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Hormones and Behavior 46 (2004) 411–419

Hormones and Behavior

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The effects of adrenalectomy and corticosterone replacement on maternal behavior in the postpartum rat

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Received 23 February 2001; revised 8 January 2004; accepted 31 March 2004
Available online 7 June 2004

Abstract

It is well known that the hypothalamic–pituitary–adrenal (HPA) axis is activated during stress. Recent work suggests it is also implicated in the regulation of "normal" behaviors. The present studies investigated the effects of adrenalectomy and of varying glucocorticoid concentrations on adult maternal behavior in primiparous rats. In two studies, rats in late pregnancy were adrenalectomized or given sham surgeries and were tested for maternal behavior. In the first study, primiparous rats were given 0, 25, 100, 300, or 500 μg/ml of corticosterone in their drinking water. In the second study, primiparous rats were given either control or corticosterone time-release pellets. Blood samples were taken to ensure that rats demonstrated levels of corticosterone in blood that were relative to doses received. In studies one and two, primiparous adrenalectomized rats showed slightly, but significantly, lower levels of some maternal behaviors, including licking and time in nest, than primiparous sham rats. Primiparous rats given higher doses of corticosterone replacement showed higher levels of these maternal behaviors than primiparous rats given lower doses of corticosterone. In conclusion, adrenalectomy decreases, but does not abolish, maternal behavior. Corticosterone replacement reverses these effects. Corticosterone is not necessary for the initiation or maintenance of maternal behavior but plays a role in the modulation of ongoing maternal behavior.
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Keywords: Adrenalectomy; Corticosterone; Maternal behavior; Postpartum; Hypothalamic–pituitary–adrenal axis; Rat

Introduction

The hormonal profile of gestation in the rat prepares the expectant mother to respond maternally toward pups immediately at parturition. Once maternal behaviors are displayed, however, these hormones are no longer necessary for the maintenance of maternal behaviors (Fleming and Li, 2002; Numan, 1994). This profile of parturitional hormones, which includes rising or elevated levels of estrogen, prolactin, and oxytocin against a background of declining progesterone (Bridges et al., 1990; Numan, 1994), is important for the initiation of maternal behaviors in new mothers, especially in female rats that have not had prior exposure to pups. These same hormones also increase the likelihood that mother rats will respond maternally to offspring by enhancing the mother's attraction to infant cues, reducing her neophobia, and reducing "fearfulness" in the context of novel pups (Fleming and Li, 2002; Fleming et al., 1999).

Other hormones that are changed during gestation and lactation are the hormones of the hypothalamic–pituitary–adrenal (HPA) axis (Neumann, 2001; Schlein et al., 1974; Windle et al., 1997). While it is clear that these hormones, more specifically corticosterone, change during gestation and lactation and affect the mother rat's stress response, it is unclear if, and how, these hormones affect the actual expression of maternal behavior. After parturition, corticosterone levels are high during baseline conditions (Zarrow et al., 1972). After stress, mother rats show a blunted HPA axis response toward neutral stressors but will show behavioral and physiological stress responses to stimuli associated with pups (Neumann, 2001; Smotherman et al., 1977; Windle et al., 1997). For example, when pups were shocked during a separation period, mother rats showed elevated levels of corticosterone upon reunion with these pups as compared to reunion with handled pups (Smotherman et al., 1977).

Stressing pups also increases the amount of maternal licking they receive when reunited with their mother (Walker

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doi:10.1016/j.yhbeh.2004.03.010

et al., 2003). This suggests that the mother rat's stress response is adapted so that the mother rat is less distracted by maternally irrelevant stimuli and very attentive to maternally relevant stimuli. The HPA axis is changed in the mother rat, and this change alters how the mother rat reacts to stressful situations. This alteration can be dependent on the presence or absence of pups and the stage of lactation (Deschamps et al., 2003).

Mother rats can show both variations in their HPA axis stress response and variations in their maternal behavior, but whether these variations are related to one another remains to be determined. One step in determining the relationship between the HPA axis and postpartum maternal behavior is to establish whether the removal and replacement of corticosterone have any effects on the expression of maternal behavior. A role for the HPA axis in maternal behavior is not unlikely. Adrenal hormones are clearly required to maintain a pregnancy, and these can be supplied to the mother rat from the fetuses' adrenal glands (Chatelain et al., 1980). During parturition, the fetuses that remain in the uteri continue to supply adrenal hormones to the mother rat until all fetuses are delivered, and these hormones continue circulating for 24 h. During this time, the mother rat behaves quite maternally, showing responses that could potentially be influenced by corticosterone.

Different studies report varying effects of glucocorticoids on maternal behavior. In some cases, the removal of the adrenal glands, the source of glucocorticoids, appears to be facilitatory (Thoman and Levine, 1970) and in other cases inhibitory or disruptive (Hennessy et al., 1977). In the Thoman and Levine (1970) study, after being adrenalectomized before mating, adrenalectomized mother rats spent more time in the home nest and more time in a lactating position, and they kept pups in the nest for a longer period of time during the postpartum period than did mother rats that received a sham surgery (Thoman and Levine, 1970).

The disparity in reported effects of adrenalectomy on maternal behavior between studies is probably due to methodological differences, such as time of adrenalectomy, use of foster pups, and length of testing period. It is important therefore to use healthy foster pups and to undertake the adrenalectomy as late in gestation as possible, two factors that could affect whether or not maternal behavior is compromised postpartum (Hennessy et al., 1977; Thoman and Levine, 1970). What also remains unclear is whether the effects of adrenalectomy on maternal behavior are due to the removal of corticosterone, other hormones that are released by the adrenal glands or hypothalamic or pituitary hormones that are affected by the adrenal hormones.

Consistent with a role of the glucocorticoids in the modulation of maternal behavior, Fleming et al. (Fleming and Anderson, 1987; Fleming et al., 1997; Stallings et al., 2001) found in human mothers that elevations in baseline cortisol on days 2 and 3 postpartum were associated with more affectionate behaviors while interacting with the infant, with greater attraction to infant odors, with greater discrimination of own versus other infant odors, and with more sympathy in response to infant cries. In the cry study, sympathy was also associated with elevated heart-rate responses (Stallings et al., 2001). Taken together, these findings suggest that the HPA axis may be involved in the mother's response to her offspring and suggest that in rats glucocorticoids could contribute to emotional responses of mother rats toward pups.

The present project is an attempt to determine the role of corticosterone in postpartum maternal behavior in primiparous rats by removing and replacing corticosterone. In an attempt to address discrepancies of previous studies and the lack of research on the role of corticosterone in maternal behavior, foster pups were used, adrenalectomies were performed in the later stages of pregnancy, and several doses of corticosterone were given. Unlike most other maternal studies where glucocorticoids were administered by injection, which is stressful in and of itself, in the first study the different concentrations of corticosterone were administered to rats in their drinking water, using a procedure that has been used successfully by others (Bodnoff et al., 1995; Deak et al., 1999; Hausler et al., 1992; McCormick et al., 1997; Watters et al., 1996). In the second replication study, an alternate mode of delivery of corticosterone was used through implantation of corticosterone pellets, as used successfully by others (Fernandes et al., 1997; Van den Buuse et al., 2002). To insure adequate delivery of corticosterone for both modes of hormone delivery, concentrations in blood of corticosterone and ACTH were assayed.

Experiment 1: Effects of adrenalectomy and corticosterone replacement in drinking water on maternal behavior

Methods

Subjects and housing

Thirty-two 60- to 90-day-old female Sprague–Dawley rats were subjects in these experiments. All of the rats were offspring from an original stock from Charles River Farms (St. Constant, Quebec, Canada) and were housed at the University of Toronto at Mississauga (Mississauga, Ontario, Canada). All rats were individually housed in $45 \times 40 \times 20$ cm^3 plastic cages under 12:12 (light/dark) conditions (lights on at 0800). Room temperature and humidity were 69/76°F and 50/60%, respectively. Tap water and food (Lab Diet: The Richmond Standard Rat Diet) were available ad libitum. All procedures involving animals were approved by the University of Toronto Animal Care Committee.

Treatment groups

Primiparous rats were either adrenalectomized (ADX) (n = 28) or received sham surgery (n = 4). Within the adrenalectomized condition, rats received corticosterone (CORT) replacement in concentrations of either 0 μg/ml (n = 4), 25 μg/ml (n = 4), 100 μg/ml (n = 8), 300 μg/ml (n = 8), or 500 μg/ml (n = 4).

Primiparous females were adrenalectomized at day 17 (+ or −1 day) of gestation. On the day of parturition, their pups were removed and a 1-day-old foster litter was provided. Also on the day of parturition, corticosterone replacement was given in the drinking water. Beginning the following day, the primiparous rats were maternally tested for 10 consecutive days.

Adrenalectomy

All adrenalectomies were done bilaterally through two dorsolateral midflank skin and muscular incisions. Sham surgeries were identical to adrenalectomies except that the adrenals were not removed. All incisions were sutured. All rats were anesthetized with isoflurane gas (Aerrane Brand) during surgery. All rats that underwent adrenalectomies were provided with 0.5% saline in their drinking water.

Corticosterone replacement

For corticosterone replacement, each concentration of corticosterone was dissolved and stirred over low heat in 4 ml of ethyl (absolute) alcohol then combined with 0.5% NaCl water, yielding final concentrations of 0 μg (no), 25 μg (low), 100 μg (medium), 300 μg (high), or 500 μg (very high) CORT (Sigma) per ml of 0.4% EtOH, 0.5% NaCl. The amount of water consumed by each rat was analyzed in a second experiment to determine whether there were any group differences.

Maternal testing

For maternal tests, six donor foster pups, from nonmanipulated donor mother rats of University of Toronto at Mississauga colony, were placed in the cage in the diagonally opposite corner of the nest site. The frequency and duration of the following behaviors were observed for 10 min: (a) retrieving (carrying a pup to the nest site); (b) licking, with separate observation of body licking and genital licking; (c) sniffing pup; (d) nest building; (e) over pups, with separate observations of hovering (being over pups and engaged in licking or other behaviors) or of crouching (usually with pups nursing); (f) mouthing (carrying pups in the cage after retrieval); (g) sniffing air; and (h) self grooming. Twenty-four hours later, the positions of the pups and the tested mother rat were noted, and the pups were removed and returned to their natural mothers that served as the donors. Within approximately 10 s of removing the pups, the nesting material in the cage was scattered by the experimenter and a fresh set of six donor pups was introduced to the diagonally opposite corner of the original nest. Observations of the same behaviors described above were made for 10 min. Nest disruption during this phase of the testing procedure was done to assess nest building behaviors. To simplify presentation, all significant differences in genital or body licking are reported as differences in licking; all differences in hovering or low crouching are reported as differences in over pups.

Results

Maternal behavior

Using a series of one-way analyses of variance (ANOVAs), the total for each maternal behavior (the sum of 10 days of maternal behavior) was analyzed. For the analysis of surgical condition, adrenalectomized rats receiving no corticosterone replacement were compared to rats given a sham surgery. For the analysis of corticosterone condition, only adrenalectomized rats were used.

Effects of adrenalectomy on quality of maternal behavior

There was an effect of surgery on the duration of licking [$F(1,10) = 5.42$, $P < 0.042$], with adrenalectomized rats showing less body licking than sham rats (see Fig. 1). Although not significant, the same pattern was shown for genital licking. There were no significant effects of surgery on the duration of other behaviors. Specifically, there were no effects on retrieval [$F(1,10) = 1.10$, $P = 0.342$]. All groups retrieved all pups during the tests.

Effects of corticosterone dose on quality of maternal behavior

There was also a main effect of CORT condition on the total duration of time over pups [$F(4,15) = 4.18$, $P < 0.018$] and time in nest [$F(4,15) = 3.77$, $P < 0.026$]. Rats given the very high (500 μg) or high (300 μg) doses of CORT spent more time over pups and time in nest than rats given medium doses of CORT (Tukey's, $P < 0.05$) (see Fig. 2). Also, there was trend towards an effect of CORT condition on the total

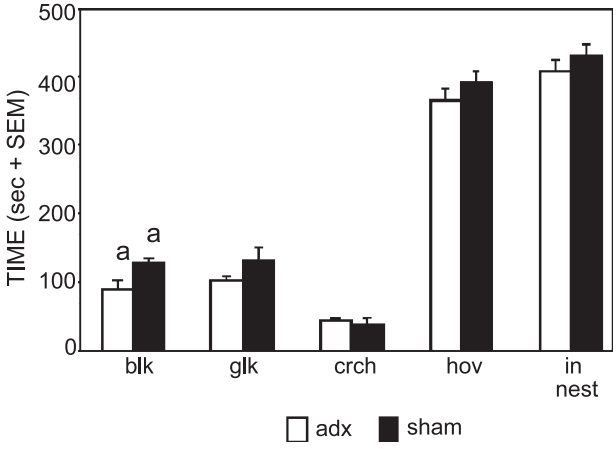

Fig. 1. Experiment 1: Average duration of maternal behaviors in seconds shown by adrenalectomized and sham primiparous rats (mean + SEM; histograms sharing letters show significant differences, $P < 0.05$) (ADX n = 4; SHAM n = 4).

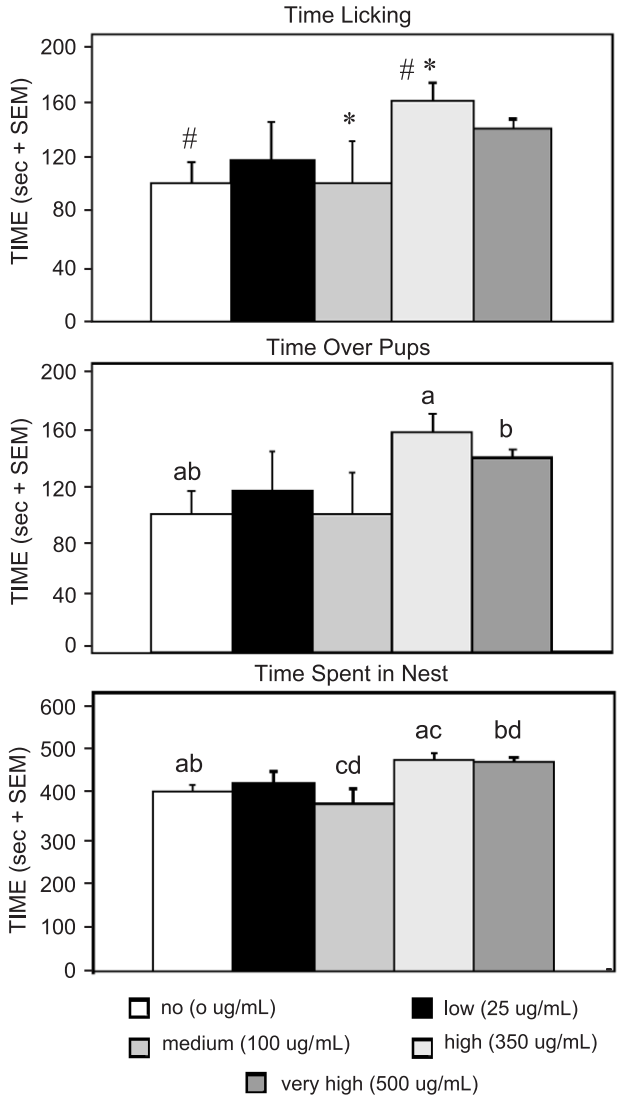

Fig. 2. Experiment 1: Average duration of maternal behaviors in seconds shown by adrenalectomized rats given different concentrations of corticosterone (mean + SEM; histograms sharing letters show significant differences, $P < 0.05$; symbols show significant differences, $P < 0.04$, 1 T) (no CORT $n = 4$; low CORT $n = 4$; medium CORT $n = 4$; high CORT $n = 4$; very high CORT $n = 4$).

duration of licking [$F(4,15) = 2.59$, $P < 0.079$; $P = 0.04$, 1 T]; the high CORT concentration produced marginally higher levels of genital licking. There were no significant effects of corticosterone replacement on the other behaviors. Specifically, there were no effects on retrieval [$F(4,15) = 0.474$, $P = 0.709$]. All groups retrieved all pups during the tests.

Amount of water consumed

Analyses were also undertaken to determine if there were any differences in the amount of water consumed among groups receiving either a high dose (300 μg/ml), a low dose (25 μg/ml), or no dose (0 μg/ml) of corticosterone in their drinking water, as described above. For 10 days, the amount of water consumed was recorded each day. There was no significant effect of corticosterone dose on the amount of drinking water consumed. This experiment demonstrated that there were no differences between amounts consumed of the different doses of corticosterone (mean ± SEM: high = 89.11 ± 2.45 ml; low = 96.19 ± 5.02 ml; no = 81.94 ± 3.82 ml), thus ensuring that the dose of corticosterone consumed was presumably relative to the dose of corticosterone given.

Concentrations of corticosterone in the blood

A separate experiment was completed to determine if different doses of corticosterone given in drinking water were related to different levels of corticosterone (Cort-a-Count Diagnostic Products, Los Angeles, CA; inter-assay = 8.5%; intra-assay = 6.8%) in the blood. Levels of ACTH were also measured (ICN Biomedicals, Inc., Costa Mesa, CA; inter-assay = 7.4%; intra-assay = 5%). Rats were adrenalectomized or given a sham surgery and given a 0.5% saline solution. In a different set of groups, rats were adrenalectomized and given, as per method described above, either a high dose (300 μg/ml, $n = 5$), a low dose (25 μg/ml, $n = 5$), or no dose (0 μg/ml, $n = 5$) of CORT in their drinking water. Drinking bottles were removed for 22 h to restrict consumption and were returned for 2 h to ensure consumption. In an earlier study, blood samples were taken without restricting consumption, and no differences in corticosterone levels were found. Using this procedure, however, it could not be determined or controlled when rats last consumed their drinking water and hence what concentration of corticosterone they have in their systems. By the rats drinking immediately before sacrifice, levels of corticosterone in blood could be accurately determined.

Using an one-way ANOVA, it was found that adrenalectomized rats had lower levels of corticosterone than sham rats [$F(1,10) = 4.91$, $P < 0.05$] (see Table 1). Also, it was found that blood levels of corticosterone reliably related to the doses of corticosterone given in the drinking water [$F(2,15) = 30.68$, $P < 0.001$] (see Table 1). As well, the highest levels of blood ACTH were associated with the no corticosterone condition and these levels of ACTH were different from levels found in the medium and high corticosterone conditions [$F(2,15) = 5.36$, $P < 0.018$] (see Table 1).

Table 1
Experiment 1: Effects of adrenalectomy and corticosterone replacement via drinking water on ACTH and corticosterone levels in primiparous rats (mean + SEM) (significant differences denoted by shared letters)

	CORT (nmol/l)	ACTH (pg/ml)
ADX	266.70 (56.56)[a] ($n = 4$)	
SHAM	544.94 (94.16)[a] ($n = 8$)	
High CORT	1422.33 (136.5)[b] ($n = 6$)	76.84 (80.9)[c] ($n = 6$)
Low CORT	458.02 (149.5)[b] ($n = 5$)	23.78 (88.5)[c] ($n = 5$)
No CORT	188.59 (126.3)[b] ($n = 6$)	426.41 (74.9)[c] ($n = 6$)

Experiment 2: Effects of adrenalectomy and corticosterone replacement in pellet form on maternal behavior

In this second study, study one was replicated but in this study corticosterone was administered in pellet form rather than in the water.

Methods

Subjects and housing

Twenty-nine 60- to 90-day-old female Sprague–Dawley rats were used in these experiments. All rats were born and housed at the University of Toronto at Mississauga. All rats were housed and fed in the same manner as in Experiment 1.

Treatment groups

Primiparous rats were divided into two groups of rats: a sham group (n = 4) and an adrenalectomized group (n = 14). Within the adrenalectomized group, rats received either a corticosterone pellet (n = 7) or a vehicle pellet (n = 7).

Primiparous rats were adrenalectomized at approximately day 17 of gestation, according to the procedures described in Experiment 1. All adrenalectomized rats were given 0.05% NaCl in their drinking water. Forty-eight hours after parturition, either a corticosterone pellet or a vehicle pellet was implanted subcutaneously. Maternal testing began 1 day after the pellet insertion and continued for eight consecutive days, from day 3 to 11 postpartum.

Corticosterone replacement

Corticosterone pellets (75.0 mg/pellet, 21 day release; Innovative Research of America) were inserted subcutaneously through a midline dorsal incision in the neck area.

Maternal testing

Using an event recorder, daily maternal testing was completed for eight consecutive days. The frequency and duration of the maternal behaviors were recorded over a 10-min test, as described above in Experiment 1. Spot checks were performed at approximately 2 h after the initial maternal test. The position of the tested rats and foster pups was noted as well as the behavior being performed at the time of the spot check.

Results

Maternal behavior

Using a series of repeated measures ANOVAs, the maternal behavior over the 8 days of testing was analyzed. For the analysis of surgical condition, adrenalectomized rats receiving no corticosterone replacement (vehicle pellet) were compared to rats given a sham surgery. For the analysis of corticosterone condition, only adrenalectomized rats were used.

Effects of adrenalectomy on maternal behavior

There was an interaction between surgical condition and days in licking [$F(1,13) = 6.73$, $P < 0.036$], time over pups [$F(1,13) = 11.73$, $P < 0.011$], and time spent in nest [$F(1,13) = 7.77$, $P < 0.027$]. In comparison to sham rats, adrenalectomized rats showed lower levels of genital licking and in nest behaviors during the early postpartum test days (test days 1–4), but there were no differences during the later postpartum days (test days 5–8) (see Fig. 3). In terms of time over pups, sham rats showed an increase in crouching over pups over time, whereas the adrenalectomized rats sustained a lower unchanging level. There was also a main effect for time over pups, where sham rats also showed more crouching than adrenalectomized rats [$F(1,13) = 6.14$, $P < 0.028$] (see Fig. 3). There were no effects of adrenalectomy on other behaviors. Specifically, there were no effects on retrieval [day: $F(1,13) = 0.355$, $P = 0.570$; day × surgery: $F(1,13) = 0.241$, $P = 0.638$; surgery: $F(1,13) = 0.437$, $P = 0.529$].

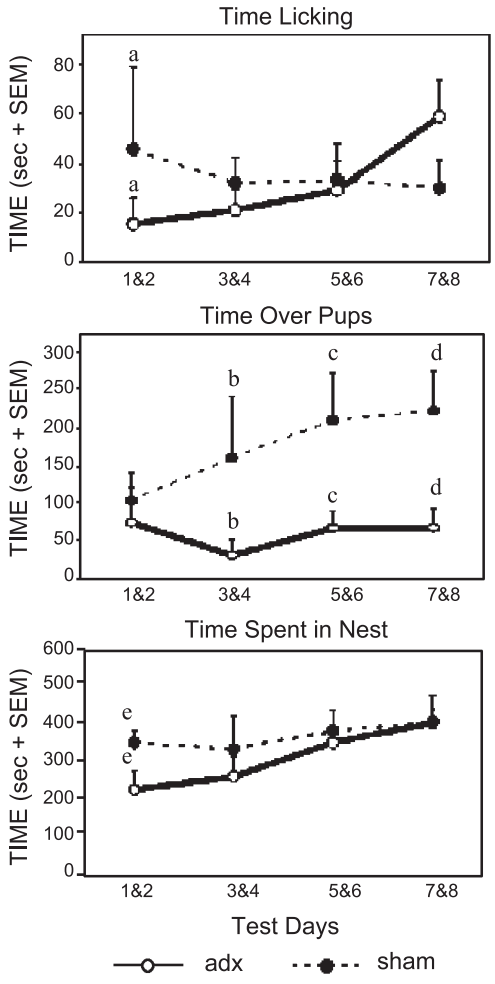

Fig. 3. Experiment 2: Duration of maternal behaviors in seconds over time shown by adrenalectomized and sham primiparous female rats (mean + SEM; histograms sharing letters show significant differences, $P < 0.05$) (ADX n = 6; SHAM n = 9).

Effects of corticosterone replacement on maternal behavior

There was an interaction between day and CORT condition on the duration of licking [$F(1,12) = 6.16$, $P < 0.029$] and time in nest [$F(1,12) = 5.36$, $P < 0.039$]. Rats given corticosterone showed higher levels of body licking on test days 5 and 6 (although not on other days). They also spent more time in the nest on the first few postpartum days than rats not given corticosterone (see Fig. 4). When all measures of over pup behaviors (hovering and crouching) were combined, there was a marginally significant effect of CORT condition on time over pups for the first 2 days of testing [$F(1,12) = 3.93$, $P < 0.071$, $P = 0.04$, 1 T], with rats given corticosterone showing higher levels than rats not given corticosterone (see Fig. 4).

There was also an effect of day on the duration of licking [body: $F(1,12) = 10.94$, $P < 0.006$; genital: $F(1,12) = 18.28$, $P < 0.001$], time over pups [$F(1,12) = 5.41$, $P < 0.038$], and time in nest [$F(1,12) = 7.76$, $P < 0.016$] with an increase in licking, crouching over pups, and time spent in nest over time (see Fig. 4). There were no effects of CORT condition on other behaviors. Specifically, there were no effects on retrieval [day: $F(1,12) = 2.98$, $P = 0.110$; day × CORT: $F(1,12) = 0.097$, $P = 0.761$; CORT: $F(1,12) = 0.083$, $P = 0.778$].

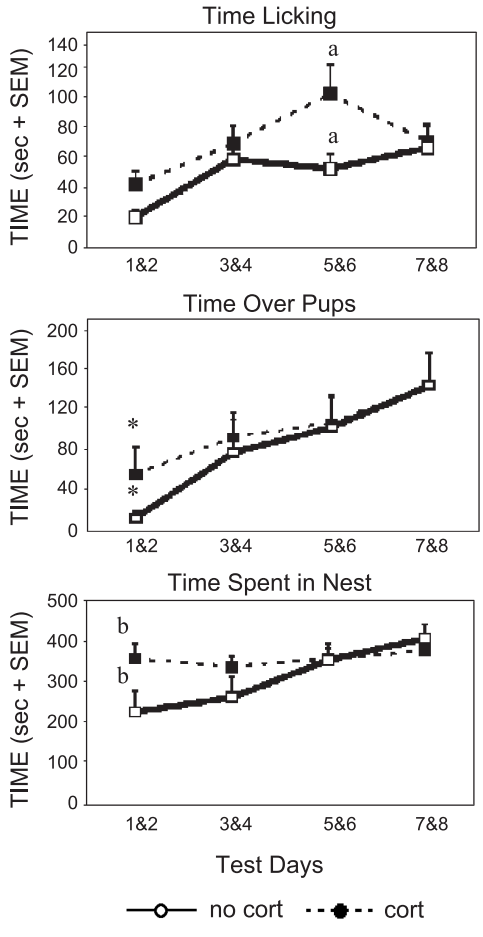

Fig. 4. Experiment 2: Duration of maternal behaviors in seconds over time shown by adrenalectomized rats given corticosterone or not given corticosterone (mean + SEM; histograms sharing letters show significant differences, $P < 0.05$; symbols show significant differences, $P < 0.04$, 1 T) (CORT $n = 8$; no CORT $n = 6$).

Table 2
Experiment 2: Effects of adrenalectomy and corticosterone replacement via subcutaneous pellets on ACTH and corticosterone levels in primiparous rats (mean + SEM) (significant differences denoted by shared letters)

	CORT (nmol/l)	ACTH (pg/ml)
ADX	163.14 (85.65)[a] ($n = 5$)	902.80 (128.62)[b] ($n = 5$)
SHAM	575.77 (117.51)[a] ($n = 4$)	129.53 (166.05)[b] ($n = 4$)
CORT	420.39 (67.71)[c] ($n = 5$)	507.34 (111.78)[d] ($n = 5$)
No CORT	163.14 (85.65)[c] ($n = 5$)	902.80 (128.62)[d] ($n = 5$)

Concentrations of corticosterone and ACTH in blood

Using only rats that were not given corticosterone replacement, the effect of adrenalectomy on blood levels of corticosterone and ACTH was analyzed (same radioimmunoassay kits as used in Experiment 1). Using a one-way ANOVA, rats that were adrenalectomized had lower levels of corticosterone [$F(1,7) = 100.49$, $P < 0.001$] and higher levels of ACTH [$F(1,6) = 120.86$, $P < 0.001$] than rats given sham surgeries (see Table 2).

Using only adrenalectomized rats, the effect of corticosterone replacement on blood levels of corticosterone and ACTH was analyzed. Using a one-way ANOVA, it was found that rats given corticosterone had higher levels of corticosterone [$F(1,11) = 5.42$, $P < 0.040$] and lower levels of ACTH [$F(1,11) = 6.77$, $P < 0.025$] than rats given vehicle pellets (see Table 2).

Correlations between maternal behavior and CORT and ACTH levels

Using a Spearman's correlation, correlations among maternal behaviors exhibited on the last day of testing or on maternal behaviors totaled over test days, and levels of corticosterone and ACTH in the blood were computed. There was a positive correlation between corticosterone and crouching on the last day of testing ($\rho = 0.499$, $P < 0.042$), as well as between corticosterone and licking (body: $\rho = 0.493$, $P < 0.045$). There was also a marginally significant correlation between corticosterone and total time spent in nest ($\rho = 0.478$, $P < 0.052$). In all cases, higher corticosterone levels were associated with higher levels of behavior.

Discussion

This experiment studied the effects of adrenalectomy and varying doses of corticosterone replacement on adult maternal behavior in the rat. Adrenalectomy decreased some maternal behaviors in the postpartum rat, and corticosterone replacement produced opposite effects. Primiparous female rats receiving higher doses of corticosterone spent more time

licking, more time over pups in some form of hovering or crouching behavior, and more time in the nest than rats given lower corticosterone concentrations.

Hormone levels change during the prepartum period and these changes enhance the immediate maternal responsiveness at parturition (Fleming and Li, 2002; Numan, 1994). Although corticosterone is also increased during pregnancy and is elevated for a period after parturition in the postpartum rat, it has been unclear as to how, or even if, corticosterone affects the expression of maternal behavior during the early postpartum period. In the present experiments, it is evident that removing corticosterone disrupts licking and time positioned over pups in a hover or crouch posture and that replacing and increasing corticosterone increases these or related maternal behaviors.

These results suggest that while adrenalectomy decreases the intensity of some behaviors, the motivation to respond is still present, and rats persist in retrieving and nursing pups. Corticosterone is therefore not necessary for the onset of the motivation to respond to pups or in the long-term maintenance of the behavior but plays a role by modulating the intensity of some behaviors. Although the behavioral effects of corticosterone administration are not large, they may well have significance for offspring development. The work of Caldji et al. (2000), Gonzalez et al. (2001), and Fleming et al. (2002) indicates that offsprings that receive less overall licking or licking-like stimulation during early postnatal development grow up to show more extreme stress responses, indicated by both increased HPA reactivity (Liu et al., 1997) and increased emotionality (Caldji et al., 1998; Francis et al., 1999), and to show decreased adult maternal behavior (Fleming et al., 2002; Gonzalez et al., 2001). Differences in intensity of behavior of the mother rat toward offspring can have long-lasting effects on the development of the offspring.

Whether corticosterone acts directly on the brain to affect these behaviors or, indirectly, by affecting peripheral or metabolic function is not known. As well, whether corticosterone itself is the relevant hormone is also not known. It is possible that the effects of removing the adrenal glands on maternal behavior are due to other adrenal hormones that are removed after surgical removal of the adrenal glands, although corticosterone is clearly a relevant hormone since its administration does increase maternal behaviors. For example, mineralocorticoids are secreted by the adrenal gland and affect electrolyte balance and reabsorption of sodium and water from the kidneys (Paillard, 1977). By removing aldosterone, a mineralocorticoid sodium regulation is in disequilibrium. Although all rats given adrenalectomies in these experiments were given 0.5% NaCl drinking water to compensate for loss of sodium reabsorption, it may be that this did not compensate for any effect that aldosterone may have on maternal behavior. For example, adrenalectomy alters water and sodium balance, and it is possible that adrenalectomized rats could compensate by increasing their anogenital licking. Through lactation, mother rats lose water as their pups suckle, but this water is regained as mother rats lick the anogenital regions of their pups (Gubernick and Alberts, 1985). It has also been suggested that low levels of anogenital licking seen in certain strains of rats may be due to their general aversion to saline, which translates to avoidance of the high salt content in pup urine (Moore and Lux, 1998).

While it is possible that disruption of water and salt reabsorption through adrenalectomy could lead to an increase in maternal licking, in the present context, it is believed that changes in corticosterone are central to the licking effects of adrenalectomy and corticosterone replacement. In the present experiments, there were no differences in ingestion of saline found between adrenalectomized and sham rats or among the groups receiving corticosterone replacement. As well, less, rather than more, licking was shown after adrenalectomies, and replacement with corticosterone (in the absence of the mineralocorticoids) reinstated licking. However, to understand more fully the role in the regulation of maternal behavior of mineralocorticoids, additional studies are clearly necessary. Finally, it is also possible that the effects of removing the adrenal glands on maternal behavior are due to other nonadrenal hormones that are altered after adrenalectomy, including andrenocorticotrophic hormone (ACTH) and corticotrophin-releasing hormone (CRH). The role of these hormones in rat maternal behavior has yet to be assessed.

Given that corticosterone is not necessary for the onset of maternal behavior, what role may it play? One possible function of corticosterone elevations may be related to a role in maternal memory. It has been shown that in the intact primiparous female rat, the maternal neural circuitry is altered when the rat behaves maternally. In terms of maternal memory, rats that interact with pups soon after birth, for even brief periods, show long-term retention of maternal behavior and enhanced memory of pup-related cues and alterations in the brain circuitry that underlies this behavior (Featherstone et al., 2000; Fleming and Korsmit, 1996; Fleming and Li, 2002; Fleming et al., 1996, 1999; Lee et al., 1999). Given the role of the HPA system in neural plasticity and in learning and memory (Anisman et al., 1998; Horvath et al., 1999; Oitzl et al., 1998; Radulovic et al., 1999), it may be that the HPA system enhances "maternal memory" perhaps by acting directly or indirectly on the maternal neural circuit or on peripheral physiology. Preliminary evidence indicates that corticosterone may indeed have this effect (Graham et al., in preparation). Although not necessary for the expression of maternal behavior, corticosterone does seem to perform either a modulating or maintaining function. It has yet to be determined, however, the mechanisms underlying these effects.

Acknowledgments

The authors would like to thank Katia Sinopoli, Radek Budin, Dean Graham, and Marg Coote for their assistance with surgeries, behavioral testing, and radioimmunoassays. This work was supported by NSERC to A.S. Fleming.

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