Relationships Between Patterns of Fecal Corticoid Excretion and Behavior, Reproduction, and Environmental Factors in Captive Black (*Diceros bicornis*) and White (*Ceratotherium simum*) Rhinoceroses

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Mortality is high in zoo-housed black rhinoceroses (*Diceros bicornis*), and the reproductive rates of captive white rhinoceroses (*Ceratotherium simum*) are unsustainably low. To determine the possible role of stress in the causation of these problems, we analyzed weekly fecal samples collected for 1 year from black (10 males and 16 females) and white (six males and 13 females) rhinoceroses at 16 zoos for corticoid metabolite concentrations. Fecal corticoid profiles were examined in relation to behavior as rated by keepers in a questionnaire, luteal phase ovarian cycles of females (Brown et al., 2001), and socioenvironmental factors. We compared individual fecal corticoid profiles by examining hormone means and variability (i.e., standard deviation (SD) and coefficient of variation (CV)). For the black rhinos, higher mean corticoid concentrations were found at zoos where rhinos were maintained in enclosures that were exposed to the public around a greater portion of the perimeter. Higher variability in corticoid excretion was correlated with higher rates of fighting between breeding partners and higher institutional mortality rates. Black rhino pairs that were kept separated exhibited lower corticoid variability and less fighting activity when they were introduced during female estrous periods compared to pairs...
that were kept together every day. For white rhinos, significantly lower mean corticoids were found for individuals that rated higher on “friendliness to keeper.” Higher corticoid variability was found in noncycling as compared to cycling white rhino females. Noncycling females exhibited higher rates of stereotypic pacing and lower frequencies of olfactory behaviors. Interindividual differences in mean corticoids in both species appeared to be related to responsiveness to humans, whereas corticoid variability was related to intraspecific social relationships. More importantly, high corticoid variability appeared to be an indicator of chronic or “bad” stress, because of its association with potentially deleterious consequences in each species (i.e., fighting and mortality (black rhino), and reproductive acyclicity (white rhino)). Our results provide evidence that social stressors may cause chronic stress in black and white rhinos, and that this contributes to the captive-population sustainability problems observed in each species. Zoo Biol 24:215–232, 2005. © 2005 Wiley-Liss, Inc.

Key words: stress; adrenal activity; olfactory behavior; ovarian activity; reproduction; mortality

INTRODUCTION

The captive populations of African rhinoceroses are currently not self-sustaining. Mortality is high in black rhinoceroses due to infant mortality and various syndromes that afflict this species [AZA Rhino Advisory Group, 2004]. Reproduction in captive white rhinoceroses is low, especially for the F1 generation, which has a reproductive rate of only 8% [Foose and Reece, 1997; Swaisgood et al., 1998]. Two previous studies of captive African rhinos suggested that stress caused by conditions in captivity may play a role in these sustainability problems [Carlstead et al., 1999a, b; Brown et al., 2001]. Carlstead et al. [1999a, b] conducted a study of 29.31 black rhinos at 23 zoos in which a methodology was developed and validated for cross-institutional comparisons of individual rhino behavior. Interrelationships among behavioral characteristics, housing conditions, social environments, reproductive success, and mortality were examined. The results showed that a high degree of exposure to zoo visitors along the perimeter of black rhino enclosures was positively associated with higher institutional mortality rates, and suggested that this environmental feature might be a source of chronic stress. Brown et al. [2001] validated fecal steroid metabolite assays to assess the reproductive status of 10.16 black and 6.13 white rhinoceroses in North America. The results confirmed earlier findings [Schwarzenberger et al., 1998; Patton et al., 1999; Hermes et al., 2002] that white rhino females exhibit numerous reproductive anomalies: some females have short cycles, some have long cycles, some have both short and long cycles, and some do not cycle at all. They suggested that stress as a cause of inconsistent gonadal activity needs to be evaluated in white rhinos.

Stress responses are characterized by increased secretion of glucocorticoids by the hypothalamic-pituitary-adrenal (HPA) axis in response to perceptions of threat, novelty, or uncertainty [Mason, 1968; Hennessy and Levine, 1979; Hennessy et al., 1979]. Glucocorticoids mobilize the metabolic resources an organism needs to respond to unusual events, social conflict, and noxious stimulation, and is a normal adaptive response of an organism to life’s challenges. However, prolonged periods of high glucocorticoid concentrations in response to chronic or frequent intermittent
stressors may have biological costs, such as immunosuppression and atrophy of tissues, decreased reproductive function, or abnormal behavior [Engel, 1967; Barnett et al., 1984; Moberg, 1985, 1990; Bioni and Zannino, 1997; Elsasser et al., 2000]. These effects form the basis for the hypothesis that chronic stress plays a role in the high mortality of black rhinos and the low reproductive rate of white rhinos in captivity. Indeed, research priorities for captive African rhinoceroses have been identified that include the development and validation of additional measures of stress, investigation of relationships between stress indicators and medical conditions, and continued investigation of social factors (e.g., group composition, compatibility, aggression, reproductive suppression, and communication), enclosure characteristics (e.g., size and complexity), and husbandry practices (e.g., keeper interaction, time in holding areas, and enrichment), that might be affecting behavior, reproduction, and health [AZA Rhino Research Advisory Group, 2004].

Longitudinal sampling of fecal corticoids has become a useful way to assess adrenal function in captive and wild species when used in a comparative or experimental research design [Whitten et al., 1998; Wasser et al., 2000; Mostl and Palme, 2002; Wielebnowski et al., 2002a; Millspaugh and Washburn, 2004]. This collection method is advantageous in that it is noninvasive and can produce a pooled sample of corticoid output over a period of hours [Whitten et al., 1998; Wasser et al., 2000]. Most noninvasive studies repeatedly sample corticoid concentrations of individuals under different conditions or in relation to social status [e.g., Wielebnowski et al., 2002; Creel et al., 1997; Wasser et al., 1997a; McLeod et al., 1996]. Data are usually described with the use of an aggregate or iterative mean value for individuals or populations, with higher mean values corresponding to greater relative levels of stress. However, for wildlife species, both the range of fecal glucocorticoid concentrations and the duration of corticoid elevations that indicate “bad” or chronic physiological stress remain unknown [Ladewig, 2000; Millspaugh and Washburn, 2004]. Research on laboratory and farm animals using serum glucocorticoids, ACTH challenges, and postmortem tissue examinations has shown that animals that are experimentally subjected to long-term chronic stressors may not differ from control animals in baseline corticoid levels. Instead, they appear to habituate to the chronically present stressor, but exhibit hyperreactivity of the adrenal cortex in response to subsequent acute stressors or to an ACTH challenge [e.g., Sakellaris and Vernikos-Danellis, 1975; Kant et al., 1985; Carlstead et al., 1993; Harris et al., 2004]. Whether long-term chronic stress caused by conditions in captivity alters adrenal hyperreactivity, and whether this change can be observed in longitudinally collected fecal corticoid concentrations remain to be determined.

In a study of reproductive function, Brown et al. [2001] validated a fecal glucocorticoid assay for black and white rhinos. A preliminary evaluation found that mean concentrations of fecal corticoids, collected over a 1–2-year period, were higher in black than in white rhinos, but no differences within species were found between sex, across seasons, or between reproductive conditions or status, and there were no correlations between mean corticoids and androgen or progestagen concentrations. However, to critically examine the significance of corticoid data as a measure of stress, it is necessary to combine hormone measures with the behavioral characteristics of individual animals to take into account the behavioral adaptations that are the primary means of coping with physiological stress [Wielebnowski, 2003]. In addition, measures of the physiological consequences or “biological costs” to
animals due to chronic stress must be incorporated into a comparative analysis of corticoid data [Moberg, 2000]. Therefore, this study expands the results of the two previous studies of African rhinos. With the use of weekly fecal samples collected from rhinos for 1 year by Brown et al. [2001], we describe patterns of stress responses for individuals of each species, using the mean and variability of corticoids combined with behavioral characteristics. To further analyze possible links between stress and “biological costs” as indicated by population sustainability problems, we compared corticoids with ovarian activity and reproductive success in white rhinos, and with environmental factors and mortality in black rhinos.

MATERIALS AND METHODS

Animals and Fecal Sample Collection

Corticoid metabolites were analyzed in weekly fecal samples collected for 1 year from 10.16 black rhino at 10 zoos and 6.13 white rhino at six zoos. Individual fecal samples (10–50 g) were collected in the morning from individually housed animals, and samples from group-housed animals were collected only from animals that were housed alone at night or after direct observation of defecation. The samples were frozen immediately after collection and stored at −20°C until they were analyzed. These samples represent a subset of samples from the study by Brown et al. [2001]. For that study, institutions and individuals were selected based on the feasibility of fecal collection and not for a balanced sample of environmental conditions or group sizes. At all 10 zoos with black rhinos, the entire zoo population of 1.1, 1.2, 1.3, 1.4, or 2.1 rhinos was monitored, except for 0.2 females at one facility that housed 2.2 individuals. Five zoos kept breeding pairs together on a daily basis, and five zoos introduced pairs only during female estrous. By contrast, all white rhinos were monitored at only four zoos with populations of 1.1, 1.2, 2.2, and 2.6, and at two other zoos only one female was monitored out of populations of 1.2 and 2.3. All six zoos with white rhinos kept potential breeding pairs together on a daily basis. Because of the small number of zoos involved and unbalanced sampling, institutional-level variables (i.e., social, environmental, and mortality) could not be analyzed for the white rhinos.

Behavioral Assessments

Keeper questionnaires were used to assess behavioral characteristics [Carlstead et al., 1999a]. Since behavioral data already existed for 9.12 of the black rhinos [Carlstead et al., 1999a], we sent questionnaires to facilities to obtain data from previously unsurveyed black rhinos and all white rhinos. The questionnaires requested the two keepers who spent the most time with the rhinos to rate individual animals on 35 behavior items, mostly on a five-point scale. The keepers rated the rhinos on the frequency and intensity of behaviors during periods of greatest social activity. We determined the interrater reliability for each behavior item by examining the rating differences of dyads of keepers for the same animal, as described in Carlstead et al. [1999a]. The number of behavior items was then reduced, and only the 13 most agreed-upon items that were also consistent with the six behavior
categories used to describe black rhinos in Carlstead et al. [1999a] were retained. The six behavior traits used in the current study are described in Table 1.

### Reproductive Function and Mortality

The “lifetime reproductive rate” for individuals of both species was calculated as the number of births or siring per year of life in captivity over the age of 4 years. In addition, white rhino females were classified as cycling or noncycling based on the progestagen data of Brown et al. [2001] (all of the black rhino females were cycling; see Table 2).

For the black rhinos, the “average mortality at zoo” was calculated from the studbook as the number of deaths of study animals and their offspring at a zoo that occurred during January 1976–2003, divided by the number of animal years (the sum of the ages of all study animals and their offspring at a zoo).

### Environmental Variables

Because of the small sample of zoos included in the study, only a few environmental variables could be statistically analyzed for the black rhinos. The variables evaluated were 1) percentage of enclosure perimeter with public access (n = 15 enclosures at eight zoos because two zoos were not open to the public); 2) enclosure area (n = 21 enclosures at 10 zoos); 3) number of males at zoo (n = 10 zoos); 4) number of females at zoo (n = 10 zoos), and 5) breeding partners housed together all or part of the day (n = 6 pairs) or separately except for breeding introductions (n = 5 pairs).

**TABLE 1. Behavioral categories used in the analyses and the rated behavior elements that comprise each trait**

<table>
<thead>
<tr>
<th>Behavior traits</th>
<th>Behavior items rated by keepers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory behavior</td>
<td>Sum of ratings (1–5) on:</td>
</tr>
<tr>
<td></td>
<td>- Flehmen: head raised, underside of upper lip curled up</td>
</tr>
<tr>
<td></td>
<td>- Footscrape: rapid alteration of hind feet on ground while rhino remains stationary, often associated with defecation</td>
</tr>
<tr>
<td></td>
<td>- Urine spray: Bursts of urine; purposeful marking of walls, doors, rocks, trees, etc.</td>
</tr>
<tr>
<td></td>
<td>- Stall Trashing: manipulates feces, etc. in stall</td>
</tr>
<tr>
<td>Friendly</td>
<td>Sum of ratings (1–5) on:</td>
</tr>
<tr>
<td></td>
<td>- Friendly to keeper</td>
</tr>
<tr>
<td></td>
<td>- Allows touching by keeper</td>
</tr>
<tr>
<td>Fighting</td>
<td>Sum of ratings (1–5) on:</td>
</tr>
<tr>
<td></td>
<td>- Hornbutt</td>
</tr>
<tr>
<td></td>
<td>- Jousting with partner: rhino jousting with horns, lateral movement of head contacting other rhino on head or horn</td>
</tr>
<tr>
<td></td>
<td>- Chasing/Charging other Rhino</td>
</tr>
<tr>
<td>Dominant to</td>
<td>Rated on a scale of 1–5 from submissive to dominant towards other rhinos</td>
</tr>
<tr>
<td>conspecifics</td>
<td>(n = 15 enclosures at eight zoos because two zoos were not open to the public)</td>
</tr>
<tr>
<td>Timid/shy</td>
<td>Sum of ratings (1–5) or EPI rankings (see [Carlstead et al., 1999a]) on:</td>
</tr>
<tr>
<td></td>
<td>- Timid/shy: reluctance to approach other rhinos</td>
</tr>
<tr>
<td></td>
<td>- Depressed: failure to seek out or respond to social interactions</td>
</tr>
<tr>
<td>Pacing/stereotypy</td>
<td>Rated on 1–5 scale. Defined as repetitive locomotion pattern in a specific area</td>
</tr>
</tbody>
</table>
The mean, standard deviation (SD), and coefficient of variation (CV) (SD/mean) were calculated for the corticoid profiles of each individual. For each species separately, Pearson correlation coefficients were used to describe relationships between corticoid profiles, behavioral characteristics, and reproductive success. We used t-tests to compare corticoids between cycling and noncycling white rhino females. For the black rhinos, the “percent perimeter exposed to the public and enclosure area” (log sq. m) was correlated with the average corticoid values of the animals residing in each separate enclosure. “Average mortality at zoo” was correlated with the average corticoid values of all study individuals at a zoo. We used t-tests to compare the average corticoids of individuals at a zoo based on “number of males at zoo,” “number of females at zoo,” and “breeding pairs together or separated.” For all analyses, an alpha of 0.05 was accepted as significant. Analyses were carried out with SAS for PC version 6.12.

RESULTS

Sample Population Summary Statistics

Summary statistics on demographics and reproductive performance for the black and white rhinos in this study are provided in Table 2. The reproductive rate of black rhinos was greater than that of white rhinos: only 23% (3/13) of females had calved at their current institution, and only 54% exhibited any ovarian cycles. The black rhino population was younger, and contained more individuals that were captive-born.

Characterization of Individual Corticoid Profiles

Three statistics were used to describe the corticoid profiles of individuals. Figure 1 illustrates how corticoid patterns differed among three representative rhinos over 52 weeks of fecal sampling. From the top to bottom in Fig. 1, individuals with high, medium, and low corticoid means are shown. The lowest SD was observed for the individual with the lowest overall mean value (bottom profile); however, the CV for this data set was similar to that for the individual with the highest mean value (top profile). The middle profile has a higher variability because the CV describes corticoid variability with the effects of mean levels removed. The middle profile

<table>
<thead>
<tr>
<th>Individuals in study (male:female)</th>
<th>Black rhino</th>
<th>White rhino</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of zoos</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>% Captive born (captive:wild)</td>
<td>44% (12:15)</td>
<td>26% (5:14)</td>
</tr>
<tr>
<td>Mean age (range) in years</td>
<td>Males: 14.0 (4–29)</td>
<td>25.3 (12–29)</td>
</tr>
<tr>
<td></td>
<td>Females: 16.2 (8–29)</td>
<td>27.3 (13–35)</td>
</tr>
<tr>
<td>% of females found to be cycling</td>
<td>Males: 100% (n = 16)</td>
<td>54% (n = 7)</td>
</tr>
<tr>
<td></td>
<td>Females: 0.24</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.04</td>
</tr>
<tr>
<td>Females: % with parturitions at current institution</td>
<td>93% (15/16)</td>
<td>23% (3/13)</td>
</tr>
</tbody>
</table>
differs from the other two because corticoids were low during the first part of the study, and then increased and remained elevated from weeks 13–49. The other two profiles varied around a constant mean throughout the year.

Black Rhinos

Of the six behavior traits examined in this study, differences between sexes were found only for olfactory behavior (males: 16.4 ± 0.5; females: 11.43 ± 0.77, T = 4.73, P = 0.0001). Therefore, correlations between olfactory behavior and fecal corticoids were calculated separately for each sex, and all individuals were combined for correlations calculated with the other five behaviors. The only behavior that was associated with corticoids was fighting, which was positively correlated (P < 0.05) with corticoid CV (Table 3) across all individuals.

The relationships between five environmental variables and the average corticoid parameters of individuals at an institution are given in Table 4. The percentage of a rhino enclosure with public access along the perimeter was positively associated (P < 0.05) with mean corticoids. The enclosure area was not correlated with any corticoid parameter, nor were the numbers of males or females at an institution. However, the variability of corticoids, as measured by the SD, was higher (P < 0.05) in breeding pairs that were kept together in the same enclosure all or part of the day, as compared to pairs that were only put together in the same enclosure when the animals were breeding (Table 4).

“Average mortality at zoo” was found to be positively correlated (P < 0.05) with the average variability of corticoids of the study animals at that institution, as
measured by the CV (\(r = 0.87, P = 0.0001\)). This relationship is illustrated in Fig. 2. (There were no correlations between lifetime reproductive rate and any of the corticoid parameters for black rhino males or females; data not reported.)

White Rhinos

Olfactory behaviors were higher in white rhino males than in females (males: \(12.44 \pm 1.1\), females: \(6.3 \pm 0.4\). \(T = 6.10, P = 0.0001\)). For females, olfactory behaviors were negatively correlated with the variability of corticoids (CV; \(P < 0.05\)) (Table 5). For both sexes together, mean corticoids were negatively associated with “friendly to keeper” \((P < 0.05)\) (Table 5), indicating that higher corticoids in white rhinos are associated with lower rates of approaching and contacting keepers.

For white rhinos, there were no significant relationships between fecal corticoids and the lifetime reproductive rate (Table 6). However, the absence of ovarian cycles was related to corticoid CV \((P < 0.05)\), with noncycling females having

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**TABLE 3. Black Rhinos: Pearson correlation coefficients for corticoid profiles and behaviors of \(n = 26\) individual black rhinos**

<table>
<thead>
<tr>
<th>Corticoid parameter: behavior</th>
<th>Mean (M: n = 10)</th>
<th>Standard deviation (SD) (M: r = -0.17)</th>
<th>Coefficient of variation (CV) (M: r = 0.45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M: (r = -0.45)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F: (r = 0.19)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pacing/stereotypy</td>
<td>(r = -0.00)</td>
<td>(r = -0.17)</td>
<td>(r = -0.28)</td>
</tr>
<tr>
<td>Friendly to keeper</td>
<td>(r = 0.09)</td>
<td>(r = 0.19)</td>
<td>(r = 0.15)</td>
</tr>
<tr>
<td>Dominant to conspecifics</td>
<td>(r = 0.19)</td>
<td>(r = 0.33)</td>
<td>(r = 0.21)</td>
</tr>
<tr>
<td>Fighting</td>
<td>(r = -0.01)</td>
<td>(r = 0.33)</td>
<td>(r = 0.46^*)</td>
</tr>
<tr>
<td>Timid/Shy</td>
<td>(r = -0.21)</td>
<td>(r = -0.21)</td>
<td>(r = -0.05)</td>
</tr>
</tbody>
</table>

*\(P < 0.05\).

**TABLE 4. Black Rhinos: Pearson correlation coefficients and \(T\) values of environmental variables and average value per zoo for each corticoid parameter**

<table>
<thead>
<tr>
<th>Corticoid parameters: Environmental and mortality variables</th>
<th>Mean</th>
<th>Standard deviation (SD)</th>
<th>Coefficient of variation (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% enclosure perimeter with public access ((n = 15) enclosures)</td>
<td>(r = 0.53^*)</td>
<td>(r = 0.45)</td>
<td>(r = 0.21)</td>
</tr>
<tr>
<td>Area of largest enclosure ((n = 21) enclosures)</td>
<td>(r = 0.24)</td>
<td>(r = 0.33)</td>
<td>(r = 0.26)</td>
</tr>
<tr>
<td>Number males at zoo ((1: n = 7; 2: n = 3))</td>
<td>(1: 43.2 \pm 6.8)</td>
<td>(1: 7.66 \pm 1.3)</td>
<td>(1: 17.4 \pm 1.9)</td>
</tr>
<tr>
<td>Number females at zoo ((1: n = 5; 2 or more: n = 5))</td>
<td>(2: 39.9 \pm 2.4)</td>
<td>(2: 9.44 \pm 1.2)</td>
<td>(2: 22.4 \pm 1.9)</td>
</tr>
<tr>
<td>Breeding pairs Together ((n = 6)) or Separate ((n = 5))</td>
<td>(2+: 40.2 \pm 2.3)</td>
<td>(2+: 9.02 \pm 1.29)</td>
<td>(2+: 21.3 \pm 2.0)</td>
</tr>
</tbody>
</table>

*\(P < 0.05\).
higher variability in corticoid concentrations (Table 6). Examples of corticoid CV over the 1-year study for cycling and noncycling females are shown in Fig. 3a and b. There was no difference in age between noncycling (mean = 25.3 ± 3.1 years) and cycling females (mean = 26.9 ± 1.6 years, P > 0.05) [Brown et al., 2001]. Four of the six noncycling females were at least primiparous, as were five of the seven cycling females. All six zoos with white rhino populations ranging from 1.1 to 2.6 had at least one cycling female.

Behavioral differences were found between reproductive classes of white rhino females. Cycling females were rated higher on olfactory behavior (P < 0.05), whereas noncycling females were rated higher on pacing/stereotypy (P < 0.05) (Fig. 4).

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**TABLE 5. White Rhinos: Pearson correlation coefficients for corticoid profiles and behaviors of n = 19 individual white rhinos**

<table>
<thead>
<tr>
<th>Corticoid parameter: behavior</th>
<th>Mean</th>
<th>Standard deviation (SD)</th>
<th>Coefficient of Variation (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory</td>
<td>M: n = 6 r = -0.04</td>
<td>M: r = -0.73</td>
<td>M: r = -0.67</td>
</tr>
<tr>
<td></td>
<td>F: n = 13 r = -0.14</td>
<td>F: r = -0.53</td>
<td>F: r = -0.78*</td>
</tr>
<tr>
<td>Pacing/stereotypy</td>
<td>r = 0.16</td>
<td>r = 0.04</td>
<td>r = 0.10</td>
</tr>
<tr>
<td>Friendly to keeper</td>
<td>r = -0.47*</td>
<td>r = -0.39</td>
<td>r = -0.01</td>
</tr>
<tr>
<td>Dominant to conspecifics</td>
<td>r = 0.29</td>
<td>r = 0.33</td>
<td>r = -0.13</td>
</tr>
<tr>
<td>Fighting</td>
<td>r = 0.34</td>
<td>r = 0.09</td>
<td>r = -0.28</td>
</tr>
<tr>
<td>Timid/shy</td>
<td>r = -0.38</td>
<td>r = -0.34</td>
<td>r = -0.04</td>
</tr>
</tbody>
</table>

*P < 0.05.
DISCUSSION

In this study, fecal corticoids were sampled longitudinally over a period of 1 year in a large number of individuals. The activity of adrenocortical hormones is controlled by a multitude of external and internal factors, including a circadian rhythm and acute elevations in hormone secretion in response to many types of environmental and social stimuli [e.g., Thun et al., 1981]. Strong individual differences are also characteristic of most species studied [Mason, 1968; Sapolsky, 1987, 1994; Carlstead et al., 1992; Wielebnowski et al., 2002a]. Obviously, a wide variety of potential stressors could have affected the corticoid profiles observed in the 46 African rhinos evaluated in this study, including seasonal changes, noises, husbandry events, pair introductions, etc. Although not all of these parameters were examined, we identified several correlations between the corticoid data and behavioral characteristics that are likely to be indicative of coping responses to stressors. By describing the corticoid data as a statistical mean, SD, and CV, we also were able to evaluate different aspects of adrenal responsiveness that took into account individual variability in secretory profiles.

We found that individual differences in mean corticoid concentrations were associated, depending on the species, with exposure to zoo visitors (black rhino) and behavior toward human caretakers (white rhino). Thus, when evaluated over an extended period of time, individual differences in mean corticoid levels appear to be strongly influenced by an individual rhino’s responsiveness to humans. By contrast, differences in corticoid variability were associated with behavioral interactions with conspecifics, such as fighting (black rhino), olfactory behavior and stereotypic pacing (white rhino), and the amount of time that pairs were kept together (black rhino). Therefore, differences in corticoid variability appear to reflect responsiveness to conspecifics. Perhaps more importantly, increased variability in adrenal activity was associated with some potential indicators of the “biological costs” of long-term or chronic stress in African rhinos (i.e., absence of ovarian cycles (white rhino) and higher mortality rates (black rhino)). The results imply, therefore, that social stressors are potential causes of chronic stress in black and white rhinos, and may be associated with biological costs that contribute to captive-population sustainability problems.

![Table 6: White Rhinos: Pearson correlation coefficients for corticoid profiles and measures of reproductive function for males and females](image-url)
The best indicator of chronic stress in animals or humans subjected to intense stressors of long duration or frequent repetitiveness is enhanced or exaggerated corticoid responses to new, acutely presented stressors. Therefore, high variability in longitudinal fecal corticoid data may be a better measure of increased reactivity to undefined or unrecorded stimuli, such as changes in the behavior of nearby conspecifics. This can be reflected through differences in either the CV, as a standardization of the SD that allows for comparison of variability regardless of the scale.

Fig. 3. Variability of fecal corticoid profiles for (a) three representative white rhino females that exhibited some ovarian cycles as reported in Brown et al. [2001], and (b) three representative white rhino females with no ovarian activity.
magnitude of mean fecal corticoid concentrations, or the SD. In our analyses, the SD and CV were not always significant together, but they tracked in the same direction. Because individual differences in mean corticoids can be caused by a variety of factors, we suggest that fecal corticoid analyses should include both standardized (CV) and nonstandardized (SD) measures of variability.

**White Rhinos**

The absence of ovarian cycles in white rhino females was related to higher variability in corticoids, increased pacing/stereotypy and reduced frequencies of olfactory behaviors. All three factors may be indicative of enhanced stress responsiveness in noncycling females. Stress-related suppression of female reproductive function is known to occur in other ungulate species [e.g., Moberg, 1985, 1990] (for review see Asa [1996]). Pacing or stereotypy is often stress-induced, although this behavior can have a variety of motivations [Mason, 1991]. In zoo animals, stereotypic pacing can be a sign of frustrated attempts to move to another area or escape from conspecifics [Meyer Holzapfel, 1968]. Decreased olfactory behavior may also be an indicator of stress, as indicated by experiments with rats in which stress was shown to suppress olfactory exploration [Garcia-Marquez and Armario, 1987; Desan et al., 1988; Irwin et al., 1989]. From studies of wild and domestic ungulates, it is known that olfactory cues may act as signaling pheromones between members of a species to influence estrous and ovulation [Booth and Signoret, 1992]. Therefore, the behavioral and corticoid profile differences observed between cycling and noncycling females indicate that stress-induced suppression of olfactory behaviors could account for the failure of some females to

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**Fig. 4.** Differences in average (+ s.e.m.) keeper ratings of six behaviors between noncycling and cycling white rhino females.
cycle because they fail to receive the olfactory cues necessary to stimulate ovarian activity. The fact that we found a cycling female in each of the six zoos lends credence to the suggestion that a dominant female in a group is the purveyor of this inhibition, and may control access to olfactory cues in subordinate females. The situation at one of the institutions in our study, with a population of 2.6 rhinos, illustrates this point. Only one female (aged 29 years) at that institution showed any ovarian cycles [Brown et al., 2001], while the other five were acyclic. All six had synchronized variation in corticoids over time. Behaviorally, the cycling female had the highest rating on “dominant to conspecifics” and olfactory behavior, and the lowest rating on pacing, compared to the other five. Mikulica [1991] examined dominance relationships in two captive groups of white rhinos (1.5 and 1.4), and found that one female in each group was more active and dominant, and males were the least dominant in both groups. In the one group that experienced reproduction, the most dominant female was the breeder.

It has been reported that captive white rhino females do not come into estrus if they are exposed to only one bull [Lindeman, 1982; Pienaar, 1994], and that the introduction of unfamiliar males stimulates reproduction in white rhinos [Patton et al., 1999]. This led Patton et al. [1999] to suggest that mate choice is important for the successful breeding of white rhinos in captivity. Our analyses provide evidence that social behaviors associated with mate choice, such as territorial marking and olfactory investigation, may have physiological impacts on reproduction in this species. There are also suggestions that these behaviors are associated with adrenocortical activity through inhibitory or possibly stimulatory pathways that are probably socially mediated. Adrenocortical activation is not always deleterious. It is a normal response to novel situations and unfamiliar conspecifics [Hennessy et al. 1979; Hennessy and Levine, 1979] that may benefit animals by enhancing learning and exploration [Weiss et al., 1989; Konarska et al., 1989; Veldhuis et al., 1982] or facilitating sexual arousal and responsiveness [Antelman and Caggiula, 1980; Carlstead and Shepherdson, 1994]. Reproduction might be hindered, or stimulated, by the physiological impacts of male territoriality, female mate choice opportunities, or developmental processes in which the F1 generation would learn to respond to complex white rhino social behavior. It is clear that future studies of white rhino reproduction should include comprehensive analyses of social behavior within and between sexes, as well as examinations of the stimulatory or priming effects of olfactory stimuli on reproductive and adrenal hormone profiles, as has been done for captive cheetahs [Wielebnowski et al., 2002b] and recently for wild white rhino males [Kretzschmar et al., 2004].

Black Rhinos

Fighting in black rhinos (hornbutt, joust, and charge/chase) was positively correlated with variability in corticoids. Black rhino pairs that were kept together 6–24 hr per day exhibited higher variability of corticoids compared to pairs that were separated at all times except for breeding introductions. Furthermore, the keeper survey responses indicated that pairs that were mostly separated tended to fight less when they were together than black rhino pairs that had daily access to each other. The average mortality also was higher at zoos with pairs that were kept together (separated = 0.007 ± 0.007, together = 0.037 ± 0.009, T = –2.32, P = 0.05). Therefore, in black rhino pairs the relationship between fighting and corticoids appears to
be related to the amount of time that individuals are forced to share the same enclosure. Fighting in black rhino pairs can lead to injuries or death in captivity and the wild [Hall-Martin and Penzhorn, 1977]. Berger and Cunningham [1998] reported that in Etosha National Park, Namibia, 50% of males and 30% of females die from combat-related wounds. In the wild, females have been known to viciously attack males before and after copulation, and males often fight each other over estrous females [Goddard, 1967]. Rhinos are large animals that in captivity are typically confined to a relatively small space. Pairings and social groupings are not the result of choices the animals have made, proximity is forced, and escape or avoidance is often not an option. Pair “incompatibility” in captive situations may be more stressful than managers realize. Therefore, one recommendation for captive management to reduce stress is to keep pairs separated except for breeding introductions.

Health problems and high mortality are the main reasons for the fact that captive black rhino populations are not self-sustaining. In this study, mortality on an institutional level was considered a rough estimate of the potential health challenges of individuals. The link between the “average mortality at zoo” and variability of corticoids provides evidence that this adrenal parameter may identify chronic stress in black rhinos. It is well known that chronic stress can compromise immune function [Engel, 1967; Cociu et al., 1974; Weiss et al, 1989; Henry, 1982; Hamilton, 1973; Blecha, 2000; Bioni and Zannino, 1997; Rivier and Rivest, 1991]. The link between corticoid variability and specific disease syndromes is an area in which further cross-institutional research is needed.

**Humans as a Source of Stress**

For both black and white rhinos, significant correlations were found between mean corticoid concentrations and aspects of the human environment. In the white rhinos, only one behavior was found to be negatively associated with mean corticoid concentrations: “friendly to keeper.” The fact that 84% of the white rhinos sampled in this study were wild-caught could account for the relationship between lack of affinity to human caretakers and higher corticoids. In black rhinos, the percentage of an enclosure perimeter that was exposed to the public was positively associated with higher mean corticoid concentrations. Thus, aversive reactions to humans, even after years in captivity, have the potential to contribute to stress responses in both black and white rhinos. A number of studies have demonstrated negative reactions of animals to caretakers [Mellen, 1991; Chamove et al., 1988; Seabrook, 1980; Hemsworth and Coleman, 1998; Hemsworth et al., 1981a, b; Hosey and Druck, 1987]. Most notably, Wielebnowski et al. [2002a] found that clouded leopards that were exposed to greater numbers of different keepers (i.e., fewer hours/keeper/animal/week) had higher mean fecal corticoid concentrations than those with fewer keepers. They also found that clouded leopards kept on public exhibit had higher corticoid concentrations than those kept off exhibit.

A variety of studies have examined rhino responses to humans, in both zoos and the wild. Mikaluca [1991] reported that white rhinos at Dvur Králové Zoo threatened visitors and keepers by rearing up and sweeping their heads from side to side, whereas Owen-Smith [1984] observed that wild white rhinos readily withdrew when humans approached. By contrast, black rhinos in the wild have been described as “irritable and explosive” toward humans. Rhinos that were previously disturbed
by humans were ready to charge, whereas those that were unfamiliar with humans were more peaceable and generally willing to retreat [Ritchie, 1968]. Berger and Cunningham [1995] found pronounced sex differences in black rhinos in terms of antipredator responses to lions, hyenas, and humans. Females were more likely to attack threatening carnivores, whereas in response to humans, females fled farther away than males.

In our study it was not clear whether there were negative consequences of aversive reactions to people, but it is possible that humans are an intermittent and predictable stressor to which most rhinos are able to behaviorally adapt during off hours and if given enough space to withdraw. Carlstead et al. [1999b] found that a high degree of exposure to visitors corresponded to increased institutional mortality rates in a sample of 23 zoos with black rhinos. In the current study, exposure to visitors corresponded to higher mean corticoids, and approached significance for corticoid SD \( (P = 0.09) \) for the 10 zoos sampled. Perhaps with a larger sample of zoos with black rhinos, exposure to visitors, corticoid variability, and institutional mortality rate could be statistically correlated. In any case, the sensitivity of rhinos to humans should be considered in the design of facilities and in husbandry practices with keepers so as to minimize aversiveness.

In summary, high variability in corticoids was associated with mortality and fighting in black rhinos, acyclicity in white rhino females, and suppressed olfactory behavior in white rhino females. These factors are potentially deleterious to rhino health and/or reproduction. Thus, it is the variability in corticoids, rather than the mean levels, that may be most informative for identifying chronic stress and associated biological costs of excessive adrenal activation in rhinos, and possibly other species. Studies of stress emphasize the importance of maintaining physiological homeostasis and the adaptive ability of the organism (for review see Broom and Johnson [1993]). When corticoids are monitored over a long period of time, it becomes apparent that individuals with more widely fluctuating levels are those that have more difficulty maintaining homeostasis. We conclude that species-specific, stress-mediated mechanisms appear to contribute to the high mortality of captive black rhinos and the reproductive failure of white rhinos. Overall, our data suggest that African rhinos are most sensitive to the social environment, both human and conspecific. Perhaps the most important result of this study is that the variability of longitudinal fecal corticoid analysis is emerging as a valuable measure of “bad” stress, that is, adrenocortical activation that has biological costs to the animal.

**CONCLUSIONS**

1. Longitudinal fecal corticoid data collected over a long period of time (e.g., 1 year) should be described by mean concentrations and variability (SD and CV).
2. Differences in mean levels of corticoids among African rhinos appear to be related, in part, to stress responses to humans.
3. Social stressors are a probable cause of chronic stress in black and white rhinos.
4. A good measure of chronic stress in rhinos (from repeated fecal sampling of individuals) is the SD or CV of corticoids. The parameters that are associated with measures of the biological costs of chronic stress are fighting and mortality in black rhinos, and female acyclicity in white rhinos.
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