Effects of restricted nursing on physiological and behavioral reactions of Brahman calves to subsequent restraint and weaning

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Abstract

The influence of restricted nursing on physiological and behavioral reactions to later restraint and weaning was examined in Brahman (Bos indicus) calves. The calves were blocked for sex and randomly assigned at birth to one of two treatments: restricted nurse (RN), or ad libitum nurse (ALN). The RN calves were separated from their dams at 21 days of age and allowed to nurse once daily (2-h duration) for 19 ± 3 days. The ALN calves remained with their dams from birth until weaning. The ALN calves were brought in and worked through handling facilities for the same amount of time each day as the RN calves. At 192 ± 4 days of age each calf was restrained while blood samples (5) were taken and heart rate monitored via telemetry. Two weeks later, the calves were weaned and blood samples and behavioral observations were taken. Mean plasma cortisol concentration (MC) increased over time for both treatments (P < 0.004) in response to restraint, but was greater for RN than for ALN calves after 15 min of restraint (P < 0.03). In response to restraint, RN calves had overall greater heart rates than did ALN calves (P < 0.02). In response to weaning, ALN calves tended to travel more (P < 0.07), and included more calves who

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ate ($P < 0.05$), and vocalized ($P < 0.05$). The effects of the restricted nursing regimen, which inherently includes handling, appears to alter the calf's later response to restraint and weaning. However, due to the lack of a uniform response further research is required to determine the meaning of and causal factors for this alteration. © 1998 Elsevier Science B.V.

**Keywords:** Calves; Stress; Nursing; Handling; Maternal deprivation

### 1. Introduction

Although an animal's experiences from birth to weaning are but a small percentage of its lifetime experiences, stress during infancy has been shown to decrease emotional behavior and/or increase the ability to cope with stressful situations in maturity for rats and mice (Denenberg and Karas, 1960; Levine et al., 1967; Levine and Mullins, 1968; Ader, 1975; Denenberg, 1975), rabbits (Kerstein et al., 1989), cats (Seitz. 1959; Wilson et al., 1965), sheep (Moberg and Wood, 1982), horses (Houpt and Hintz, 1983; Heird et al., 1986), and cattle (Boissy and Bouissou, 1988). These research projects used various 'stressors' including handling, restraint, and maternal deprivation. Early research on social deprivation (e.g., Harlow, 1958; Harlow and McKinney, 1971) has illustrated the importance of maternal contact in the rhesus macaque. This species showed pathological type behavior when deprived of maternal contact during infancy. The physiological basis for alterations in behavior due to experiencing 'stress' during development remains unclear. Adrenal hormones released in response to stress during infancy have been found to modify the brain and it has been suggested that this modification causes differences in the emotional behavior of these individuals in maturity (Denenberg and Zarrow, 1971). The decreased emotionality in maturity found in rodents and livestock may improve the ability of an animal to handle subsequent stress. The actual mechanisms involved have not been elucidated but there appears to be a difference between acute and chronic stressors. For example, Levine (1962) and Halmeyer et al. (1967) found that rats handled in infancy responded to acute noxious stressors with a greater corticosteroid release, but chronic stressors elicited less of a reaction. The handling used by these researchers contained a component of maternal deprivation. Indeed, this has been a major issue in early stress research. One cannot handle an animal without depriving it of its dam. Thus it is important to consider that stressors are multifaceted and it is not clear if maternal deprivation stress will have the identical effect of handling in livestock.

A restricted nursing regimen, a potential stressor to a calf, is sometimes used to help initiate estrus cycles soon after parturition (Browning et al., 1994). In order to restrict a calf's nursing, the calf must be herded in and out of pens when the dam is introduced and then removed; this manipulation of the calf will be referred to as 'handling'. Handling is an essential component of a restricted nursing procedure, and manipulations by humans of the neonatal animal have been shown to have an affect on that animal's subsequent reactions to stress (e.g., Boissy and Bouissou, 1988; Hilakivi-Clarke et al., 1991). A restricted nursing regimen could have short term negative effects on the calf due to a decrease in milk intake and lack of maternal care. However, if a restricted nursing regimen decreases the stress or emotional response displayed by calves in
response to stressors experienced by the calf as it matures, then welfare and production of those calves could be enhanced.

2. Animals, materials and methods

The following experiment was conducted to determine what effect an early restricted nursing/handling regimen had on a calf’s heart rate, plasma-cortisol concentration, and behavior in response to stress at weaning. This experiment was performed under the guidelines of the Animal Care and Use Committee of Texas A&M University. At birth, we blocked for sex and randomly assigned 38 Brahman (Bos indicus) calves to one of two treatments: restricted nursing (RN), or ad libitum nursing (ALN). The RN calves were separated from their dams for 19.0 ± 2.6 days at 21 days of age and allowed to nurse only once daily for a 2-h duration. These calves were maintained in a 12 m × 15 m dry-lot paddock. They were offered concentrate (75% corn, 25% solvent-extracted soybean meal), coastal bermuda grass hay, and water. The pen also contained a 3 m × 5 m shelter. After the approximate 20-d separation period, the RN calves were returned to normal ad-libitum nursing from their dam until weaning. The ALN calves remained with their dams from birth until weaning; therefore, they were allowed to have ad-libitum access to nurse.

Because the dams of RN calves were allowed access to their calf each day, after the 2-h access both dams and calves had to be herded into an alley and cut into separate pens to remove the dams. This manipulation of the calves was considered ‘handling’. Because the dams of the ALN calves were blood sampled, along with the dams of the RN calves, they too had their calves herded into the alley and cut into a separate pen. This separation of the ALN calves from their dams lasted no longer than 45 min. Additionally, both the RN and the ALN calves were weighed once a week. Consequently, both RN and ALN calves were subjected to the same amount of handling. The calves assigned to both the RN and ALN treatment were handled from 21 days of age until they were approximately 120 days of age. During the 100 days of handling, observations by research personnel manipulating the cattle indicated that the calves became accustomed to this handling. This judgement was made based on the decreased flight distance of the calves, fewer escape-avoidance reactions, and an increased ease of movement. The design of this experiment isolated the effect of restricted nursing from the effects of ad-libitum nursing by keeping the effects of handling that are inherently associated with a restricted nursing regimen constant.

Temperament scores were assigned to each calf based on daily interactions between the calf and the observer (Neuendorff) during the 100 days of routine handling procedures. A calf received a score of 1 if it approached, touched, pushed, and licked the observer; a score of 2 if it approached the observer but not close enough to touch and would not tolerate human touch; or a score of 3 if the calf did not approach the observer and retreated when approached. This scoring system has proven to be a reliable indicator of temperament and has been shown to be positively correlated with plasma cortisol (Lay et al., 1992).
In the spring, when the calves were 191 ± 3 days of age, their behavioral and physiological responses to restraint and handling were determined. Each calf was separated from the herd and loosely restrained in a squeeze chute for 20 min while vocalizations, defecation, urination, and tail switching were recorded. Heart rate and blood samples were also taken at this time. This was the first time that any of the calves had been restrained in a squeeze chute. Nineteen calves were sampled on each of two consecutive days. To control for diurnal rhythm effects, calves from alternating treatments (every other) were restrained throughout the day.

Heart rates were recorded at 5, 7.5, 10, 12.5, 15, 17.5, and 20 min relative to when the calf entered the squeeze chute. These heart rates were recorded at these intervals for a duration of 30 s, the mean of this 30-s sample was recorded. At time 0 min, a stainless steel 22 mm surgical staple (Biomedical Research Instruments, Rockville, MD) was clipped to the left side of the calf in front of the shoulder, while a second staple was clipped on the right side of the calf over the ribs, directly behind the shoulder. Electrode leads were attached to each staple with small alligator clips. A battery-powered transmitter telemetrically relayed the heart beats to a Telonics TR-2 receiver controlled by an Apple IIe computer, the computer recorded each beat and tabulated a per minute rate using a modification of Datacol (Stuart and Friend, 1989).

Five 7-ml blood samples were collected by jugular venipuncture, into vacutainers containing 0.07 ml of a 15% Na EDTA solution. Each calf’s head was restrained with a halter during venipuncture to facilitate sampling. The blood samples were collected at: 0, 5.5, 10.5, 15.5, and 20.5 min relative to when the calf was caught in the squeeze chute. Blood samples were placed in an ice bath and centrifuged at 0° to 5°C within 2 h of sampling. The plasma was then frozen at −25°C until cortisol concentrations were determined.

Cortisol concentrations were determined on duplicate samples using commercially available coated-tube RIA kits (Pantex, Santa Monica, CA). Samples were re-assayed if the duplicates differed by more than 5%. The intra-assay CV for cortisol was 7.6%. The recovery rate for cortisol added to bovine plasma was 98%. Cross reactivity of the antiserum was as follows: cortisol, 100%; prednisolone, 40%; 11-deoxycorticisol, 13.3%; corticosterone, 10.5%; cortisone, prednisone, and dexamethasone were less than 3.1% (analysis by Pantex).

Two weeks after the calves were restrained in the squeeze chute, their response to weaning was determined. Five trained observers were used to collect behavioral data from randomly assigned calves. Training consisted of having each observer watch the same calf to record its behavior. This procedure was repeated until each observer was recording the same behaviors. Because the recorded behaviors were discrete (eating, walking, vocalizing) consistency between observers was easily accomplished. The weaning protocol is presented in Table 1. Each calf was separated from its dam and herded into a working chute for weighing and one 7-ml jugular blood sample was collected via vacutainer. The calf was first weighed and then blood sampled. It was then placed into a pen with other calves from the same treatment. Once a group of 9 or 10 calves was gathered they were transported 8 km and placed in a 21 × 4 m pen. These calves were then observed for the first 15 min after entering the pens. One to two calves were assigned to one of five observers who counted vocalizations, traced the movement
of the calf onto a scaled drawing of the pen for later determination of distance travelled (DT), and recorded time spent eating. Every 30 min a group of calves from an alternate treatment was then separated from their dams, transported, and placed in an adjacent pen until all 38 calves (4 pens) had been weaned within 2 h. The calves had tactile contact with adjacent pens, and visual, auditory, and olfactory contact with all of the calves on the study. Cortisol, heart rate, body weight, and distance travelled data were analyzed using the GLM procedure of SAS (1985). Split plot models were used to describe the data with day, time, day × time, calf identification nested in treatment, sample number, calf identification nested in treatment × sample number, sex, temperament, weight, and interactions incorporated as main factors. The day × time error was used to test day and time effects, the calf identification nested in treatment × sample number error was used to test for treatment and sample number effects, and the residual error was used to test for sex, temperament and weight effects. If the treatment × sample number interaction was significant \((P < 0.05)\), then least square means for the interaction were used to elucidate differences at individual sample times. The variation of the repeated data was equal for each animal; therefore, a repeated measures analysis is not presented in this manuscript because the factor calf identification nested in treatment was included in the model. This factor accounts for repeated measures using the assumption that the variation between the data points is equal for each animal. Behavioral data collected during restraint and the data for the number of calves that vocalized and/or ate during the 15-min behavior observations at weaning were analyzed using the Chi-square test (SAS, 1985). Because the calves were housed together during weaning, some behavioral measures may not be entirely independent. Therefore, the data should be viewed with this in mind. Taking into consideration that all of the calves from both treatments had auditory, visual, and olfactory contact during weaning, any treatment differences would be masked, not created. The data for the number of vocalizations and the duration of time spent eating during the 15-min behavioral observations were subjected to the 2-sided Kolmogorov–Smirnov two-sample test (Siegel and Castellan, 1988).
3. Results

No treatment differences were found (\( P > 0.2 \)) for the number of calves who: vocalized (4 RN vs. 6 ALN), defecated (3 RN vs. 2 ALN), tail switched (10 RN vs. 7 ALN), or urinated (7 for each treatment) while being restrained in the squeeze chute.

Restraint in the squeeze chute elevated mean plasma cortisol concentrations (cortisol concentrations) in both treatments during the entire 20 min sampling procedure (\( P < 0.004 \), Fig. 1). However, the RN calves had greater cortisol concentrations at 15 and 20 min (\( P < 0.03 \) and 0.002, respectively) than the ALN calves. Although 20 min is approximately 10 min short of when we would expect to see maximal plasma cortisol concentrations, this quick response difference indicates that the hypothalamic–pituitary–adrenal axis of RN calves was more adept at responding to the restraint stress. Furthermore, previous research (Lay et al., 1996) examining different doses of ACTH (roughly comparable to experiencing different degrees of stress) indicate that maximal concentrations of plasma cortisol are reached at 30 min even when ACTH concentrations are relatively low.

The RN calves also had overall greater heart rates than the ALN calves (79 vs. 74 ± 0.7 beats/min, respectively; \( P < 0.02 \)) while being restrained. Because the calves were not trained to the restraint procedure (as this would negate the stress test) no baseline heart rates were obtainable which makes these relatively high rates difficult to interpret as typical or due to the restraint procedure. However, previous research using similar aged calves (Lay et al., 1992) found minimal heart rates of approximately 65 beats/min and maximal heart rates (after hot iron branding) of 80 beats/min. The treatment differences found in this study were significant (\( P < 0.02 \)) and indicate

![Graph showing cortisol response to restraint](image)

Fig. 1. Mean (± SE) plasma cortisol response to restraint. Bars with different letters within each sampling time differ (\( P < 0.03 \)). Mean plasma cortisol concentrations for both treatments were affected by sampling time (\( P < 0.004 \)).
Table 2
Behavioral data collected during weaning for ad libitum (ALN) and restricted nursed (RN) calves

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>Time</th>
<th>No. of calves vocalizing</th>
<th>Mean no. of vocalizations</th>
<th>No. of calves eating</th>
<th>Mean duration of eating (min)</th>
<th>Distance travelled (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ALN</td>
<td>RN</td>
<td>ALN</td>
<td>RN</td>
<td>ALN</td>
</tr>
<tr>
<td>Day 0</td>
<td>1</td>
<td>1200</td>
<td>11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.8</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1530</td>
<td>14</td>
<td>15</td>
<td>7.3</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2000</td>
<td>19</td>
<td>18</td>
<td>15.3</td>
<td>14.2</td>
</tr>
<tr>
<td>Day 1</td>
<td>4</td>
<td>1200</td>
<td>18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.9</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1530</td>
<td>19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.1</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2000</td>
<td>17</td>
<td>15</td>
<td>10.7</td>
<td>12.4</td>
</tr>
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</table>

Data were collected (sequentially by pens) during 15-min observation periods.
The column headed by ‘Time’ indicates when observers started the collection sequence.
<sup>a,b,c,d</sup>Numbers in the same row within a behavioral category with different superscripts differ ($P < 0.05$; $P < 0.08$).

Inherent differences between calves who had experienced RN compared with the normal suckled calves.

During weaning, more ALN calves vocalized during the 15-min observation periods at sampling period 1, 5 ($P < 0.05$), and 4 ($P < 0.08$) than did the RN calves, although overall mean vocalization rates by treatment did not differ ($P < 0.10$, Table 2). More

Fig. 2. Mean ($\pm$ SE) plasma cortisol response to weaning. Cortisol concentrations did not significantly differ ($P = 0.15$) between treatments in response to weaning (Day 0, 1600; Day 1, 1100; and Day 1, 1600 samples). Mean plasma cortisol concentrations for both treatments were affected by sampling time ($P > 0.002$).
ALN calves tended to eat at sampling period 3 and 4 than did the RN calves (P < 0.05 and 0.07, respectively). The RN calves spent more time eating at sample 6 (P < 0.009, Table 2). The ALN calves tended to travel further overall than the RN calves (77.9 ± 3.6 vs. 68.6 ± 3.7 m, P < 0.07) in response to weaning. Weight gain during weaning did not differ between ALN and RN calves (16.7 vs. 16.6 ± 1.1 kg, P > 0.50), with overall weights averaging 167.4 ± 3.0 and 173.8 ± 3.3 kg, respectively.

Cortisol concentrations did not significantly differ (P = 0.15) between treatments in response to weaning (Day 0, 1600; Day 1, 1100; and Day 1, 1600 samples). Cortisol concentrations on Day 5 were not used in that analysis because the calves were expected to have recovered from weaning by 5 days, and that assumption is supported by visual examination of Fig. 2. Cortisol concentrations were affected (P < 0.002) by time after weaning with an overall increase in cortisol concentrations peaking on day 0 at 1600, and decreasing to basal concentrations by 5 days after weaning (Fig. 2).

Temperament score did not affect cortisol concentrations (P > 0.35) or heart rates (P > 0.40) during restraint, nor did temperament affect behavior (P > 0.2) during restraint or weaning. However, temperament did affect overall cortisol concentrations during weaning (P < 0.02), with temperament scores of 1, 2, and 3 having cortisol concentrations of 14.0 ± 1.2, 14.5 ± 1, and 20.4 ± 3 ng/ml, respectively. The sex of the calf did not affect behavioral or physiological responses to restraint or weaning (P > 0.10).

4. Discussion

Generally, the RN calves had greater mean plasma cortisol concentrations and overall heart rates than the ALN calves in response to restraint (Table 3). During weaning, eating by RN calves was decreased; however, the RN calves appeared to be less stressed, as indicated by a lower distance travelled and number of calves that vocalized. Because a restricted nursing regimen has components of maternal deprivation, handling, and altered milk intake; the importance of each factor on differences due to the restricted nursing regimen is unclear. Studies on handling and maternal deprivation of young or neonatal livestock (e.g., Moberg and Wood, 1982; Houpt and Hintz, 1983) have shown

![Table 3](image)

<table>
<thead>
<tr>
<th>Restraint</th>
<th>Weaning</th>
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<tr>
<td></td>
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<tr>
<td>Cortisol</td>
<td>Heart</td>
</tr>
<tr>
<td>rate</td>
<td>rate</td>
</tr>
<tr>
<td>RN calves</td>
<td>↑</td>
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</table>

Direction of the arrows indicate the response of the restricted nursed (RN) calves in relation to the response of the ad libitum nursed calves (P < 0.08). A dash line indicates that no differences between treatments were found.
similar results to research with rodents (Ader, 1975; Denenberg, 1975), but the ‘handling’ stimulus was quite different between the experiments. Handling of rodents usually consisted of holding the animal for a short duration and returning it to the nest, whereas handling in livestock requires that the animal be herded through a chute or working area and the animal may never be touched by humans.

The data obtained in this experiment lacked a uniform response within treatments which could be due to the different personal experiences of each animal. Denenberg (1975) has suggested that it is the amount of stimuli (amount of handling, etc.) that is beneficial to the animal, not the actual type of stimulation per se. This is important when we consider that the laboratory rats referred to in this manuscript were maintained in a relatively stimulus-free environment when compared with our calves who were raised in a large herd and experienced seasonal changes. One possible component contributing to the lack of homogenous experiences of each calf was learning from their dam. Temperament between dams could differ; therefore, when the dams reacted to being herded, the calves could learn the dam’s response pattern. It is highly likely that the behavioral response and possibly the physiological response of each calf to handling is correlated with its dam’s responses. For this reason, we assigned temperament scores to each calf and included this variable in the experimental analysis. In this study, even after the restricted nursing regimen was completed for the RN calves, both the RN and ALN calves were separated daily, by the same personnel, and kept in a paddock while their dams were blood sampled as part of another study on reproduction (Browning et al., 1994) until they were approximately 120 days of age. This separation lasted no longer than 45 min. Both the RN and the ALN calves were weighed once a week. Consequently, both RN and ALN calves were subjected to the same amount of handling. Differences should, therefore, be due to the deprivation of nursing and maternal contact. If greater cortisol and heart rates indicate a lessened coping ability, then maternal deprivation decreased the coping ability of restricted nursed calves to respond to restraint, but may have increased their ability to adjust to weaning. Harlow (e.g., Harlow, 1958) completed a series of very elegant experiments which clearly showed that maternal deprivation and social isolation caused pathological type behavior in rhesus macaque monkeys. This is contradictory to the research on rodents, rabbits, cats, sheep and the current research presented here. There are several possibilities for this discrepancy. First, the monkeys were usually maintained in total social isolation, not just maternal isolation. Secondly, compared with the species listed, monkeys are much more altricial and, therefore, require more care during infancy. Thirdly, it would appear that the highly social nature, the group structure, and the long juvenile period require that these primates learn more of their social behaviors from social situations compared with many other animals. Hilakivi-Clarke et al. (1991) concluded that early handling, but not maternal deprivation, caused an increased coping ability of the rats that they studied.

There are many uncontrollable factors that can enter a calf’s life as it is raised on a pasture, including variations in temperature, food, and human contact. If the bovine hypothalamic-pituitary-adrenal axis is influenced by environmental factors during its maturation process, as occurs in the rodent adrenal gland, different environmental stress demands could cause differential changes in the adrenal gland. This experiment was conducted in the spring; therefore, the calves had been born in the fall and lived through
the winter. Environmental and seasonal changes are uncontrollable and could influence adrenal responsiveness to stressors (maternal deprivation and handling).

This study was part of a project on reproduction (Browning et al., 1994) which found that restricted nursing benefited reproductive efficiency of the dam. If this procedure also helped the calf to adjust to stress more appropriately in maturity, then the restricted nursing procedure would have proven even more beneficial as a management tool for increasing production. The restricted nursing regimen used in this study appeared to cause calves to have a greater stress response during restraint, but may have benefited the calves during weaning. However, due to a lack of a uniform response further research is required to determine the meaning of and causal factors for this alteration.

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