Cortisol Response to Relocation Stress in Garnett's Bushbaby (*Otolemur garnettii*)

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Relocation of research animals, either within a facility or from one facility to another, is assumed to be stressful. Development of appropriate research methodologies may be facilitated by understanding the extent and duration of the physiological response to relocation stress and whether the stress can be buffered by environmental or social factors, such as the presence of a cagemate. To characterize the response to relocation stress in Garnett's bushbaby, we assessed cortisol concentrations in nine female and six male bushbabies during relocation to a different facility; six of the animals were pair-housed at the time of the move and were moved with their respective cagemates. Fecal cortisol was assessed at three time points: 1) baseline (1 day prior to moving); 2) relocation (the day the animals were relocated); and 3) post-relocation (7 days after relocation). Cortisol concentrations were higher at the relocation time point than at baseline and post-relocation, which did not differ. Cortisol concentration did not differ as a function of having a cagemate during relocation. Although relocation resulted in a significant ($P < 0.05$) increase in cortisol excretion concentration, the levels returned to baseline within 7 days after the stressor.

Relocation of laboratory animals may be necessitated by a variety of factors, such as a change in the principal investigators institutional affiliation, the building of a new animal research facility, or the acquisition of animals from a vendor. Relocation of research animals, within a facility or from one facility to another, is presumed to be stressful for the animals. However, for many species there is little empirical evidence documenting the severity of the stress at the time of relocation or the duration of the stress response after relocation. In addition, there is little information regarding factors that might effectively buffer or protect the animal from potential deleterious effects associated with relocation stress.

The relocation experience exposes an animal to a novel, changing environment and climate. The animal cannot predict or control the changes that are occurring in its immediate environment. Thus, the animal may perceive such a situation as a threat to its survival or well-being. The physiological and behavioral sequelae associated with uncontrollable and unpredictable environmental events have been well-studied in both humans (1) and laboratory animals (5, 6, 8).

Within the same facility, neither a change in cage size nor in housing room significantly elevated urinary cortisol levels in pigtail macaques (*Macaca nemestrina*) (4) or longtail macaques (*M. fasicularis*) (3). However, there is little known about cortisol response in primate species after relocation from one facility to another. Given the potential deleterious effects of chronically elevated plasma cortisol concentrations, it is important to understand the extent and duration of the stress response to relocation of experimental animals. The primary purpose of this study, then, was to assess cortisol concentrations prior to, during, and 1 week after the relocation of 15 Garnett's bushbabies (*Otolemur garnettii*) from one university to another. Bushbabies have been used as animal models to investigate a variety of behavioral and physiologic mechanisms, including cognitive function (11), reproductive mechanisms (10), and laterality (9). Moreover, as the bushbaby may well be poised to become an important adjunct primate model, it would be useful to gain information on whether environmental or social factors, such as the presence of a cagemate, might provide an effective buffer against some of the deleterious consequences associated with prolonged and uncontrollable stress. Therefore, another purpose of the study was to compare cortisol levels of animals transported individually with those of animals transported in pairs.

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Materials and Methods

Animals. Hormone concentrations were assessed in nine female and six male bushbabies during relocation from one university facility (Jackson State University, Jackson, Miss.) to another facility at a university located approximately 90 miles away (University of Southern Mississippi, Hattiesburg, Miss.). Of the 15 animals, 6 were pair-housed (three housed with same-sex cagemates and three housed with opposite-sex cagemates). The same-sex pairings were mother–daughter dyads, and the opposite-sex pairs were established mates. All pairs had been housed together for more than 1 year prior to the move. All bushbabies were laboratory-born and had been resident at the initiating laboratory for as long as 4 years. The bushbabies had physical examinations before and after relocation, and all animals appeared clinically healthy prior to, during, and after the relocation process. All animals were treated in accordance with federal guidelines and had Institutional Animal Care and Use Committee (IACUC) approval from both universities.

Relocation procedure. Relocation began at approximately 9:00 a.m., and animals were safely secured in the new housing by 4:30 p.m. that same day. Ambient temperature during the relocation averaged 23°C (range, 17 to 28°C). Transportation of animals was consistent with USDA regulations as outlined in the Animal Welfare Act (Sections 3.86 through 3.92). Specifically, the animals were removed from their home cage, placed in a standard animal transport carrier (i.e., "pet taxi"), and transported in a temperature-controlled van to the new facility. Pair-housed animals were moved in the same animal carrier. The home cages also were moved, and the animals were replaced in the same home cages in the new facility. The home cages were cleaned with detergent and water prior to moving but were not otherwise modified. Therefore, the animals' primary enclosure remained familiar.

Fecal hormone assay. Hormonal fluctuations were monitored by fecal steroid analysis. This noninvasive method of hormone assessment

provides information about the hormonal milieu of the bushbabies without the stress of capture and blood sampling. The use of fecal steroids, including cortisol, as a noninvasive index of physiologic stress in nonhuman primates, including prosimians, has been confirmed in studies showing that fecal and urinary cortisol responses to stressors are significantly correlated (2, 10). We collected fecal samples (10) to allow assessment of cortisol concentrations at three time points: 1) baseline (1 day prior to relocation); 2) relocation (on the day the animals were relocated); and 3) post-relocation (7 days after the relocation). The baseline fecal sample was collected on the morning of relocation, prior to preparing the animals for move, the relocation fecal sample was taken the morning after relocation was completed, and the post-relocation fecal sample was taken on the morning of day 8. Each animal was manually removed from the home cage, and a fecal sample was collected at that time. Samples were not used from any animals that urinated while defecating. All samples were collected between 9:00 and 11:00 a.m. and were kept frozen until assay. Fresh samples were stored on dry ice until transfer to a –62°C freezer. The baseline sample was transferred to the freezer within 6 h of collection; samples for the remaining two time points were frozen within 2 h of collection. The samples were analyzed for levels of cortisol at the Center for Reproductive Endocrinology, Emory University (Atlanta, Ga.), by using the procedure below.

Fecal extraction. A 0.1-g sample of feces was homogenized in 2 ml methanol:acetone (8:2, vol/vol) and filtered through a 0.2-µl nylon centrifuge filter (Centrex MF; Scheicher and Schuell, Keene, N.H.). The filtrate was extracted on Sep-Pak VAC C18 columns (500 mg; Water Corp. Milford, Mass.). An equal volume of distilled water was used to dilute the sample, which then was layered onto a column primed according to manufacturer's instructions. The column was washed with 5 ml distilled water, and the steroid fraction was eluted with 3 ml methanol.

Cortisol radioimmunoassay. A micro-radioimmunoassay procedure with an active range 10 times lower than commercial assays (12) was used. The assay was a modification of the Double Antibody ^{125}I Cortisol Kit (DPC KCOD, Diagnostics Products Corporation, Los Angeles, Calif.) for serum determinations. The working buffer was distilled water. Tracer–antiserum solution was prepared by mixing equal parts of ^{125}I cortisol and cortisol antiserum; 50 µl of this buffer was added to 25-µl aliquots of standards (diluted 1:10 to give concentrations of 1 to 50 ng/ml), samples (concentrated 4:1), and controls (diluted 1:10). Each reaction mixture was vortexed and incubated at 37°C. After 45 min, 250 µl of cold precipitating solution was added, and the incubates were vortexed, incubated an additional 5 min at room temperature, and centrifuged at 3000 g for 15 min at room temperature. After decanting of the supernatant, the radioactivity of the precipitate was determined by counting for 5 min in a gamma counter.

Statistical analysis. Data were analyzed using a mixed-model analysis of variance with housing (pair- or single-housed) as the between-subjects variable, and the three time points as the within-subjects variable. Post hoc analyses were performed using Tukey's post hoc test. All statistical analyses were performed using SPSS for Windows (SPSS for Windows, Rel. 11.0.1. 2001. Chicago: SPSS Inc.).

Results

As expected, the animals exhibited a statistically significant increase in cortisol concentrations on the day the animals were relocated compared to baseline cortisol levels ($F(2, 26) = 4.73$, $P = 0.047$; Fig. 1). The nearly 10-fold increase in cortisol levels during the relocation period gives some indication of the severity of the distress experienced by the animals during relocation. There was no significant difference in cortisol between the baseline and post-relocation time points. In other words, although relocation resulted in a significant cortisol increase, levels returned to baseline within 7 days after the stressor. As reflected in the standard error bars in Fig. 1, there were substantial differences in cortisol response to relocation. In contrast, the variance in post-relocation cortisol levels was negligible. Cortisol concentrations did not differ between male and female bushbabies at any time point.

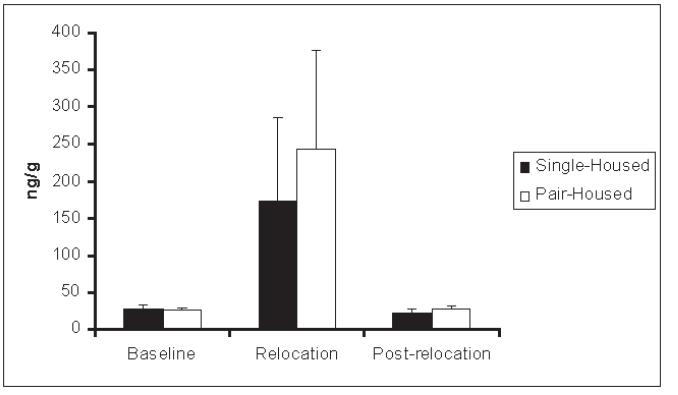

Figure 1. Cortisol levels (mean ± standard error of the mean) at baseline, in response to stress during relocation, and post-relocation for singly and pair-housed bushbabies. Cortisol was significantly increased ($F(2,26) = 4.73$, $P = 0.047$) versus baseline value) during relocation, but baseline and post-relocation concentrations did not differ from each other.

Discussion

Using a fecal steroid assay (2), we were able to document the baseline cortisol concentrations of the study animals, a physiologic response to a known stressor and the return to baseline adrenal functioning. Although relocation involves changes on several dimensions (e.g., capture, travel, environmental change), the relocation per se may be conceptualized as a specific event. Therefore, the present results provide support for the use of the fecal cortisol assay in assessing the physiologic response to a specific stressful situation (i.e., relocation stress) rather than simply indicating a state of non-specific or generalized anxiety. IACUC members at institutions that house and use primates are aware of the requirement for "psychological well-being," yet objective standards of well-being in Garnett's bushbabies and other primate species have not been well characterized. The specificity of the cortisol response observed here, as well as its return to baseline within 7 days, provides an objective method and criterion for assessing the stress response associated with relocation of animals. In addition, the fecal assay method has the advantage of being noninvasive.

Despite the perception that singly housed primates experience more stress than pair-housed animals, we found no significant difference in cortisol concentrations between animals that were transferred individually versus animals that were relocated in established pairs. Garnett's bushbabies are nocturnal prosimians and are considered "solitary foragers" (7). Given their relatively solitary nature, it is perhaps not surprising that the effects of the relocation stress were not diminished by the presence of a cagemate in this species. Whether the presence of a cagemate might attenuate the stress response in a gregarious prosimian species is unknown.

It would be useful to investigate cortisol levels across a number of time points after relocation to assess how rapidly the cortisol response returns to baseline. This information could be used to assess the degree to which the health status or psychological well-being of animals might be affected during relocation. The animals were relocated within 8 h, so relocation might be considered an acute stressor. Unlike chronic cortisol elevations, an acute response may have protective rather than deleterious effects.

We advocate a science-based approach to characterizing the effects of relocation and other stressors on experimental animals as well as determining the validity of institutional and national regulations in the care and housing of laboratory animals. Although numerous laboratory experiments have been published on acute and chronic stressors in a wide variety of vertebrate species, far fewer attempts have been made at "translational" research that attempts to apply these findings to ameliorate potential health threats to laboratory animals.

Acknowledgments

We wish to thank Patricia Whitten and James Millette at the Center for Reproductive Endocrinology, Emory University, Atlanta, Ga.

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