Urinary cortisol levels of gray-cheeked mangabeys are higher in disturbed compared to undisturbed forest areas in Kibale National Park, Uganda

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Abstract

Habitat disturbance due to anthropogenic activities is a source of acute and chronic energetic stress in wild animals, including primates. Physiological responses to stress can compromise growth and reproduction, increase susceptibility to infection and lead to deleterious effects on health and conservation efforts. However, physiological measures of energetic stress in association with habitat disturbance are uncommon, especially for wild primate species. Here, we report differences in the stress hormone cortisol in two subpopulations of wild gray-cheeked mangabeys (Lophocebus albigena) inhabiting disturbed and undisturbed forest areas of Kibale National Park, Uganda. Cortisol levels were assessed via opportunistically and noninvasively collected urine samples using previously validated methods. We hypothesized that mangabeys in disturbed forest (DF) areas would experience greater stress and therefore exhibit higher average cortisol levels than conspecifics in nearby relatively undisturbed forest areas (UF). As predicted, mangabeys in the disturbed area had significantly higher cortisol levels (unpaired t-test of log transformed data, $t=4.88$, d.f. $=108$, $P<0.0001$). Mangabeys in undisturbed forest exhibited expected diurnal patterns of cortisol excretion while those in disturbed areas did not, suggesting alteration of the circadian pattern of hypothalamic-pituitary-adrenal function (DF, $r=0.12$, $P=0.43$; UF, $r=0.35$, $P=0.005$). Reasons for differences are unclear, but could include altered food availability and distribution, human contact or other anthropogenic effects. Noninvasive measurements of urinary hormones are useful for quantifying animal energetic stress in the wild and assessing the effects of conservation efforts to attenuate anthropogenic stress in wild populations.

Introduction

Deterioration of environmental quality can threaten the well-being of wild animal populations. Ecological factors that influence environmental quality include climate, diet, predation or habitat disturbance, while social factors include competition, dominance relationships or reproductive access or opportunities. Perturbations of ecological or social factors may disrupt homeostasis and force immediate physiological adjustments to cope with such stimuli (Reeder & Kramer, 2005). The habitats of wild nonhuman primates vary in the degrees of disturbance and/or conservation protection, ranging from large swaths of closed canopy forests to patchy, fragmented habitats under threat by human activities (Johns & Skorupa, 1987; Bicca-Marques, 2003; Marsh, 2003). Contrasting undisturbed versus disturbed habitats allow researchers to investigate how environmental conditions influence energetic stress. To this end, the noninvasive collection of biological samples such as urine and feces from wild animal populations for the measurement of stress biomarkers such as glucocorticoid levels is a valuable research method (Wikelski & Cooke, 2006; Romano et al., 2010).

Cortisol (C) is a glucocorticoid produced by the adrenal cortex under stimulation by adrenocorticotrophic hormone in response to environmental challenges and conditions that require the mobilization and utilization of circulating glucose (Griffin & Ojeda, 2004). Challenges and conditions that stimulate cortisol increases include food shortages, social instability, infection and injury, unusual events and habitat disturbance (reviewed in Anestis, 2010). However, chronically elevated cortisol causes disruptions in reproductive function, immunocompetence, growth and neurological function (Sapolsky et al., 1990; Chrousos, Torpy & Gold, 1998; Habib et al., 2000). Levels of cortisol in urine reflect the amount of the hormone circulating in both conjugated and unconjugated forms, and can be assessed noninvasively under adverse field conditions (Whitten, Brockman &
Stavisky, 1998). Only limited attention has been given to how habitat disturbance influences cortisol levels and what this means for conservation strategies, despite the endangered status of many primate species (Chapman & Peres, 2001).

Gray-cheeked mangabeys (Lophocebus albigena johnstoni) are large-bodied cercopithecines that are common in Kibale National Park, Uganda, where their abundance is highest in old growth forest and other areas where fruit abundance is high (Olupot et al., 1997; Teelen, 2007). Mangabeys in disturbed habitats have lower body masses and smaller groups than those in old growth forest, but little detailed information on the impact of habitat quality and fruiting tree density on mangabey socioecology is available (Olupot et al., 1994; Olupot, 2000; Worman & Chapman, 2005). Building on these findings by using hormonal biomarkers of energetic stress provides crucial information on more subtle effects of habitat disturbance on growth, reproduction and overall health. We collected behavioral and hormonal data on gray-cheeked mangabeys at two Kibale sites, one recently subjected to heavy human disturbance and the other relatively undisturbed, to examine the relationship between habitat disturbance and energetic stress in this species.

**Study site and methods**

Kibale National Park, Uganda (766 km²) is a moist, evergreen medium altitude forest with a mosaic of old growth forest, swamp forest and regenerating forest of varying ages (Struhsaker, 1997). Mainaro, on the southeastern edge of the park (Fig. 1), experienced severe encroachment by local farmers between the 1960s and the early 1990s despite Kibale’s protected status (Van Orsdol, 1986). Replanting of endemic tree species was initiated in 1994, and the site now has a mosaic of old growth forest patches interspersed with patches of previously disturbed vegetation at various stages of succession, and a large area of replanted forest (Verweij & Emmer, 1998; Mucunguzi et al., 2007). The Ngogo research site is in the center of Kibale (Fig. 1). Ngogo was not subjected to mechanized logging while Kibale was a forest reserve (Struhsaker, 1997) nor did it experience recent conversion of forest to farmland. The Ngogo habitat contains large areas of old growth forest plus areas of regenerating forest, swamp forest and grassland (Lwanga, Butynski & Struhsaker, 2000). Ngogo is much more remote; human presence is more conspicuous at Mainaro because it is accessible via a large road cut. Average tree diameter at breast height (dbh, measured in trees > 10 cm dbh across multiple vegetation transects) is similar at both sites [Mainaro: 24.2 cm ± 16.31 standard deviation (sd), n = 3447 trees; Aronsen & Teelen unpubl. data; Ngogo: 25.0 cm ± 24.0 sn, n = 2600 trees; Chapman unpubl. data]. However, Ngogo has more large trees, given its lack of recent human disturbance (Kolmogorov–Smirnov Z = 2.02, P = 0.001).

With the help of two field assistants (one at Mainaro and one at Ngogo), N. A. J. collected behavioral data on five mangabey groups at Ngogo and six at Mainaro between June and August 2009 and used validated field methods (Knott, 1997) to collect and process fresh urine samples for eventual hormonal analysis. Mangabeys at Mainaro were assigned to the disturbed forest group (DF) category, while Ngogo animals were assigned to the undisturbed forest (UF) forest category. N. A. J. and field assistants walked along existing forest transects to find mangabey groups; on encounter, they counted group sizes, then followed the group until they lost it or until the end of the day. During follows, they recorded data on activity state (travel, forage/feed, rest, vigilance) at 2-min intervals using scan sampling (Altmann, 1974; Mainaro = 321 scans, Ngogo = 667 scans).

To avoid potential additional chronic stress associated with exposure to human presence, we tried not to resample the same group on consecutive days by spacing out collection locations by distances greater than 1 km from wherever animals had been sampled on the previous day (Kibale mangabey day ranges average about 1 km; Aronsen unpubl., data; Olupot et al., 1997). Urinary cortisol values reflect circulating levels 4–8 h in the past (Whitten et al., 1998), thus this sampling regime reduced the impact of observational contact with researchers. We made every effort to avoid repeated sampling of any one individual in any group, but note that no tags or collars are on these animals.
Urine was collected from leaves in the ground layer of vegetation with plastic disposable pipettes, then immediately placed into 1.5-mL snap tubes that were labeled with sample identification numbers and information on individual sex and age (when known), date and time of day. To minimize risk of sample cross contamination, urine was collected only when it looked fresh and it was clear that multiple individuals had not urinated in the same area. Global Positioning System location was noted for each sample collection. No samples were collected on days when it rained. We collected 121 samples from Mainaro (DF) and 78 from Ngogo (UF), over 15-day periods at each site. Cortisol assays were done on 66 samples from Ngogo, 64 of which were included in the analysis reported here, and on 71 samples from Mainaro, 46 of which were included. Samples that were excluded either had levels below detection limits of the assay or had insufficient volume.

Field handling and storage followed validated protocols (Knott, 1997). Urine samples collected in the field were transferred to filter paper in camp. Each piece of filter paper was placed on aluminum foil and labeled with a sample identification number and with information on individual sex and age (when known), date and time of day. Fifty microliters of urine were evenly pipetted onto the filter paper, in duplicate, when there was sufficient volume. To protect samples against mold contamination, the filter paper was squeezed against the side of the tube and placed in a small ziplock bag that was in turn placed on aluminum foil resting over a layer of silica. These ziplock bags were placed in the shade and left undisturbed for 2 days (Campbell, 1994; Shideler et al., 1995). Once dried, samples were wrapped in foil, labeled with sample number and placed in plastic slide sheets for storage and transport back to the Yale University Reproductive Ecology Laboratory.

Urinary cortisol was assayed as follows. Elution of filter paper was conducted by insertion into labeled 16×100 mm borosilicate tubes. Five milliliters of 100% methanol was added to each test tube, and tubes were capped with parafilm and refrigerated overnight. On the following morning, the filter paper was squeezed against the side of the tube using sterilized forceps. Sample tubes were dried, reconstituted with 1 mL of distilled water, vortexed for 2 min, and capped with parafilm until they were assayed, following previous studies (Knott, 2005; Marshall & Hohmann, 2005; Dittami et al., 2008).

Creatinine values were assessed via Jaffe's reaction (Taussky, 1954). Creatinine correction was calculated by dividing cortisol value by creatinine value. Immediately after creatinine assays were completed, samples were assayed in duplicate using an unmodified high-sensitivity salivary cortisol enzyme immunoassay suitable for detecting the range of reconstituted urine hormone values (Catalogue 1-3102, Salimetrics, State College, PA, USA; Anestis & Bribiescas, unpubl. data). Coefficients of variation for internal high- and low-quality control were 4.38% and 2.77%. Blanks read below detection levels.

Statistical analysis

Mean differences in urinary cortisol were determined using unpaired t-tests of log transformed data due to non-Gaussian distributions and significant differences in variances in the raw data. Associations between cortisol and collection times were determined using standard linear regression. Behavioral differences were examined using χ² and Mann–Whitney U tests. Statistical analyses were conducted using Instat 3.0b for Macintosh (GraphPad, Inc., San Diego, CA, USA). Alpha was set at 0.05.

Results

C levels were significantly higher in the disturbed area (Mainaro, n = 46) than in the undisturbed areas (Ngogo, n = 64; unpaired t = 4.88, P < 0.0001; Fig. 2). Levels were negatively associated with time of day at Ngogo (r = -0.38, P < 0.003), reflecting the expected circadian pattern of cortisol secretion. However, no diurnal pattern was evident at Mainaro (r = 0.19, P < 0.23), suggesting that the expected pattern was perturbed (Coe, Savage & Bromley, 1992). The overall cortisol secretion slopes were not significantly different between the sites (F = 0.26, P = 0.85; Fig. 3).

Mean group size was significantly smaller at Mainaro (14.1; se 2.0, n = 6 groups) than at Ngogo (20.0; se 2.5, n = 5 groups; Mann–Whitney U = 26, P = 0.05). Activity budgets differed significantly between the sites (χ² = 81.9, d.f. = 4, P > 0.001): compared to Ngogo, Mainaro mangabey groups traveled slightly more (25% vs. 20%), rested less (13% vs. 24%), foraged and fed less (25% vs. 39%) and were far more vigilant (29% vs. 7%).

Discussion

Compared to conspecifics in undisturbed habitats, primates in disturbed habitats often differ in variables such as group size, activity budgets and predation risk (Onderdonk & Chapman, 2000; Mitani et al., 2001; Boinski et al., 2003).

Figure 2 Urinary cortisol levels at Mainaro (disturbed forest) and Ngogo (undisturbed forest; log transformed, unpaired t = 4.88, P < 0.0001). Box plots show mean values and ranges between sites. Raw values are presented for illustrative purposes only.
Associations between habitat quality and hormone profiles occur in many wild primate populations. For example, Rangel-Negrín et al. (2009) found that spider monkeys (Ateles geoffroyi) in fragmented forests had significantly higher mean fecal cortisol levels than those in protected, intact forests. They concluded that forest fragmentation and proximity to humans had cascading social and ecological effects (e.g., increases in aggression and in time spent foraging) that increased stress. Wild howler monkeys (Alouatta pigra) in forest patches in southern Mexico had significantly higher levels of stress, as measured by fecal cortisol, than those in continuous forest (Martínez-Mota et al., 2007). In a protected Belizean reserve, A. pigra exposure to tourists was associated with significantly increased levels of fecal cortisol independently of food availability (Behie, Pavelka & Chapman, 2010). Both the noise and disturbance of human activity and the unpredictability of human contact are likely stimulants of cortisol increases (Sapolsky, 1992).

In Africa, recent work on Tana River mangabeys (Cercocebus galeritus) indicates that parasite load increases and fecundity decreases as habitat disturbance increases (Mbora et al., 2009). Other studies of wild primates in and around Kibale have found detrimental effects of habitat disturbance on immunocompetence (Gillespie & Chapman, 2008; Goldberg et al., 2008). Chapman et al. (2006) found that fecal cortisol levels for groups of red colobus monkeys (Piliocolobus tephrosceles) in intact forest were associated with parasite richness and nematode egg presence in fecal samples. Similarly, parasite richness was associated with fecal cortisol levels in wild chimpanzees (Pan troglodytes) at Ngogo (Muehlenbein, 2006).

This preliminary study suggests that noninvasive studies of primates in habitats with varying levels of disturbance can provide an important ‘first look’ at associated stress via measurement of overall cortisol levels and of daily variation in cortisol secretion. Potential sources of energetic stress for mangabeys at Mainaro include habitat fragmentation, increased visibility to predators, higher intra- and intergroup agonism, lower diet quality and increased distance between food patches. Downstream results of energetic stress, such as reduced birth weight, lower breast milk quality and decreased body mass may ultimately lead to population decline (Honess & Marin, 2006; Busch & Hayward, 2009). Longer term research can assess these variables by examining whether food availability and individual energetic status is generally better at Ngogo, and whether predation risk, foraging costs and parasite loads are higher at Mainaro. Our preliminary results show measurable variation in energetic stress between habitats with different histories of human disturbance, and we suggest that multivariate studies of habitat quality, cortisol levels and variables such as fecal parasite load can provide important data on local and regional primate population condition.

Acknowledgments

We appreciate the two anonymous reviewers whose comments improved our paper. The authors acknowledge...
funding support from the Yale Institute for Biospheric Studies Center for Human and Primate Reproductive Ecology, the Yale STARS II Program (to N.A.J.), the L.S.B. Leakey Foundation, the Great Ape Trust of Iowa and the Yale University Department of Anthropology. We thank the Ugandan President’s Office, the Uganda Wildlife Authority and the Uganda National Council for Science and Technology for their permission to work in Kibale National Park. We thank the UWA/FACE Project Director (W. Chemutai), the Ngogo Chimpanzee Project Manager (J. Lwanga) and the camp staff of Ngogo and Mainaro for logistic support in the field. NAI and GPA especially thank P. Birungi, D. Kamekune, P. Mwesigwa, F. Kamekune and J. Turyasingura for their field assistance during this project.

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