RESEARCH ARTICLES

Behavioral and Adrenocortical Responses to Environmental Changes in Leopard Cats (*Felis bengalensis*)

Kathy Carlstead, Janine L. Brown, and John Seidensticker

National Zoological Park, Smithsonian Institution, Washington, DC

Indicators of environmental adequacy relevant to the well-being of small felids are developed by examining, in 4 captive leopard cats, interrelationships between behavioral and adrenocortical responses to changes in housing conditions. Singly housed cats were moved from their barren home cage (Cage 1, baseline) sequentially to 2 new, barren housing situations (Cages 2 and 3; =10 weeks/cage). Urinary cortisol concentrations, stereotypic pacing, and hiding frequencies were transiently increased for 1 week after translocation to Cage 2. After translocation to Cage 3, cortisol concentrations and hiding also were increased for the first week. However, conditions in Cage 3 were determined to be aversive to the cats, as evidenced by cortisol concentrations that remained chronically elevated for the entire 10-week period. Exploratory behavior was suppressed during this period. When Cage 3 was enriched with a complex of branches and hiding places, urinary cortisol concentrations and stereotypic pacing decreased, and exploration increased. Concealment locations that camouflage were more often used for lying down when urinary cortisol was elevated. These results suggest that reduced exploratory behavior is an indicator of chronic exposure to aversive environmental conditions. Stereotypic pacing may not necessarily increase when adrenocortical activity increases. The results also suggest that enrichment facilitates coping with aversive stimulation by providing behavioral options to confined felids. To promote the welfare of small felids, appropriate camouflage hiding places should be provided and enrichment programs developed to stimulate exploratory behavior. © 1993 Wiley-Liss, Inc.

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INTRODUCTION

The captive propagation of small felids is characterized by reproductive results that vary greatly from location to location. In addition, abnormal behaviors, including

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Address reprint requests to Kathy Carlstead, National Zoological Park, Smithsonian Institution, Washington, DC 20008.

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stereotypic pacing, excessive grooming, self-plucking of hair, and tail/paw sucking, may occur in nondomestic felids in captivity. Such evidence suggests that captive conditions are often suboptimal for felids and that environmental factors may be responsible for poor reproductive success. Indeed, Mellen [1991] found that the quality of caretaking by humans is closely tied to the number of litters produced by small felids in zoos. In domestic laboratory cats, stress-inducing caretaking procedures have been shown to reduce reproductive hormone secretion (LH), suppress exploratory and play activity, and increased hiding [Carlstead et al., submitted]. It is well known that stress can suppress reproductive function [Rivier et al., 1986; Moberg, 1985, 1990] and alter immune function and disease status [Engel, 1967; Riley, 1981; Selye, 1976] in a variety of species. Thus, assessment of the adequacy of housing and management procedures for small felids in zoos is a requisite for promoting the health and well-being of the animals and, ultimately, for conducting successful breeding programs.

The primary objective of this study was to develop an understanding of the interrelationships between behavior and adrenocortical responses elicited by potentially stress-inducing changes in housing conditions in the leopard cat (*Felis bengalensis*), a small (3–7 kg) Asian species. The activity of the hypothalamic-pituitary-adrenal (HPA) axis is particularly sensitive to psychological stressors resulting from an organism’s perceptions of threat, novelty, or uncertainty from its environment [Mason, 1968, 1971; Hennessy et al., 1979a; Levine, 1985]. Increases in glucocorticoid secretion from the adrenal cortex vary with the intensity of stimulation, and fluctuations of circulating glucocorticoid levels can be used as a measure of changes in emotional state [Hennessy et al., 1979b; Misslin et al., 1982]. In felids, exposure to psychological stressors such as translocation, unpredictable caretaking routines, and handling by humans, can be detected as increases in adrenocortical output [Carlstead et al., in press]. The latter can be assessed noninvasively via radioimmunoassay of cortisol in samples of voided-urine collected once daily at a fixed time [Carlstead et al., 1992].

In the present study, fluctuations in urinary cortisol concentrations and concomitant behavioral changes have been used to determine the behaviors that might be indicators of aversive environmental conditions for leopard cats. In this regard, stereotypic pacing in confined animals is often associated with suboptimal environments and poor welfare [Mason, 1991], but in small felids its relationship to HPA-axis activity and to environmental stimulation is not known. A second objective of this study was to examine the potentially stress-mitigating effects of environmental enrichment by comparing behavior and adrenocortical responses under barren and enriched caging conditions.

**MATERIALS AND METHODS**

**Animals and Housing**

Two male and two female leopard cats, captive born and ranging in age from 3–9 yr, were the subjects of this study.

Throughout the two experiments described below, leopard cats resided in 3 different caging situations.

**Cage 1.** The cats had been housed at the National Institutes of Health (NIH) for 1–3 yr. Cages were concrete and wiremesh (3.0 × 1.5 × 2.7 m), containing a wire
shelf (75 × 15 cm), a wire holding cage (75 × 90 × 125 cm) elevated above the ground with a metal ramp leading to the entrance, and 3 metal pans for food, water, and urine collection. The cats had auditory, olfactory, and limited visual contact with conspecifics, and with pigs, dogs, and domestic cats housed in the same building. Keepers and other personnel passed through the building at random throughout the day.

Cage 2. Located in a quarantine area of the Propagation Building at the National Zoological Park (NZP), cages were elevated, constructed of stainless steel with a mesh front, and measured 2.5 × 1.3 × 1.3 m. Cage furnishings included a 60 × 30 × 40 cm plastic transport carrier and food, water, and urine pans. No other animals were housed in the area. Cats had auditory, olfactory, and visual contact with each other. A keeper entered the building once per day to tend to the cats.

Cage 3. Located in the Lion and Tiger Building at NZP, the cages were concrete and wiremesh measuring 2.4 × 3.0 × 2.7 m. The cages contained the same food, water, and urine pans and carriers used in Cage 2. Also housed in this facility were several other large felids (lions, tigers, pumas, jaguar) with which the leopard cats had auditory and olfactory but no visual contact. Keepers were in the building almost continually between 7:00 A.M. and 3:30 P.M.

In all 3 environments, a temperature of 24°C was maintained year round, and artificial light was provided 12 hr per day. Natural light from windows entered the buildings to varying degrees. Cats were fed ~ 0.25 kg Nebraska brand feline diet once daily; on some days 1 or 2 mice or chicks also were given. Cages were hosed daily by keepers who entered the cage (except cage 2). Feeding and cleaning times varied with the facility, but generally occurred between 8:30 A.M. and 1:30 P.M.

Procedures

Experiment 1. Translocation to novel, barren cages. Over a 4-week period in Cage 1, video recordings were made of each cat for 24-hr periods for 7 days. Each morning when available, urine was collected with a syringe from the cage floor or aluminum pan (the time of urine excretion could not usually be determined and the sample was most likely a pool of several urinations). Cats were then transferred to Cage 2, where 24-hr video recordings were made 3 times weekly for 8 weeks (25 recordings) and urine was collected each morning. After 10 weeks in Cage 2, the cats were moved to Cage 3 where they remained for the duration of Experiments 1 and 2. Urine was collected each morning and 24-hr video recordings were made ~ 3 times per week throughout the first 8 weeks (21 recordings).

Experiment 2. Physical enrichment of a barren cage. After 10 weeks in Cage 3 under barren conditions, the cats were shifted to a holding area for one day while their cages were “enriched” by adding the following furnishings: 3 or 4 tree branches crossing the length of the cage; 5 m of synthetic rope, 7 cm in diameter, draped across and around the branches; 3 wooden shelves, 30 × 15 cm, anchored to the wire mesh in front and at the back of the cages at various elevations > 1.8 m, and around each shelf, a cluster of bamboo leaf was placed to provide a screen.; 2 cardboard boxes, anchored in the branches or on the wall; 3 litter pans on the floor, filled with mulch, sand, or dirt; and 2 hollow stumps or logs placed on the floor.

The transport carrier, food and water bowls, and aluminum urine pan also remained. Objects on the floor did not obstruct the pacing paths of the cats. Urine was collected each morning (if available) for 6 months. For a three-day period every 14
days, behavioral observations were made via 24-hr video recordings for the first 3 months.

In the 4th month of enriched conditions, a shift door in each cage was left open and the cat was given access to a 2 m runway behind the cage. One week later, a solid shift door at the end of the runway was replaced with a wire screen so that each cat had access to its neighbor through the screen when both cats were in their respective runways. Urine was collected when available and video recordings made for a 3-day period every month for 2 months.

Data Analysis and Statistics

From the video recordings, the following, mutually exclusive behaviors were registered: Stereotypie Pacing (repetitive movement in a fixed pattern), Exploration (nonrepetitive, seemingly random movement about the cage, orienting toward objects and sounds in and outside of the cage), Eating, Defecation/Urination, Lying Down (awake or asleep), Grooming, and Sit/Stand (in an attentive posture). The percent time (min/hr observed) of each behavior was scored; the locations of the latter 3 behaviors and keeper presence in the cage area were also noted.

Urine samples were analyzed for cortisol concentrations, using a steroid extraction and radioimmunoassay procedure previously described [Carlstead et al., 1992]. Assay sensitivity was 2 ng/ml; intra- and interassay coefficients of variation were < 10%. Cortisol/creatinine concentrations from urines collected during the weeks and/or months after a change had been made to the cats' housing conditions were averaged for each cat.

In Experiments 1 and 2, comparison of cortisol concentrations was carried out using a one-way analysis of variance, repeated measures design. Likewise, behavior frequencies were compared using a nonparametric Friedman two-way analysis of variance. Posthoc comparisons were made between postmanipulation periods and the baseline condition [Siegel and Castellan, 1988], established in Experiment 1 in Cage 1 and in Experiment 2 during the last 4 weeks in Cage 3 under barren conditions. In Experiment 1, the first week after translocation to a novel cage (2 and 3) was analyzed separately from the subsequent 2–8 weeks in each cage. This is because, in a pilot study, cortisol concentrations after cage translocation were found to be elevated for only 1 week in a Geoffroy's cat (Felis geoffroyi) and two pumas (Felis concolor) [Carlstead et al., 1992].

RESULTS

Behavioral and adrenocortical responses to translocation to novel, barren cages were examined in Experiment 1. Mean urinary cortisol concentrations and mean behavior frequencies during the baseline period in Cage 1 and after translocation to Cages 2 and 3 are shown in Figure 1. Urinary cortisol concentrations were significantly elevated ($F = 7.15$, DF = 4,16, $P = 0.002$) the first week after relocation to Cage 2 and to Cage 3. However, although urinary cortisol concentrations during the second to eighth week in Cage 2 did not differ from baseline levels, in Cage 3 they were elevated throughout the 8-week period. Behavioral responses to the new environments also differed between Cages 2 and 3. Although relocation to each cage resulted in increased time spent "in carrier" (the only concealment spot in the cage) ($F_r = 14.6$, $P < 0.01$) during the first week, other behaviors showed more variable
patterns. Stereotypic pacing was increased ($F_r = 10.6, P < 0.05$) only for the first week in Cage 2. Exploration was significantly decreased in Cage 3 for the 2–8 week period subsequent to relocation ($F_r = 0.4, P < 0.05$). The other behaviors recorded from video did not change significantly under any of the caging conditions (data not shown).

The effects of physical enrichment of a barren cage were examined in Experiment 2. The elevated urinary cortisol concentrations observed during the last 4 weeks the cats were housed in barren Cage 3 (baseline) were significantly decreased throughout the 3 months after the cage had been provided with furnishings ($F = 11.67, DF = 2.6, P = 0.009$) (Fig. 2). Stereotypic pacing also significantly decreased after the environment was made more complex ($F_r = 6.5, P = 0.05$). Exploration was increased postenrichment ($F_r = 6.5, P = 0.05$). After 4 months, exposure to a neighboring cat through a screen had no significant effect on either behavior. Compared to baseline under barren conditions, “in carrier” behavior was reduced after enrichment and during exposure to a neighbor, but only at a 0.10 significance level ($F_r = 6.0, P = 0.10$). Other behaviors measured in this experiment were unaffected by the enrichment manipulations.

The locations of “lying down” behavior were examined to determine preferences for resting and concealment spots. When no furnishings are provided other than a carrier, most lying down takes place on top of the carrier. Under enriched conditions, cats used 8 of at least 14 options for resting and/or concealment, but the most preferred appeared to be the mulch pan on the floor of the cage. The coat of the cats blended in with this substrate so that the animals were substantially camouflaged by it; pans with sand or dirt were never used for resting. Elevated positions offering some camouflage or covering, but from where cats could see outside the cage, also were frequently used (shelf with branches in front of it, open tree trunk). Locations utilized under bare conditions (i.e., on carrier, in carrier, on floor) were seldom used under enriched conditions.
A further analysis of resting and concealment behavior was carried out posthoc. Throughout the 5 months of enriched conditions, urinary cortisol concentrations were elevated on some days. These elevations may have been due to the occurrence of random, unpredictable events in the animals' environment that were perceived as threatening or aversive. We wished to determine whether the cats' preferences for resting or concealment locations varied with these perceptions. For this analysis, data on the frequency of concealment behavior were examined on days when behavior was recorded and urine was obtained the following morning. For each cat, days were divided into those with low urinary cortisol concentrations (below the median value for the cat) and those with high concentrations (above the median value). The percentage of total time concealed (lying down) in either the carrier, the mulch pan, or on an elevated shelf are given for each cat in Figure 3 for low and high cortisol days. The mulch pan was used significantly more often on days when cortisol was found to be relatively high than when it was relatively low (Mann Whitney U = 0, P = 0.014).

Because changes in stereotypic pacing in Experiment 1 did not vary in a consistent manner with changes in cortisol or other behaviors, pacing must be modulated by environmental stimuli that are specific to a given caging situation. In order to further examine environmental influences on this behavior, an analysis of the temporal pattern of pacing was carried out. Video recordings made 24-hr/day revealed that the frequency of stereotypic pacing and sit/stand (an attentive posture) was highly influenced by the activity of keepers and/or the light/dark cycle. Most pacing or-
Fig. 3. For 4 leopard cats in Cage 3 under enriched conditions, the percent of total time lying down in 3 locations on days when cortisol concentrations were low or high. Asterisk indicates $P < 0.05$ for mulchpan use on high cortisol days compared to low cortisol days.

Fig. 4. The average min/hr in stereotypic pacing (solid line) and sit/stand (dashed line) for the 24 hr of the day. The percent of total observations in which the keeper entered the cage area during a given hour is represented by the solid bars.

curred during the nighttime hours, and this was found to be true in all cages, barren or enriched. Figure 4 shows the average hourly frequencies of pacing and sit/stand in Cage 3 (all conditions and individuals combined) and the average frequency of the Keeper being in the area. Pacing dropped to its lowest frequency at 7:00 A.M. when keepers arrived to collect urine from the cages. After feeding and cleaning around 11:00 A.M., pacing picked up briefly for an hour at noon when keepers were on lunch break. At 3:30 P.M., when the keepers leave the building, pacing increased slightly until the twilight hours at 5:00 P.M., when it rose to an average of 14 min/hr. Changes in sit/stand followed a pattern opposite to pacing, with highest frequencies occurring when keepers were active in the building.
As with many longitudinal evaluations of zoo-housed animals, the small number of experimental subjects limits the conclusions that can be drawn from this study. In these experiments, behavior and adrenocortical activity could not be controlled for possible seasonal fluctuations, nor for changes in behavior and physiology caused by long-term habituation to any of the environments. However, we attempted to minimize these effects by comparing the responses to each translocation or manipulation to a “baseline” value established immediately prior to the environmental change.

Three conclusions can be drawn from the results of translocating cats to novel cages: (1) the initial response is characterized by increased adrenocortical activity and increased hiding behavior, (2) failure to adapt to a new environment, as determined by chronically elevated glucocorticoid secretion, is associated with suppressed exploratory behavior, and (3) changes in pacing behavior are specific to the caging situation. Thus, changes in behavior and physiology differed with the environmental conditions to which leopard cats were subjected and with the amount of time exposed to each new environment.

The differing nature of the aversive stimuli in each caging situation may account for the varied adrenocortical and behavioral responses. Both Cages 2 and 3 were unfamiliar to the cats, and the novelty of the new environments may have elicited the initial increase in hiding and in urinary cortisol concentration in the first week after each translocation. The cortisol response to the Cage 2 translocation was transient, returning to baseline levels after 1 week, whereas moving to Cage 3 resulted in a prolonged, chronic increase in adrenal output lasting throughout the 10 weeks they were housed under these conditions. The latter may have been related to the presence of potential predators in the Lion and Tiger Building (Cage 3) where the leopard cats had very limited visual contact with other large cats in the building, but considerable olfactory and auditory contact. The natural ranges of most small felid species overlap with larger cats that may predate upon them; tigers and leopards are found within the range of leopard cats, both of which are known to consume other carnivores as prey [summarized by Kitchener, 1991]. In addition, keepers were present in the building for 8 hr/day, producing sounds that might be perceived by the cats as threatening or uncertain. In the presence of unlocalizable dangers/predators, it would be adaptive to respond by suppressing activity. In keeping with such a response strategy, exploration remained significantly reduced for the duration of time the cats were housed in Cage 3 under barren conditions. Similarly, in other species, active investigatory behavior is suppressed when animals are subjected to a chronic stressor [Henry and Stephens, 1977; Koob and Bloom, 1985; Berridge and Dunn, 1989].

"Coping" may be defined as a pertinent behavioral and/or psychological strategy developed to reduce physiological activation elicited by a stressor [Dantzer, 1989; Friend, 1991]. After 10 weeks of elevated cortisol concentrations in Cage 3 under barren conditions, enriching Cage 3 with physical structures coincided with a reduction in the cats’ urinary cortisol secretion. Thus, enrichment may have facilitated coping with the aversive stimulation in the Lion and Tiger Building. The data, as discussed below, suggest that under enriched conditions, as compared to barren conditions, the cats have at least 2 more behavioral options for responding to aversive stimulation in their environment.

First, adding concealment places to the barren conditions provided the leopard
cats with an appropriate behavioral response, i.e., hiding, for coping with the perceived threat of predation. Seeking concealment may be an effective coping response for confined small felids in uncertain surroundings. In an investigation of domestic laboratory cats subjected to a chronically stressful, unpredictable caretaking routine, hiding was the predominant behavioral response. Individuals that spent much time attempting to hide behind their litter pan had lower urinary cortisol concentrations than those that spent less time [Carlstead et al., in press]. In the present study, fluctuations from day to day in adrenocortical output were associated with daily differences in concealment behavior. On days when cortisol output was relatively high, indicating possibly that uncontrolled aversive events had taken place in the building during the day, there was a greater tendency for the cats to lay in the mulch pan where they were camouflaged against the substrate. Leopard cats in the wild prefer resting and moving on the ground in thick cover, denning in hollow trees and small caves under tree roots [Inoue, 1972; Rabinowitz, 1990]. The tawny or grey background color of leopard cats is covered with many black spots, sometimes forming bands along the flanks. Such markings allow cats to conceal themselves from prey in the dappled light falling through vegetation [Kitchener, 1991]. The preference for a mulch substrate over sand or dirt suggests that the type of hiding places provided for small cats in captivity should take into consideration the pelage markings of the species.

Second, having objects to investigate may also have contributed to coping with the aversive stimuli in the Lion and Tiger Building. The reduction in adrenocortical activity concomitant with enrichment of Cage 3 corresponded with an increase in exploratory behavior. Environmental stimuli that promote active behaviors may allow the animal to cope with an aversive situation by directing attention away from the source of aversion (displacement activities) [Dantzer, 1985, 1989]. In rats or pigs subjected to a stressor (e.g., electric shock, novel environment, frustration), but at the same time provided with opportunities for activities such as chain pulling, drinking, fighting, or wheel-running, pituitary-adrenal activation was inhibited compared to stressed animals not given such opportunities [Heybach and Vernikos-Danellis, 1979; Levine et al., 1979; Dantzer and Mormede, 1981; Dantzer, 1989].

The results of Experiment 1 suggest that a depressed exploration rate is a behavioral indicator of chronic exposure to an aversive, HPA-axis-activating situation. Experiment 2 suggests that the converse is also true, that high exploration rates correspond to more optimal levels of physiological responsiveness to environmental stimulation. In contrast, stereotypic pacing was found to be modulated by several environmental factors that may not necessarily be associated with changes in adrenocortical activity. Although pacing and cortisol excretion increased transiently when the cats were moved from Cage 1 to Cage 2, this was not the case when they were moved to Cage 3.

In many captive carnivore species, stereotypic pacing is thought to be predominantly the expression of frustrated foraging or predatory behavior, especially the appetitive, search, and locate phase [Hughes and Duncan, 1988; Carlstead et al., 1991; Terlouw et al., 1991]. In the wild, leopard cats patrol 0.6–1.2 km/day through their home range in search of a diverse diet of small animal prey, are active ~ 50 percent of the time, and exhibit arrhythmic activity patterns [Rabinowitz, 1990]. Movement patterns motivated by appetitive, home range-patrolling tendencies may become overly rigid and repetitive in a small, physically barren cage environment
Carlstead et al. [e.g., in lions; Clarey and Farnsworth, 1983], resulting in stereotypic pacing. A stereotypy is a movement pattern that is considered abnormal, because it is morphologically invariant, and performed repetitively with no apparent goal or function. The leopard cats of this study generally paced the perimeter of their cage, and when access to the runway behind the cage was allowed, this area was also incorporated into the movement pattern. Two observations from the present study also support the hypothesis that pacing is the expression of frustrated appetitive behavior: (1) providing physical structures that stimulated investigation and exploration of the cage environment corresponded with a reduction in pacing, and (2) pacing occurred mainly during the evening and night when there was a lack of “food availability” signals. The sounds keepers make throughout the day are the same as those they make prior to delivering food to the cats; keeper sounds may thus become signals to the cats for “prey availability” (i.e., potential food delivery). It is during the keeper’s working hours that pacing is reduced and sitting/standing in an attentive posture predominates. If the latter is indicative of predatory vigilance and visual or auditory investigation, the absence of “food availability” signals from the keepers during nonworking hours might frustrate such vigilance, and be expressed as pacing instead.

CONCLUSIONS

1. Reduced exploration is a behavioral indicator of chronically elevated adrenocortical activity in leopard cats.
2. Repetitive, stereotypic pacing does not necessarily increase in situations that elicit increased adrenocortical activity.
3. Enriching the environment of felines with physical structures may provide increased behavioral opportunities (hiding, exploring) for coping with aversive stimulation.
4. The use of concealment locations that provide for camouflage increases when adrenocortical output is elevated. Appropriate hiding places that camouflage should always be provided to small cats.
5. Environmental enrichment stimulates exploratory behavior and reduces stereotypic pacing. Therefore, attempts should be made to provide small felids with environmental stimulation that maximally promotes exploratory behavior.

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