

The effects of adrenalectomy and corticosterone replacement on induction of maternal behavior in the virgin female rat

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Abstract

Maternal behavior of the sensitized virgin rat is affected by approach–avoidance systems as well as by hypothalamic–pituitary–adrenal (HPA) axis, which is also activated during stress. The present experiments investigated the effects of adrenalectomy and of varying corticosterone concentrations on the onset and expression of maternal behavior in sensitized virgin rats. In the first experiment, latency to onset of maternal behavior and time spent licking once maternal were positively related to endogenous levels of corticosterone. However, few rats showed licking. In the second experiment, virgin rats were adrenalectomized or given sham surgeries before being sensitized and being given 0, 25, 100, 300, or 500 µg/mL of corticosterone in their drinking water. In the third experiment, virgin rats were adrenalectomized or given sham surgeries and given either control or corticosterone time-release pellets after being sensitized. Maternal behavior was then tested. Adrenalectomy increased licking in the second experiment and time over pups in the third experiment. Corticosterone replacement reduced licking in the second experiment and both licking and time over pups in the third experiment. In conclusion, exogenous corticosterone had an inhibitory effect on the expression of maternal behavior in the sensitized virgin rat, unlike the facilitatory effect previously found in the postpartum rat.

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Introduction

Following parturition, mother rats show immediate maternal responsiveness to their offspring through the actions of circulating maternal hormones such as estrogen, progesterone, oxytocin, and prolactin (Fleming et al., 1999). Another hormone that modulates maternal behavior in the rat is corticosterone, although its presence is not necessary for the expression of maternal behavior (Rees et al., 2004). In this case, the removal of the adrenal gland, the source of corticosterone, decreases maternal licking and crouching in the postpartum rat, while the replacement with a high concentration of corticosterone increases these maternal behaviors (Rees et al., 2004). In addition to an effect on maternal licking and crouching, there is also evidence to show that corticosterone enhances mother rats' memory for pups during the postpartum

period, when other parturitional hormones are no longer exerting effects (Graham et al., *in press*).

In contrast to the rapid onset of maternal behavior shown at parturition by the new mother rat, virgin female and male rats can become maternal through continuous exposure to pups over several days, a procedure called maternal sensitization (Rosenblatt, 1967). This provides an example of maternal behavior that is based on experience rather than on maternal hormones, such as estrogen and progesterone. Maternal sensitization is thought to be a product of changes in a biphasic process involving approach and withdrawal behavior (Numan and Insel, 2003). Initially, virgin rats find pups aversive and will actively avoid them (Fleming and Luebke, 1981). Once virgin rats habituate to pups, approach behaviors can occur. Virgin rats are also more emotional than postpartum rats and show longer emergence latencies and less ambulation in a novel environment (Fleming and Luebke, 1981). With time and continuous exposure to pups, however, virgin rats come to display all components of the maternal response except lactation, although

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the quality of behavior is not identical to that seen in postpartum rats (Lonstein et al., 1999). While this procedure has been well studied and is known to occur independently of the hormones normally associated with parturition, little research has determined the role of corticosterone in sensitized maternal behavior. When exposed to pups, corticosterone levels are increased in non-maternal virgin rats and decreased in maternal virgin rats (Koranyi et al., 1977). This is similar to corticosterone changes after repeated exposure to a novel object (Dal-Zotto et al., 2003), suggesting that habituation to pups during maternal sensitization may be very similar to the habituation that occurs with other stressors.

Corticosterone also plays a role in other avoidant behaviors. For example, rats with increased corticosterone levels, both basal and stress-induced, showed more freezing behavior during a punished drinking test (Nunez et al., 1996). Elevated corticosterone is also associated with the development and expression of freezing/fear behavior (Takahashi and Rubin, 1993; Moriceau et al., 2004). This may be due to a change in how a stressful stimulus is being perceived. Bhatnagar et al. (2000) have suggested that corticosterone acts by altering or magnifying the salience of stimuli, both positive and negative; hence, the hormone augments the prepotent valence of stimuli. For example, corticosterone administration increases saccharin consumption, especially after deprivation, and sensitivity to rewarding brain stimulation; these are both positive stimuli (Barr et al., 2000; Bhatnagar et al., 2000). Corticosterone administration also affects the perception of negative stimuli; administration of corticosterone increases fear-conditioned freezing (Thompson et al., 2004). As well, there is a positive relation between corticosterone levels and fear-related behavioral inhibition (Cordero et al., 1998).

In the case of sensitization to pups in the virgin rat, corticosterone may enhance the salience of pup stimuli such that on initial exposure when pups are still novel, corticosterone may increase the avoidant-like behavior, perhaps by activating the fear withdrawal system. However, what the effect of corticosterone once the virgin rat has become maternal is unclear. Corticosterone could continue to have an inhibitory effect once virgin rats have become sensitized and once they express maternal behavior or it could instead enhance positive responses to pups, much as it has been shown to do in the postpartum rat (Rees et al., 2004).

The purpose of the following studies was to determine the role of corticosterone in the onset and expression of maternal behavior in the sensitized virgin rat. In the first experiment, the relation between endogenous corticosterone, the latency to exhibit maternal behavior, and the quality of maternal behavior shown was first established. In the second and third experiments, the effects of adrenalectomy and corticosterone replacement on virgin maternal behavior were assessed. Corticosterone was administered either in the drinking water (experiment two) 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), or in pellet form (experiment three), also successfully used by others (Fernandes et al., 1997; van den Buuse et al.,

2002). The present experiments will attempt to determine whether corticosterone would be inhibitory, as suggested by Leon et al. (1975) where adrenalectomy decreased maternal latencies in sensitized rats, or facilitatory to the expression of maternal behavior, as found in the postpartum rat (Rees et al., 2004). It could also have both effects at different stages of the sensitization process.

General methods

Subjects and housing

Overall, eighty four 70-day-old female Sprague–Dawley rats were subjects in these experiments (28 rats in experiment one; 44 in experiment two; 12 in experiment three). 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 $45 \times 40 \times 20$ cm³ plastic cages under 12:12 (light/dark) conditions (lights on at 08:00). 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.

Maternal sensitization

Maternal sensitization procedures were used in experiments one and two to assess the latency to become maternal. Rats were sensitized to become maternal in experiment three, but maternal latency was not assessed. On the day prior to first exposure to pups, virgin rats were placed in a large maternal cage and provided with nesting material. On the following day, virgin rats were exposed to six 2- to 6-day-old foster pups. These foster pups were replaced daily as the virgin rats were not lactating. Until each virgin rat became maternal (maternal criterion: retrieved all pups to nest site on two consecutive days within 2 h of foster pups being replaced), it was continually exposed to pups. Once the maternal criterion was achieved and all testing was completed, rats were sacrificed for blood sampling. In experiment one and three, maternal sensitization procedures began on PND 70, while, for experiment two, sensitization procedures began on PND 75, 5 days following surgical procedures.

Maternal testing

For maternal testing in experiments one and two, virgin rats were tested using a spot-check technique (i.e. one-zero time sampling which involves recording ongoing behavior every 5 s for 8 min to determine a frequency measure of behaviors) in a maternal retrieval test. For maternal testing in experiment three, an event recorder (BEST, Behavioral Evaluation Strategies and Taxonomies, S and K Computer Products, Toronto, Ontario) was used to record the frequency and duration of maternal behaviors during a 10-min maternal retrieval test. Maternal behaviors were observed immediately following the replacement of six donor foster pups aged 2 to 6 days, obtained from non-manipulated donor mother rats of University of Toronto at Mississauga colony. At 2 h following these retrieval tests, one 5 s spot-check was used to determine if all foster pups had been retrieved to the nest. If a virgin rat had retrieved all pups to the nest during the retrieval test or by the spot-check on two consecutive days, then that rat would be considered maternal. This testing procedure was continued each day until rats became maternal. For experiment one, rats were tested each day until the maternal criterion was reached, while, for experiments two and three, tests were undertaken daily for 10 and 8 consecutive days, respectively, once the maternal criterion was reached.

The frequency and duration of the following behaviors were recorded: 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 (arched back posture over pups while

not engaged in other activities); f) mouthing (carrying pups in the cage after retrieval); g) sniffing air; h) self grooming. For analysis in experiment one, behaviors shown on the day before the maternal criterion was reached and behaviors shown on the day after maternal criterion was reached (last day of testing) were observed.

Twenty-four hours later, the position 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 2- to 6-day-old donor pups was introduced to the diagonally opposite corner of the original nest. Another maternal retrieval test was then performed. Nest disruption during this phase of the testing procedure was done to assess nest building behaviors. To simplify presentation, if there was a significant difference in body licking or in genital licking, it was referred to as a difference in “licking” in the figures. Similarly, if there was a significant difference in hovering or low crouching, it was referred to as a difference in “over pup” behavior in the figures.

Adrenalectomy

In experiment two, on PND 70, virgin rats were adrenalectomized and, 5 days later, exposed to donor pups for maternal sensitization. In experiment three, once virgin rats had become maternal, they were adrenalectomized. All adrenalectomies constituted the bilateral removal of the adrenal glands through two dorso-lateral mid-flank skin and muscular incisions. Sham surgeries were identical to adrenalectomies except that the adrenal glands were not removed. All incisions were sutured. Rats were anesthetized during the surgical procedures with isoflurane gas (Aerrane Brand). Following surgery, adrenalectomized rats were provided with 0.5% saline in their drinking water. In experiment two, 20 rats were adrenalectomized, while 4 rats were given sham surgeries. In experiment three, eight rats were adrenalectomized, and four rats were given sham surgeries.

Corticosterone replacement

In experiment two, once adrenalectomized virgin rats became maternal (two consecutive days of retrieval), they were given corticosterone and then tested for maternal behavior over the following 10 days. 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 μ g (no), 25 μ g (low), 100 μ g (medium), 300 μ g (high), or 500 μ g (very high) of corticosterone (Sigma) per milliliter. The amount of water consumed by each rat was analyzed in a second experiment to determine whether there were any group differences. In a previous study using corticosterone replacement in drinking water, higher concentrations of corticosterone administered related to higher levels of corticosterone in blood of primiparous rats and lower concentrations of corticosterone administered related to lower levels of corticosterone in blood of primiparous rats (Rees et al., 2004). Furthermore, adrenalectomy results in lower levels of corticosterone (Graham et al., in press; Rees et al., 2004). In experiment two, adrenalectomized rats received no ($n = 4$), low ($n = 4$), medium ($n = 4$), high ($n = 4$), or very high ($n = 4$) concentrations of corticosterone.

In experiment three, 48 h after adrenalectomies were performed on maternal virgin rats, either a corticosterone pellet ($n = 4$) or a vehicle pellet ($n = 4$) was implanted subcutaneously. Maternal testing began 1 day after the pellet insertion and continued for eight consecutive days. 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. Blood samples were obtained to determine levels of corticosterone in blood. In previous studies, primiparous rats given corticosterone pellets had higher levels of corticosterone compared to primiparous rats given vehicle pellets (Graham et al., in press; Rees et al., 2004).

Blood sampling

In experiment one, once the maternal criterion was reached, rats were sacrificed, and trunk blood was collected after decapitation. In a separate set of rats, levels of corticosterone were analyzed for adrenalectomized and sham rats

as well as for rats given corticosterone replacement in their drinking water and for rats given corticosterone replacement through pellets. For each of these treatments (1. adrenalectomy; 2. corticosterone replacement in drinking water; 3. corticosterone replacement in pellet form), separate radioimmunoassays were completed. Trunk blood was also collected for these rats. Blood samples were stored in a -80°C freezer until analysis. Corticosterone was analyzed for all rats (Cort-a-Count Diagnostic Products, Los Angeles, CA; inter-assay = 8.5%; intra-assay = 6.83%).

Data analysis

For experiment one, in order to determine relations between endogenous corticosterone levels and maternal latency and behaviors, correlations using Spearman's rho were calculated. Furthermore, a series of Spearman correlation coefficients were computed relating corticosterone levels to the latency to become maternal and to behaviors expressed on the day before full retrieval and the day after first full retrieval.

For experiment two, since there were no effects of test day, maternal behaviors were averaged across testing days. To determine the effects of adrenalectomy on maternal behavior and maternal latency, multiple one-way analysis of variances (ANOVAs) were computed for experiment two, and 2 (surgery) $\times$ 8 (days) repeated measures ANOVAs were computed for experiment three to compare the behaviors of rats that were adrenalectomized and given no corticosterone replacement to rats that were given sham surgeries. To determine the effects of corticosterone replacement on maternal behavior and maternal latency, multiple one-way ANOVAs were computed for experiment two, and 2 (surgery) $\times$ 8 (days) repeated measures ANOVAs were computed for experiment three including only those rats that were adrenalectomized in the analyses. This allowed for comparison between dosages of corticosterone replacement.

Results

Experiment one: the relation between corticosterone, maternal latency, and maternal behavior

Maternal Latency

As shown in Fig. 1, virgin rats take from 5 to 20 days of continuous exposure to pups to begin exhibiting maternal behavior. By the fifth day of testing, 11% of the rats had become maternal, while by days 10 and 15, 39% and 50% had become maternal, respectively. On day 20, the final testing day, only 14% (four of the 28 rats) had not become maternal and, their latencies were recorded as the total number of testing days (see Fig. 1). The mean level of corticosterone was 528.82 ng/mL (SEM $\pm$ 74.30).

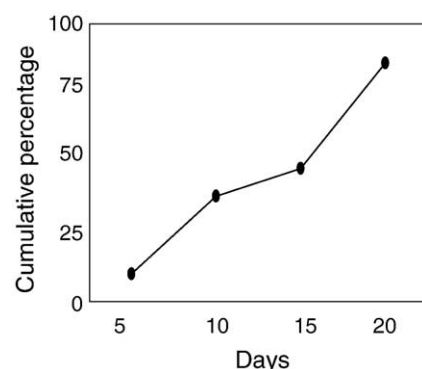


Fig. 1. Experiment one: cumulative percentage of rats becoming maternal on each test day ($n = 26$).

Correlations between corticosterone and maternal latencies

There were no significant relations between corticosterone levels, the latency to achieve the maternal criterion, or the latencies to show individual maternal behaviors such as retrieving, licking, or crouching. There was, however, a trend showing that rats that took longer to become maternal tended to have higher corticosterone levels ($\rho = 0.372$, $P = 0.061$) (see Fig. 2).

Correlations between corticosterone and maternal behaviors

Correlations between corticosterone and behaviors exhibited once maternal showed a significant positive relation between corticosterone levels and time licking on the last day of testing (genital licking: $\rho = 0.389$, $P < 0.050$) (see Fig. 2). Few rats showed genital licking, however, those that did showed a positive relation between licking and corticosterone levels. There were no significant relations between corticosterone and other behaviors on the last day of testing (see Table 1).

Experiment two: the effects of adrenalectomy and corticosterone replacement in drinking water on maternal latency and maternal behavior

Maternal latency

There were no latency differences between adrenalectomized and sham rats or among groups of adrenalectomized rats receiving different concentrations of corticosterone.

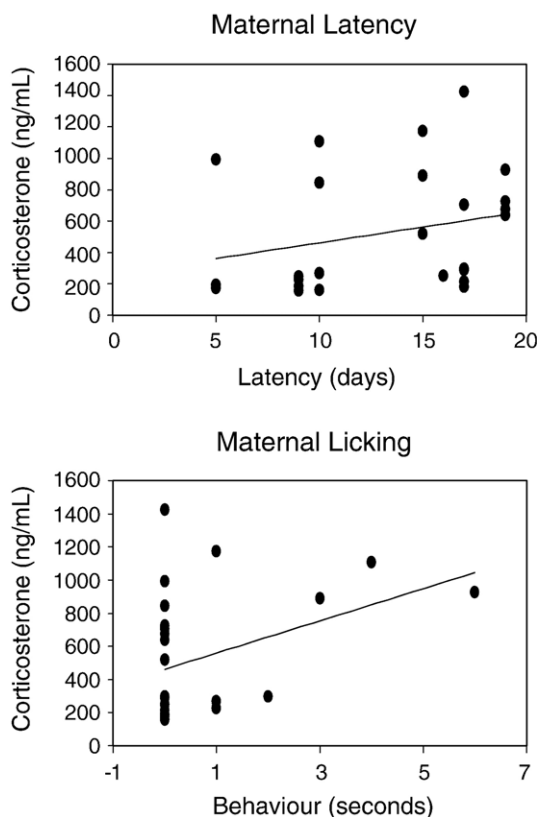


Fig. 2. Experiment one: scatterplot showing the relationship between corticosterone and measures of maternal latency (top) and behaviors (bottom) in virgin rats ($n = 26$).

Table 1

Experiment one: correlations between corticosterone and duration of various maternal behaviors on (a) the day before full retrieval and (b) the day after full retrieval (2nd day of maternal behavior) (symbols indicate significant correlations, $P < 0.05$)

	Retrieving	Body licking	Genital licking	Hovering
<i>Behavior before retrieval</i>				
CORT	$\rho = 0.348$ $P < 0.081$	$\rho = 0.200$ $P < 0.337$	$\rho = -0.092$ $P < 0.662$	$\rho = 0.517$ $P < 0.007^*$
<i>Behavior after retrieval</i>				
CORT	$\rho = 0.103$ $P < 0.624$	$\rho = -0.289$ $P < 0.161$	$\rho = 0.389$ $P < 0.050^*$	$\rho = 0.134$ $P < 0.523$

Maternal behavior

Effects of adrenalectomy. There was an effect of surgery on the time spent licking (body licking: $F(1,10) = 7.02$, $P < 0.024$), with adrenalectomized rats showing more body licking than sham rats. There were no significant effects on other behaviors measured (see Fig. 3).

Effects of corticosterone dose. There was an effect of corticosterone condition on the time spent licking (body licking: $F(4,23) = 2.94$, $P < 0.042$) with rats given no corticosterone showing the most body licking as compared to the other groups (see Fig. 4). There were no significant effects on other behaviors measured.

Amount of water consumed

Analyses were also undertaken to determine if there were any differences in the amount of water consumed among adrenalectomized groups receiving either a high dose (300 $\mu\text{g}/\text{mL}$), a low dose (25 $\mu\text{g}/\text{mL}$), or no dose (0 $\mu\text{g}/\text{mL}$) of corticosterone in their drinking water. For 10 days, the amount of water consumed was recorded each day. There was no significant difference among any of the groups in terms of amount of water consumed (mean $\pm$ SEM: high = 72.53 mL $\pm$ 12.25; low = 86.78 mL $\pm$ 1.84; no = 69.48 mL $\pm$ 3.00). Virgin rats given just saline consumed less than virgin rats given either dose of corticosterone. This experiment demonstrated that there were no differences in the amount consumed among the

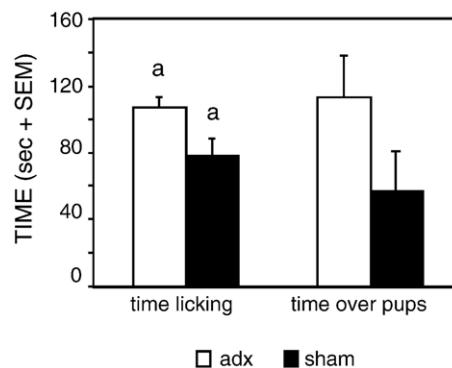


Fig. 3. Experiment two: average duration of maternal behaviors in seconds shown by adrenalectomized and sham rats (mean $\pm$ SEM; histograms sharing letters show significant differences, $P < 0.05$) (ADX $n = 4$; SHAM $n = 4$).

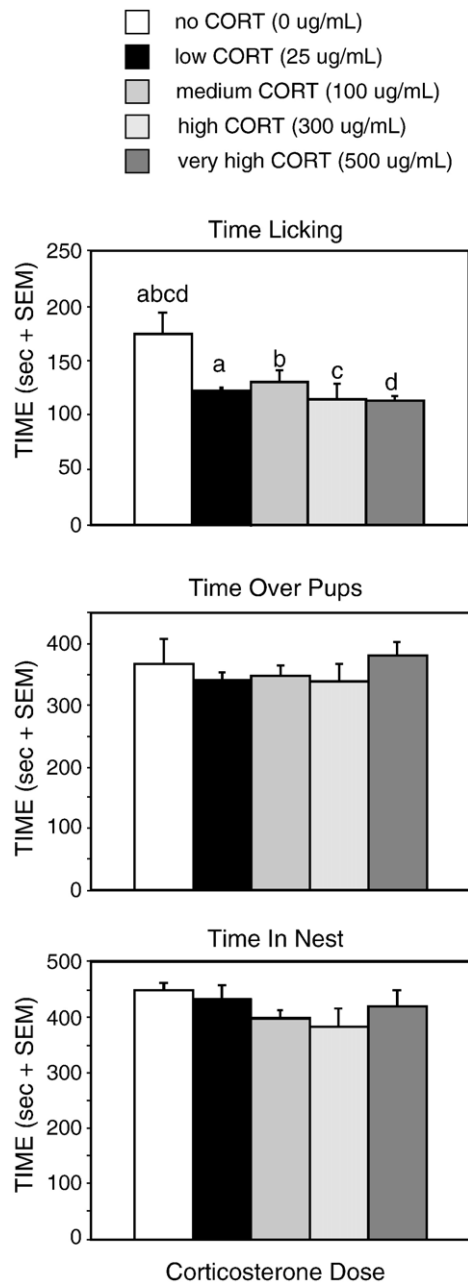


Fig. 4. Experiment two: average duration of maternal behaviors in seconds shown by adrenalectomized rats given different concentrations of corticosterone (mean + SEM; histograms sharing letters show significant differences, $P < 0.05$) (no CORT $n = 4$; low CORT $n = 4$; medium CORT $n = 4$; high CORT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

different doses of corticosterone, thus ensuring that the dose of corticosterone consumed was presumably relative to the dose of corticosterone given.

Experiment three: the effects of adrenalectomy and corticosterone replacement in pellet form on maternal behavior

Maternal behavior

Effects of adrenalectomy. There was a main effect of surgery on time over pups (high crouching: $F(1,7) = 6.36$, $P < 0.040$;

low crouching: $F(1,7) = 3.36$, $P < 0.040$). Adrenalectomized rats showed higher levels of high and low crouching than sham rats (see Fig. 5). There was also an effect of day on time spent licking (body licking: $F(1,7) = 16.77$, $P < 0.005$), time over pups (hovering: $F(1,7) = 9.25$, $P < 0.019$), and time spent in nest ($F(1,7) = 9.94$, $P < 0.016$), with increasing levels across time in most of these behaviors. There were no day by surgical condition interactions.

Effects of corticosterone. There was also an interaction between day and corticosterone condition on time spent licking (genital licking: $F(1,7) = 12.24$, $P < 0.010$). On the last 2 days of testing, there was an increase of genital licking for those rats not given corticosterone and a decrease of genital licking for those rats given corticosterone (see Fig. 6). Furthermore, there was a significant corticosterone effects for time spent over pups (high crouching: $F(1,7) = 5.35$, $P = 0.054$) with rats given corticosterone showing lower levels of high crouching (see Fig. 6).

Levels of corticosterone in blood samples

There was a significant effect of surgery on corticosterone levels ($F(1,7) = 12.57$, $P < 0.009$), with adrenalectomized rats having lower levels of corticosterone than rats given sham surgery (see Table 2). To determine corticosterone levels in rats receiving corticosterone in their water, in a separate group of ADX rats, water bottles with corticosterone concentrations were removed for 22 h to induce water deprivation. After the 22 h, water bottles with appropriate corticosterone concentrations were returned for 2 h. There was a significant effect of corticosterone dose with rats given high levels of corticosterone having higher levels of

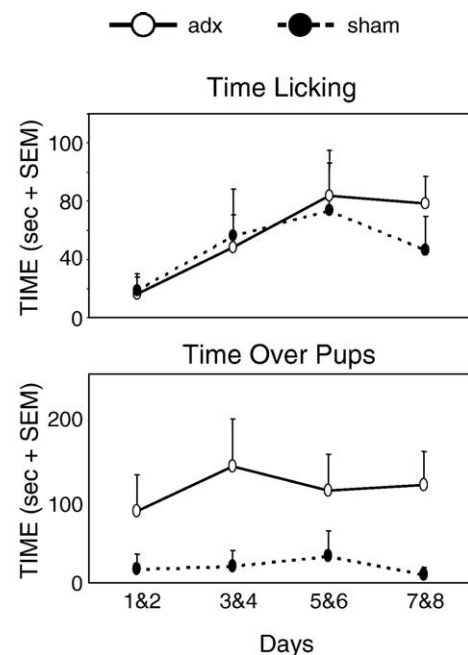


Fig. 5. Experiment three: duration of maternal behaviors in seconds over days shown by adrenalectomized and sham virgin female rats (mean + SEM; main effect of group, $P < 0.05$) (ADX $n = 4$; SHAM $n = 4$).

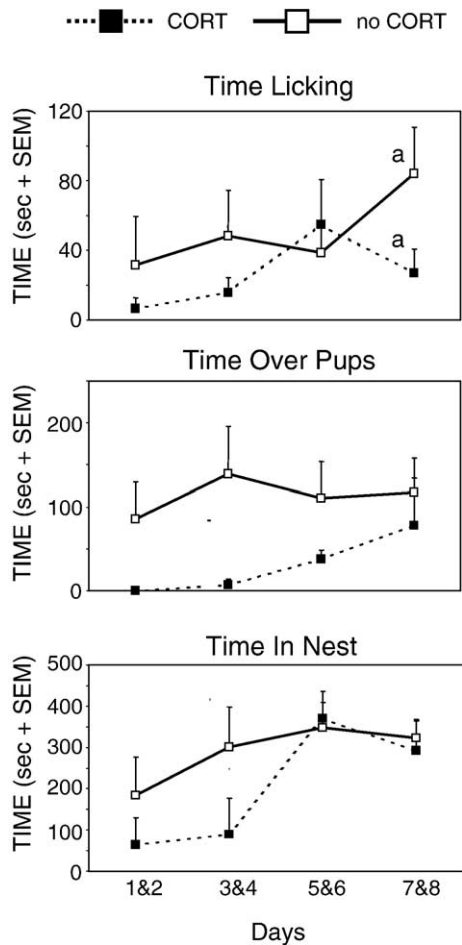


Fig. 6. Experiment three: duration of maternal behaviors in seconds over time shown by adrenalectomized rats given corticosterone or not given corticosterone (mean + SEM; histograms sharing letters show significant differences, $P < 0.05$; main effect of group, $P < 0.05$) (CORT $n = 4$; no CORT $n = 4$).

corticosterone in blood than rats given no corticosterone ($t_{10} = 2.77$, $P < 0.020$), and, although not significant, there was a graded difference between the high, low, and no doses of corticosterone ($F(2,15) = 3.33$, $P = 0.064$) (see Table 2). There was also a significant effect of corticosterone dose on ACTH levels with rats given high doses having lower levels of ACTH than rats given no dose (see Table 2).

In a separate set of ADX rats given corticosterone in pellet form, there was a significant effect of corticosterone replacement ($F(1,0) = 92.23$, $P < 0.000$) with rats given corticosterone pellets showing higher levels of corticosterone than rats given vehicle pellets (see Table 2). There was also a significant effect on ACTH levels with rats given corticosterone pellets showing lower levels of ACTH than rats given vehicle pellets (see Table 2).

Discussion

These experiments investigated the role of corticosterone on maternal behavior in the sensitized virgin rat. Contrary to expectations, in experiments one and two, corticosterone was marginally related to, but did not significantly affect, the latency

to become maternal. In experiment one, endogenous corticosterone was positively related to elevated licking once rats had become maternal, much as has been found for the postpartum rat (Rees et al., 2004). However, when corticosterone was removed through adrenalectomy, or administered exogenously, a different pattern emerged. In experiment two, adrenalectomy increased licking, and corticosterone replacement, at every dose, decreased licking. In experiment three, adrenalectomy increased crouching, and corticosterone replacement decreased genital licking (on final test days) and crouching. This inhibitory effect of corticosterone contrasts with what was seen in the postpartum rat (Rees et al., 2004).

There are a number of possible explanations for this parity difference in response to HPA manipulations. The primiparous rat undergoes many changes during pregnancy and following parturition that are not experienced by the virgin rat, including differences in underlying endocrine profile and hypothalamic–pituitary–gonadal function (Fleming et al., 1999), differences in feedback sensitivities and responsiveness of the HPA axis (Deschamps et al., 2003; Neumann, 2003; Windle et al., 1997), differences in brain activity (Numan and Numan, 1994), and differences in emotionality (Fleming and Luebke, 1981). Specifically in terms of the HPA axis, postpartum rats show a blunted response to stimuli that are not pup-related (Neumann, 2003; Windle et al., 1997) and higher basal ACTH and corticosterone levels (da Costa et al., 2001; Stern et al., 1973; Zarrow et al., 1972) when compared to virgin rats. Postpartum rats, however, show a normal HPA response to pup-related stressors (Deschamps et al., 2003; Smotherman et al., 1977). Modulation of maternal behavior by the HPA axis may be dependent on the actions of maternal hormones, such as estrogen, progesterone, oxytocin, and/or prolactin, that interact with the HPA axis and that differ between postpartum and virgin rats. Alternatively, modulation of maternal behavior by the HPA axis may depend more simply on basic differences in the HPA axis expression between postpartum and sensitized virgin rats. For example, postpartum rats show increased basal corticosterone levels (Atkinson and Waddell, 1995; Fischer et al., 1995; Stern et al., 1973; Zarrow et al., 1972), while sensitized

Table 2
Effects of adrenalectomy and corticosterone replacement on levels of corticosterone in blood (shared letters indicate significant correlations, $P < 0.05$; shared symbols indicate significant correlations, $P < 0.08$)

	CORT (ng/mL) (+SEM)	ACTH (pg/mL) (+SEM)
ADX	284.78 (155.8) ^a	708.13 (155.8)
SHAM	964.83 (220.35) ^a	510.43 (220.3)
<i>In drinking water</i>		
High (300 µg/mL)	843.58 (140.2) [*]	0 (0) ^{bc}
Low (25 µg/mL)	448.18 (172.9)	61.90 (40.6) ^b
No (0 µg/mL)	288.44 (136.3) [*]	276.29 (75.6) ^c
<i>In pellets</i>		
CORT	160.17 (15.4) ^d	360.8 (171.6) ^c
No CORT	11.5 (1.54) ^d	1430.13 (240.2) ^c

maternal virgin rats have decreased basal corticosterone levels (Koranyi et al., 1977) when compared to naive virgin rats.

Although in experiments two and three adrenalectomy increased, and corticosterone replacement decreased, maternal behavior in the virgin rat, there were some discrepancies between experiments two and three. In both experiments two and three, corticosterone decreased time spent licking, and, although adrenalectomy only increased licking in experiment two, a similar trend was seen in experiment three. Adrenalectomized rats in experiment three showed increased time spent over pups that was marginally and transiently decreased in rats given corticosterone replacement, while there were no effects on time spent over pups in experiment two (although a similar trend was seen). A possible reason for these inconsistencies lies in differences in methodology. In experiment two, virgin rats were adrenalectomized then sensitized until they became maternal then given corticosterone and tested for maternal behavior; in experiment three, virgin rats were first sensitized to become maternal then adrenalectomized and given corticosterone replacement and then retested for maternal behavior. Virgin rats in experiment two became maternal under conditions of low corticosterone, while virgin rats in experiment three became maternal under conditions of normal fluctuating levels of corticosterone. Alternatively, licking and crouching may be differentially modulated by corticosterone in the sensitized virgin rat. Overall, exogenous corticosterone consistently decreases licking in the maternal virgin rat. Adrenalectomy, however, did not consistently affect maternal behavior in the maternal virgin rat, although, when it did have an effect, it enhanced maternal behavior.

Another question that arises from these experiments is why there are differences in the relationship between corticosterone and licking in experiment one (endogenous corticosterone) and in experiments two and three (exogenous corticosterone). In evaluating this discrepancy, it is important to note that the effects of adrenalectomy and corticosterone replacement may not be due solely or entirely to direct effects of corticosterone but rather may also involve changes in other hormones of the HPA axis, such as CRH and/or ACTH. For example, when a rat is adrenalectomized, CRH levels will rise due to the disruption of negative feedback from corticosterone. This can be reversed with corticosterone replacement (Watts et al., 2004). Presumably, in the adrenalectomized rats in the experiments described above, a similar effect would have occurred. If CRH is the hormone relevant to maternal behavior in virgin rats, then it is possible that in experiments two and three increased CRH levels in adrenalectomized rats enhanced maternal behavior. This would be consistent with results from experiment one, where the high levels of corticosterone were likely associated with high levels of CRH. Unfortunately, very little research has addressed the effects of CRH and/or ACTH on the expression of maternal behavior. Although the administration of CRH to hormonally primed virgin rats increases cannibalism (Pedersen et al., 1991) and inhibits maternal aggressiveness in postpartum mice (Gammie et al., 2004), from this evidence, it is unclear what would be the effects of CRH in the non-primed sensitized virgin rat.

It is also possible that the HPA axis effects on maternal behavior are in part peripherally mediated. Anogenital licking is important to rat pups for defecation and urination. Anogenital licking is also beneficial for the mother rat. A lactating female rat will provide nutrients to the pups through her milk, and, in order to replenish water and electrolytes loss through this milk letdown, the lactating female will ingest the pups' urine and feces (Gubernick and Alberts, 1985). Lack of mineralocorticoids, hormones that affect sodium and water balance in the kidney (Paillard, 1977), through adrenalectomy, could alter the electrolyte balance such that adrenalectomized rats would show increased genital licking to regulate an imbalance. While it is possible that disruption of water and salt reabsorption through adrenalectomy could lead to an increase in maternal licking, it is likely that the lack of corticosterone rather than adrenal mineralocorticoids is important for the effects of adrenalectomy on maternal behavior. As well, corticosterone had no effect on saline intake but did depress licking, suggesting that corticosterone rather than mineralocorticoids is important for the effects of adrenalectomy and corticosterone on maternal behavior. Additional studies are required to further clarify the role of mineralocorticoids and CRH, as well as interactions between maternal hormones and the HPA axis, in the regulation of maternal behavior in both postpartum and virgin maternal behavior.

How these experiments relate to the role of glucocorticoids in human maternal behavior remains unclear. In postpartum human mothers, increased basal cortisol on days two and three postpartum was 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 (Fleming and Anderson, 1987; Fleming et al., 1997; Stallings et al., 2001), consistent with the positive effect of corticosterone on maternal behavior in the postpartum rat (Rees et al., 2004). Furthermore, postpartum women who were more sympathetic to infant cries showed higher cortisol levels compared to both postpartum women who were less sympathetic and to non-postpartum women. Non-postpartum women had lower cortisol levels after listening to infant cries than postpartum women, regardless of how sympathetic they were to cry stimuli (Stallings et al., 2001). This suggests that cortisol may also be related to the perception of infant-related stimuli. This evidence, along with the evidence that the role of corticosterone in maternal behavior of the rat depends on parity, suggests that glucocorticoids play a different role in non-postpartum females than in postpartum females. As well, teen postpartum mothers engage in more instrumental behavior and less affectionate behavior and have higher basal cortisol levels than mature postpartum mothers (Krupan et al., 2005), suggesting that cortisol levels may be associated with differences in behavior. How this relates to maternal behavior of adoptive human mothers (the analogue of the maternal virgin rat?) remains unclear, but it does raise some intriguing questions.

In conclusion, postpartum and virgin rats show differences in HPA axis expression and activation (da Costa et al., 2001; Windle et al., 1997) that may cause the effects of corticosterone

removal and replacement on the maternal behavior to differ between postpartum and virgin rats. While primiparous rats show a decrease of the intensity of maternal behavior with the removal of corticosterone and an increase with high levels of replaced corticosterone (Rees et al., 2004), maternal virgin rats, in general, show an opposite pattern of effects—although there seems to be more variability in the relation between corticosterone status and maternal behavior in the virgin than previously reported for the postpartum mother. Whether adrenalectomy and corticosterone replacement effects are due to direct effects of corticosterone on maternal behavior or to indirect or interactive effects of other hormones, such as CRH, ACTH, and/or the maternal hormones, remains unclear.

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