incisions. Sham rats underwent the same procedure with the exception that the adrenal glands were left intact.

Adrenalectomized rats were assigned to a corticosterone group (75 mg/pellet; 21-day release; Innovative Research of America) or a no corticosterone group (empty silastic tubing approximately the same size as the corticosterone pellet). A dorsal incision was then made between the shoulder blades immediately after adrenalectomy, and either the pellet or vehicle tubing was inserted subcutaneously. Both sham groups were given vehicle tubing. After both surgeries, rats were placed in a new cage lined with paper towels. All adrenalectomized rats received 0.9% saline after surgery, while the sham rats continued receiving tap water. All animals were monitored throughout the study to insure that they were healthy, active, showed no noticeable weight loss, and had clean fur. All animals were retained in the study and appeared active and healthy.

Pup exposure and isolation

At parturition, rats were divided into a brief experience group (approximately 15 min spent with pups) and a 4-h experience group (4 h in contact with pups). Rats in the brief experience group had pups removed at 15-min intervals until the completion of birth so that exposure to pups was minimized. Rats in the 4-h experience group had 4 h of interaction with pups starting from the beginning of birth. After the 4-h exposure period, pups were removed. All rats were then isolated in normal housing cages for ten full days without pups.

Maternal testing

On the tenth isolation day, rats were moved from their cage and placed into a larger "maternal" (51 × 41 × 20 cm) cage that contained one torn up paper towel. This allowed them 1 day of habituation to their new environment. The next day, six foster pups (three male and three female) ranging in age from postnatal day (PND) 0 to 6 were placed in the cage corner opposite the nest site, and a 10-min maternal observation/retrieval test was performed. Following the 10-min test, pups were left with the rat being tested, and paper and pencil spot-checks were done throughout the day to determine if maternal behaviors were being performed at other times than during the test. On the next day, a spot-check was done before the pups were replaced with new donor pups, and maternal testing was again performed.

Frequency and duration of the following behaviors exhibited during the 10-min observation/retrieval testing were recorded: (1) retrieval (the act of moving pups from the opposite corner to the nest); (2) high crouching (standing in a lactating posture over all the pups in the nest, typified by an arched back); (3) low crouching (being in a crouch without an arched back); (4) hovering (moving around the nest over pups often, but not in a stationary crouching posture); (5) anogenital licking (licking a pup's anogenital region); (6) body licking (licking a pup's body); (7) time in nest (the time the mother rat spends in the nest). The nest site is defined as the location where the mother rat sleeps and nurses the pups and where nesting material is gathered. Maternal behavior is defined as the occurrence of retrievals of all six pups as well as a crouch or lactating posture accompanied by pup-licking, during the observation period or during spot-checks. In addition, other behaviors that were observed include (8) mouthing (the action of moving a pup and depositing it within an inch of pick-up point, not considered retrieval); (9) nest building (moving wood chips or paper towel pieces to make a nest); (10) sniffing pups (exploring the scent of the pups); and (11) sniffing air. Behaviors were recorded using a computer-based event recorder (BEST, Behavioral Evaluation Strategies and Taxonomies, S and K Computer products, Toronto, Ontario).

If retrieval and crouching were present for two consecutive days, testing ceased, and the rat was considered maternal, thus 2 days of consecutive maternal behavior fulfilled the maternal criterion. Furthermore, the latency for becoming maternal after the re-introduction of pups was recorded. This maternal latency was considered to be the first of the two consecutive days that retrieval and crouching were exhibited during the testing period. If maternal behaviors were not present on the first 2 days of re-exposure to pups, spot-checks were done every day until maternal behaviors were observed. Once retrieval and crouching were present, maternal behavior tests were again performed until two consecutive days of maternal behaviors were displayed during testing. If two consecutive days of maternal behaviors were not present after 10 days with pups, the rat was considered non-maternal, and testing for that rat was discontinued. Non-maternal rats were assigned maternal latencies of 11 days for statistical purposes.

Perfusions and blood sampling

Once the maternal criterion was met, or after 10 days had passed and testing had ceased, rats were perfused, and blood samples for corticosterone were taken from the atrium. Rats were anesthetized with a 1.0 cm^3 overdose (with 0.2 cm^3 boost as needed) of Somnotol (MTC Pharmaceuticals, Cambridge, ON), and blood was taken immediately after rats went down, the moment before perfusion commenced. Perfusions were performed using saline (0.9%) and paraformaldehyde (4%) solutions, and brains were collected for future procedures.

Radioimmunoassays for levels of corticosterone in blood

Levels of corticosterone were measured in all rats to determine if corticosterone changed as a function of surgical and experience manipulations (Cort-a-Count Diagnostic Products, Los Angeles, CA; inter-assay = 8.5%; intra-assay = 6.8%).

Statistical analysis

Latencies to express maternal behavior were compared across all six groups (ADX/CORT/4HR Exp, ADX/CORT/Brief Exp, ADX/No CORT/4HR Exp, ADX/No CORT/Brief Exp, Sham/4HR Exp, Sham/Brief Exp) and were analyzed using a between group Kruskal–Wallis Test followed by two group post-hoc comparisons using the Mann–Whitney U test ($n = 6$ for each group).

Durations and frequencies of behavior were also analyzed, using analyses of variance (ANOVAs). The first analysis included a series of 2 (corticosterone replacement: CORT vs. No CORT) × 2 (pup experience: 4HR Experience vs. Brief Experience) ANOVAs on maternal behaviors for the first 2 days of pup re-exposure ($df = 1,20$) and the first 2 days of maternal behavior for maternal rats ($df = 1,15$). These were followed by Tukey's post-hoc tests, using $P < 0.05$. The second set of analyses was an independent samples t test comparing the two sham groups for all the behaviors on the first 2 days of pup re-exposure

(df = 1,10) and on the first 2 days of maternal behavior (df = 1,9). The third set of ANOVAs (corticosterone replacement × experience) assessed levels of corticosterone in blood samples for each group (df = 1,30). The final set of analyses was a Spearman 'rho' coefficient relating levels of corticosterone to duration and frequency of maternal behaviors after rats had become maternal.

Results

Maternal latency

Fig. 1 shows the cumulative percentage of rats becoming maternal for each of the four adrenalectomy groups. As this figure indicates, the two groups given corticosterone (ADX/CORT) had a higher percentage of rats becoming maternal across days than did the groups not given corticosterone (ADX/No CORT).

As shown in Fig. 2, there was a main effect of group on latencies to exhibit maternal behavior (Kruskal–Wallis Test, χ^2 = 11.471, df = 3, P < 0.009). In terms of the effects of adrenalectomy on maternal latency, adrenalectomized rats had longer latencies than sham rats, but only when comparing adrenalectomized rats that had the limited brief experience with pups (ADX/No CORT/Brief Exp) to sham rats had 4-h experience with pups (Sham/4HR Exp) (Mann–Whitney U = 3, P < 0.026).

As predicted by previous work on the maternal experience effect, rats receiving adrenalectomy and corticosterone replacement that were given 4 h of experience with pups (ADX/CORT/4HR Exp) had lower latencies than adrenalectomized rats given corticosterone and only a brief experience with pups (ADX/CORT/Brief Exp) (Mann–Whitney U = 6, P = 0.044). In contrast, 4-h experienced adrenalectomized rats not given corticosterone (ADX/No CORT/4HR Exp) did not differ from the briefly experienced adrenalectomized rats not given corticosterone (ADX/No CORT/Brief Exp). This suggests that, for the 4-h experience to have an effect, corticosterone must be present.

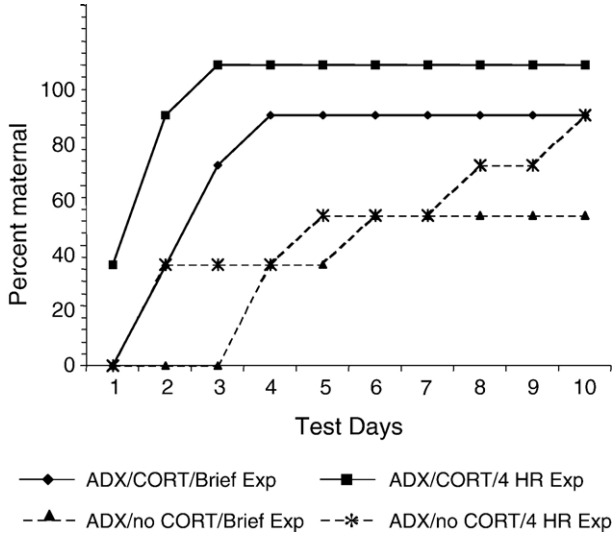

Fig. 1. Cumulative percentage of adrenalectomized rats becoming maternal on each test day (ADX/CORT/Brief Exp: n = 6; ADX/CORT/4HR Exp: n = 6; ADX/No CORT/Brief Exp: n = 6; ADX/No CORT/4 HR Exp: n = 6).

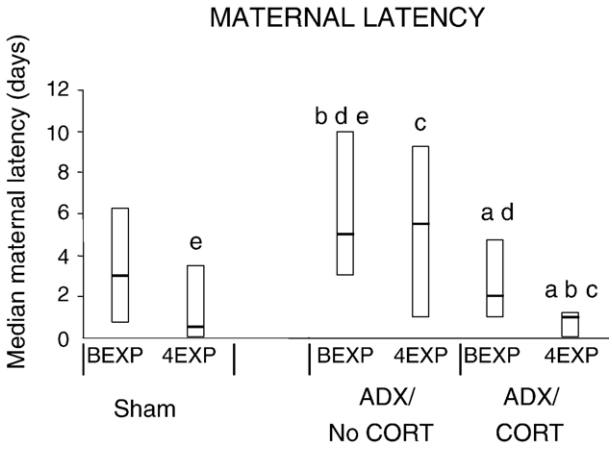

Fig. 2. Latency (in days) to reach the maternal criterion shown by all adrenalectomized and sham rats. Lines reflect medians, and the surrounding box reflects the interquartile range. Histograms sharing letters are significantly different from one another (P < 0.05) (SHAM/Brief Exp: n = 6; SHAM/4 HR Exp: n = 6; ADX/No CORT/Brief Exp: n = 6; ADX/No CORT/4 HR Exp: n = 6; ADX/CORT/Brief Exp: n = 6; ADX/CORT/4HR Exp: n = 6).

Corticosterone also facilitated the onset of maternal behavior, independent of pup experience. This was seen when adrenalectomized rats given corticosterone had lower latencies than adrenalectomized rats that were not given corticosterone regardless of the extent of pup experience (ADX/CORT/4HR Exp vs. ADX/No CORT/4HR Exp: Mann–Whitney U = 5, P = 0.031; ADX/CORT/Brief Exp vs. ADX/No CORT/Brief Exp: Mann–Whitney U = 5, P = 0.039). When comparing maternal latencies of rats that showed a maternal response during the testing period, similar results were found (Kruskal–Wallis Test, χ^2 = 8.421, df = 3, P = 0.038).

Behaviors exhibited during the initial tests upon re-exposure to pups

The average duration of licking, crouching, and time in nest upon re-exposure to pups was analyzed. To compute this average, the duration of each behavior was averaged across the first 2 days of testing.

Licking

The two types of licking recorded during testing (anogenital and body licking) were combined into one category called licking. As can be seen in Fig. 3A, there was a main effect of corticosterone replacement (F(1,20) = 4.453, P < 0.048) where adrenalectomized rats given corticosterone (ADX/CORT) showed more licking than did adrenalectomized rats not given corticosterone (ADX/No CORT). There were no main effects of pup experience or interactions between corticosterone replacement and pup experience (see Fig. 3A). There were no differences between the two sham groups for licking on the first 2 days of testing.

Crouching

The two types of nursing postures, also known as high and low crouching, shown on the first 2 days of re-exposure to pups were combined into one behavior called crouching. As

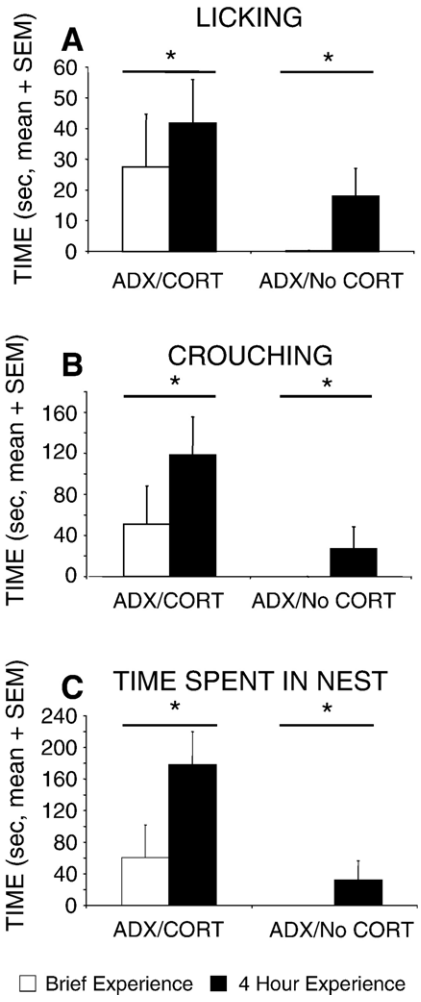

Fig. 3. Average duration of maternal behaviors upon re-exposure to foster pups, in seconds, shown by adrenalectomized rats with and without corticosterone and with and without pup experience (mean ± SEM; histograms sharing symbols show significant differences, $P < 0.05$) (ADX/CORT/Brief Exp: $n = 6$; ADX/CORT/4HR Exp: $n = 6$; ADX/No CORT/Brief Exp: $n = 6$; ADX/No CORT/4 HR Exp: $n = 6$).

shown in Fig. 3B, there was a main effect of corticosterone replacement ($F(1,20) = 6.25$, $P < 0.021$) with adrenalectomized rats given corticosterone (ADX/CORT) showing higher levels of crouching than did adrenalectomized rats not given corticosterone (ADX/No CORT) upon re-exposure to pups. This was not affected by pup experience. The 4-h experienced and briefly experienced sham groups also did not differ in duration of crouching on the first 2 days of pup re-exposure.

Time in nest

When comparing adrenalectomized rats, there were significant main effects of corticosterone replacement and pup experience. As seen in Fig. 3C, adrenalectomized rats given corticosterone (ADX/CORT) spent more time in the nest than did those not given corticosterone (ADX/No CORT) ($F(1,20) = 10.509$, $P < 0.004$). Adrenalectomized rats that had 4-h pup experience (ADX/4HR Exp) spent significantly more time in their nest than those that had brief pup experience (ADX/Brief Exp) ($F(1,20) = 5.5$, $P < 0.029$). There were no interactions between corticosterone replacement and pup experience. There were no differences between the two sham groups.

Behaviors exhibited when maternal criterion was achieved

To determine if the groups differed in the quality of maternal behavior they displayed once they met the maternal criterion, the three critical behaviors were each averaged across first 2 days of full maternal behavior. Since not all rats became maternal within the 10-day testing period, only those that performed maternally on two consecutive days were included in the analysis.

Licking

As shown in Fig. 4A, within the adrenalectomized groups, there was a marginal main effect of corticosterone replacement ($F(3,19) = 4.270$, $P = 0.057$), with those rats receiving corticosterone showing increased licking and a corticosterone replacement × experience interaction ($F(1,15) = 10.05$, $P < 0.006$). Interestingly, rats with corticosterone that had the limited brief experience showed the highest levels of licking than did all other groups. Four-hour experienced and briefly experienced sham rats did not differ in amount of licking they showed once maternal.

Crouching

There were no main effects or interactions among adrenalectomized groups for duration of crouching on the first two maternal days (see Fig. 4B). Furthermore, no significant differences were found between the two sham groups.

Time in nest

As in the case of crouching, there were no significant differences among the adrenalectomized groups or between the sham groups.

Corticosterone levels

Levels of corticosterone were compared across the four adrenalectomy groups and two sham groups. An ANOVA revealed a group effect on corticosterone levels ($F(2,33) = 120$, $P < 0.000$). There were significantly lower levels of corticosterone in both adrenalectomy groups (ADX/CORT and ADX/No CORT) than in the two sham groups (Sham) (Tukey's, $P < 0.05$ for both). Hence, exogenous corticosterone replacement did not achieve endogenous corticosterone levels. However, there were significantly higher levels of corticosterone in adrenalectomized rats that received corticosterone replacement (ADX/CORT) compared to those that did not (ADX/No CORT) (Tukey's, $P < 0.05$) (see Fig. 5). To determine whether corticosterone replacement and pup experience affected corticosterone levels, a corticosterone replacement (CORT vs. No CORT) × pup experience (4HR Exp vs. Brief Exp) ANOVA was computed. There was a significant main effect of corticosterone replacement ($F(1,20) = 74$, $P < 0.000$), with adrenalectomized rats given corticosterone

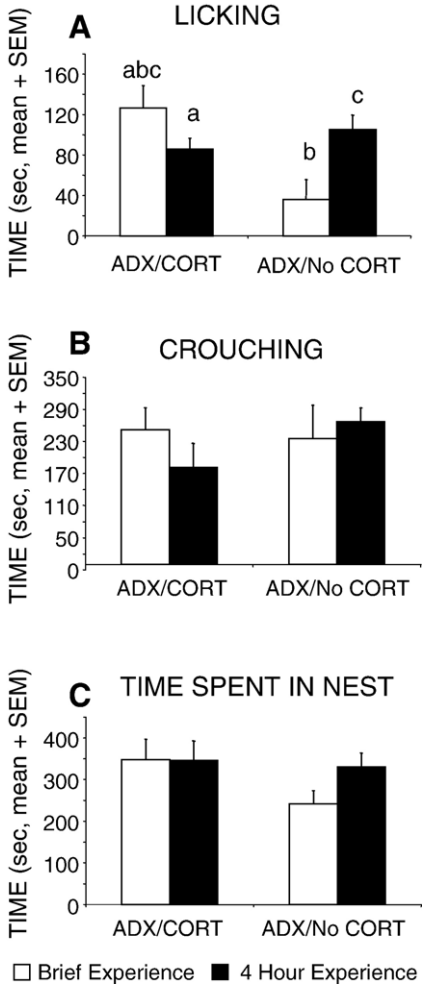

Fig. 4. Average duration of maternal behaviors once maternal, in seconds, shown by adrenalectomized rats with and without corticosterone and with and without pup experience (mean ± SEM; histograms sharing letters show significant differences, $P < 0.05$) (ADX/CORT/Brief Exp: $n = 5$; ADX/CORT/4HR Exp: $n = 6$; ADX/No CORT/Brief Exp: $n = 3$; ADX/No CORT/4 HR Exp: $n = 5$).

(ADX/CORT) having higher levels than adrenalectomized rats not given corticosterone (ADX/No CORT) but no effect of pup experience or interaction between corticosterone replacement and pup experience.

Discussion

In this study, mother rats without corticosterone (ADX/No CORT) were compared to rats with either endogenous (Sham) or exogenous (ADX/CORT) corticosterone to determine the effect that this adrenal hormone had on the maternal experience effect. Results showed that the maternal experience effect depended on the presence of adrenal corticosterone. Rats with corticosterone and 4-h pup experience (ADX/CORT/4HR Exp) required significantly less time to exhibit maternal behaviors on re-exposure to pups compared to rats with corticosterone and a brief pup experience (ADX/CORT/Brief Exp). Furthermore, rats given corticosterone and 4-h pup experience (ADX/CORT/4HR Exp) showed shorter latencies than rats not given corticosterone and with 4-h pup experience (ADX/No CORT/4HR Exp). In contrast, the effect of experience seen in the adrenalectomized rats given corticosterone was not found between the two adrenalectomized groups lacking corticosterone (ADX/No CORT/4HR Exp and ADX/No CORT/Brief Exp). These two groups did not differ in their latencies to become maternal on re-exposure to pups. These results are consistent with our previous observations that adrenalectomy disrupts the maternal experience effect seen in intact rats given 12 h of pup experience (Graham and Fleming, unpublished data).

In addition to this specific effect on maternal experience, in this study, corticosterone enhanced the expression of maternal behavior regardless of experience; primiparous rats with corticosterone (ADX/CORT) were altogether more maternally responsive, and, after a period of separation from pups, they reached the maternal criterion more quickly and displayed higher levels of maternal behaviors on retest than did those rats not given corticosterone (ADX/No CORT). The facilitatory effects of corticosterone on behavior after a 10-day separation from pups are consistent with previous findings on the effects of corticosterone on the expression of maternal licking earlier in the postpartum period (Rees et al., 2004). However, this study only partially replicated a corticosterone-dependent enhancement of licking behavior shown previously in postpartum maternal rats (Rees et al., 2004). There were no significant differences in maternal behavior between corticosterone and no corticosterone groups that had had a longer postpartum maternal experience (ADX/CORT/4HR Exp and ADX/No CORT/4HR Exp). Among briefly experienced rats, rats given corticosterone (ADX/CORT/Brief Exp) showed higher levels of licking than did rats not given corticosterone (ADX/No CORT/Brief Exp).

These data show that corticosterone can reduce the amount of time it takes to become maternal and can also affect the quality of maternal behaviors displayed. The present results depart somewhat from recent findings by Rees et al. (in press) on the effects of corticosterone replacement in adrenalectomized virgin rats, where there were no facilitatory effects of corticosterone on latency to become sensitized. In all

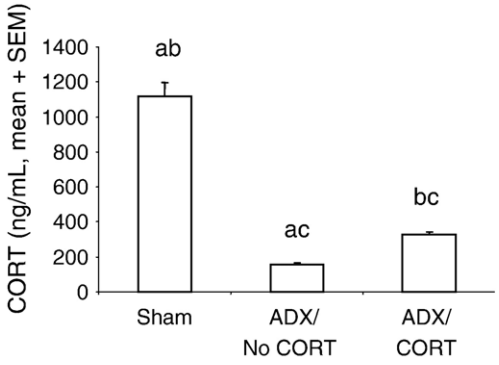

Fig. 5. Levels of corticosterone (ng/ml, mean ± SEM) for adrenalectomized rats without corticosterone (both 4-h experience and brief experience), for adrenalectomized rats given exogenous corticosterone (both 4-h experience and brief experience), and for sham rats (both 4-h experience and brief experience) (mean ± SEM; histograms sharing letters show significant differences, $P < 0.05$) (SHAM: $n = 12$; ADX/No CORT: $n = 12$; ADX/CORT: $n = 12$).

probability, the endocrine profile of the day 10 postpartum rat after separation from pups is a different one from that of the virgin rat.

Unfortunately, in the present study, among the sham rats, the maternal experience effect was not as robust as is usually the case in our laboratory primarily because the briefly experienced rats had such low latencies themselves (Li and Fleming, 2003; Orpen and Fleming, 1987). In fact, in the present study, all briefly experienced groups had considerably lower re-induction latencies than originally found in the Orpen and Fleming (1987) study. The differences in onset latencies of so-called briefly experienced rats, across these studies, are quite clearly due to the fact that in the Orpen and Fleming (1987) study the briefly experienced rats received NO experience of pups since they were c-sectioned and hence never encountered pups; their onset latencies were high and similar to virgins. In an attempt to reduce the trauma of surgery and to insure an endocrine state normally associated with parturition, in this and other more recent studies, pups of brief experience rats were removed at parturition every 15 min; hence, brief experience rats necessarily received some contact with pups. This brief contact and experience may explain the very low maternal onset latencies in the brief experience rats of adrenalectomized and sham-operated groups. At the same time, corticosterone levels were higher in sham rats than in adrenalectomized rats given corticosterone, suggesting that perhaps a "floor" effect on maternal latencies may be related to high corticosterone levels. As well, briefly experienced rats were exposed to distal pup cues from other mother rats and their litters that were housed in the same room. Despite the absence of a significant maternal experience effect in the sham rats, the differences between 4-h experience and briefly experienced rats were in the predicted direction in these sham rats and were robust and significant in the adrenalectomized rats.

Consistent with these effects of corticosterone on maternal memory, many studies have found a role of glucocorticoids in both consolidation and retrieval in other memory systems. For example, glucocorticoids facilitate the consolidation of memories in contextual fear conditioning (Cordero et al., 1998, 2002). Generally, the effects of corticosterone on the consolidation of memories follow a U-shaped function in that high and low levels impair consolidation (Luine et al., 1994), while medium levels enhance memory consolidation (Luine et al., 1996; Roozendaal and McGaugh, 1996; Sandi et al., 1997; Shors et al., 1992). With the present study, it was unclear if such a function exists in maternal memory as only one dose of corticosterone was used. Furthermore, corticosterone affects memory retrieval but in a context-specific and time-dependent fashion (De Quervain et al., 1998; Okuda et al., 2004; Roozendaal et al., 2004). Since corticosterone was present both at parturition and during testing in the present study, whether corticosterone affects consolidation of the maternal experience or its retrieval remains unclear. Generally, the present study suggests that corticosterone plays a role in maternal learning. However, this effect may be limited to the primiparous rat as adrenalectomy and corticosterone replacement have no effect on the rate of maternal sensitization in virgin rats (Rees et al., in press). This suggests an important interaction between corticosterone and other maternal hormones such as estrogen, progesterone, prolactin, and/or oxytocin.

Support for the possible importance of this hormonal interaction lies in the fact that adrenalectomized rats without corticosterone but with 4-h pup experience (ADX/No CORT/4HR Exp) did not differ in terms of maternal latency from adrenalectomized rats without corticosterone and with limited pup experience (ADX/No CORT/Brief Exp). This suggests that, for pup experience to lower maternal latency, corticosterone needs to be present during the initial pup exposure. Thus, corticosterone may play an important role in the initial consolidation of pup cues, in the presence of maternal hormones. The analysis of corticosterone levels after testing showed that the level of corticosterone in rats given corticosterone exogenously (ADX/CORT) differed significantly from the amount found naturally within the sham rats (Sham). The levels of corticosterone in rats given corticosterone also differed from the level of corticosterone in the rats that lacked corticosterone altogether (ADX/No CORT). This finding shows that corticosterone levels lower than the endogenous circulating levels were adequate to significantly lower the maternal latency while at the same time increasing maternal memory and maternal behaviors.

Alternatively, corticosterone may not be the hormone driving the changes in maternal latency and behavior of primiparous rats. Other hormones that influence both the stress system and maternal behavior may be involved. For example, increases in CRH indirectly increase corticosterone levels, which, in turn, decrease CRH levels through negative feedback loops (Makino et al., 2002). When the adrenal glands are removed and corticosterone levels are decreased, CRH levels are increased, and, when corticosterone replacement is given, CRH levels return to normal (Watts et al., 2004). Hormonally primed virgin rats given CRH will show increased pup killing (Pedersen et al., 1991), suggesting that high CRH levels disrupt the assimilation of pup cues by rats with high levels of maternal hormones. The fact that adrenalectomized rats not given corticosterone (ADX/No CORT), regardless of pup experience, in the present study showed higher latencies to become maternal and decreased maternal behaviors upon re-exposure than did adrenalectomized rats given corticosterone and with 4-h pup experience (ADX/CORT/4HR Exp) suggests that it may be that high levels of CRH are inhibiting the onset and memory of maternal behavior. Furthermore, in maternal adrenalectomized rats with brief pup experience, the lack of corticosterone, or possibly high levels of CRH, decreased maternal behavior. Little research has addressed the direct role of CRH in maternal behavior in rats, although CRH does inhibit maternal aggression in postpartum mice (Gammie et al., 2004). As well, in a description of differential neural effects of exogenous CRH in virgin and lactating females (Da Costa et al., 1997), Numan and Insel (2003) concluded that "these differentially activated neural regions are known to be involved in mediating fearful behavior, such as the corticomedial amygdala which is also involved in inhibiting maternal behavior".

Other hormones/neurotransmitters involved in the stress system and maternal behavior that also may play a role in corticosterone's effects on maternal memory include oxytocin, opioids, prolactin, and the catecholamines (Byrnes and Bridges, 2000; Byrnes et al., 2002; Insel, 1990; Modney et al., 1990; Moffat et al., 1993; Pedersen, 1997; Pedersen et al., 1994). For example, the interaction between the noradrenergic system and the HPA axis in maternal memory may be especially interesting as epinephrine has been found to be closely interrelated with the regulation of the HPA axis (Habib et al., 2001) and to play a role in maternal memory (Moffat et al., 1993).

In conclusion, corticosterone enhanced maternal memory and maternal behavior upon re-exposure in rats with 4-h postpartum pup experience. Whether corticosterone enhances the consolidation or retrieval of memories formed during postpartum pup experience remains unclear, but both consolidation and retrieval of other types of memory, such as object recognition (Okuda et al., 2004), are dependent on training experience prior to retention tests. Other hormones and neurotransmitters, such as CRH and norepinephrine, may also be modulating the effects of corticosterone and pup experience on maternal memory. The exact mechanisms underlying the HPA axis' role in maternal memory remain to be determined.

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