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ORIGINAL PAPER

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Characterization of pituitary–adrenocortical activity in the Malayan flying fox (*Pteropus vampyrus*)

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Abstract Pituitary–adrenocortical and gonadal endocrine activity was investigated in a captive colony of *Pteropus vampyrus*, a highly social Old World fruit bat. Both cortisol and corticosterone were present in plasma, at a ratio of approximately 5:1, respectively. Glucocorticoid but not testosterone levels significantly increased prior to and concomitant with the evening active period. Restraint stress for 15–60 min resulted in a significant and rapid increase in plasma levels of adrenocorticotropic hormone (ACTH) and glucocorticoids. ACTH levels quickly returned to baseline following restraint whereas glucocorticoid levels remained elevated for at least 30 min after restraint ended. Plasma ACTH levels after stress were similar to levels reported after stress in other mammals. Stress-induced glucocorticoid levels were several-fold greater than those reported for most mammals. Restraint for 15 min significantly inhibited testosterone levels. Restraint stress did not affect hormone levels on the morning following restraint. Brief capture, handling, and release of the animals did not elicit increases in these hormones. The physiological responsiveness of the pituitary and adrenal glands, along with *P. vampyrus*'s documented seasonality and range of social behaviors, makes these bats an excellent model for exploring the general physiology of the hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axes, as well as social influences on these axes.

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Successful reproduction in vertebrates depends on the endocrine system, which must have the capacity to function within and adapt to a changing physical and social environment. Although there are many different neuroendocrine components involved in regulating reproduction and the response to changing environments, the hypothalamic–pituitary–adrenal (HPA) axis and the hypothalamic–pituitary–gonadal (HPG) axis are particularly important (Romero 2002; Wingfield and Sapolsky 2003; Reeder and Kramer 2005). Glucocorticoid hormones (cortisol and corticosterone) are important regulators of energy balance, and elevations in glucocorticoid hormones are considered a hallmark of the vertebrate stress response (Reeder and Kramer 2005). The HPA and HPG axes interact with one another in complex ways and, in part, serve to regulate behavior and fertility (Handa et al. 1994; DeVries 2002; Viau 2002).

We have been interested in the endocrinology of Chiroptera for several reasons. Bats, which are the second most specious order of mammals (Wilson and Reeder 2005), are vital links in many different ecosystems, acting as seed dispersers, pollinators, and predators of insects. Thus, an understanding of factors that govern reproduction and survival of bats is critically important (Barclay and Harder 2003). The regulation and functional characteristics of the HPA axis in some insectivorous bat species resembles those observed in laboratory animals (Widmaier et al. 1994; Reeder et al. 2004b). However, in one pteropodid species (*Pteropus hypomelanus*), circulating glucocorticoid levels are among the highest ever reported in vertebrates (up to 3,000 ng/ml in individual animals 1 h following handling and blood sampling; Widmaier and Kunz 1993; Widmaier et al. 1994; Reeder et al. 2004a), perhaps even

exceeding those of squirrel monkeys (*Saimiri*; Brown et al. 1970) and other New World primates (Chrousos et al. 1982). Despite such high steroid levels, pteropodid bats, like the New World primates do not exhibit adverse health consequences (e.g., suppressed immune system) that might be expected in other mammals exposed to chronically elevated glucocorticoids, and are known to thrive in captivity. This observation suggests the possibility of relatively uncommon control mechanisms in bats that may be of relevance in understanding pathologies of HPA activity in humans and other mammals.

Whether the unusual glucocorticoid levels in *P. hypomelanus* are specific to this species, or found throughout the genus *Pteropus* is largely unknown, although we have demonstrated that two related species, *P. pumulis* and *P. vampyrus*, also have exceptionally high circulating glucocorticoids (Widmaier and Kunz 1993; Widmaier et al. 1994; Reeder et al. 2005). *P. vampyrus* is a large, socially gregarious, Old-World fruit bat weighing ~1.1 kg with a wingspan of ~1.5 m. They roost in groups of tens to thousands throughout Southeast Asia and are highly seasonal and synchronous breeders, with most females giving birth to a single pup annually (Kunz and Jones 2000). Both breeding and non-breeding males and females exhibit significant seasonal rhythms in baseline glucocorticoid levels, but the peaks and nadirs of these rhythms differ by sex (Reeder et al. 2005). Other pteropodid species are either gregarious (e.g., *P. hypomelanus*) or exhibit infrequent social interactions (*P. pumulis*), which will permit us in future studies to determine the influence of changing population dynamics (as might occur with habitat destruction) on HPA and HPG activity. Before that is possible, however, it is necessary to establish basic characteristics of these two endocrine axes in pteropodid bats. The objectives of this study were to characterize basal and circadian pituitary–adrenocortical function in *P. vampyrus*, the magnitude and pattern of the pituitary–adrenocortical response to a standard stressor (restraint), and the effect, if any, of this stressor on HPG activity as measured by circulating testosterone levels in males. We also asked whether exposure to stress resulted in changes in the pattern of basal glucocorticoid levels the following day, since prior stress has been reported to alter subsequent HPA activity in laboratory rats (Dallman et al. 2000).

Materials and methods

Animals and animal care

Subjects included 24 male and 24 female *Pteropus vampyrus*. Animals were housed in captivity at the Lubee Bat Conservancy, Gainesville, FL, USA. All of the research described in this paper was approved by the Lubee Bat Conservancy IACUC. Animals were housed in octagonal, double wire enclosures, measuring approximately 11 m in diameter and 2 m high. Enclosures contained an outdoor portion that encircled a smaller inside roost (the nighthouse; 3 m in diameter and temperature controlled), and were designed to maximize the ability of bats to feed, rest, and fly freely. Eight of the subjects (all male) were wild-caught, captured either in 1990 (3) or in 2000 (5) in Indonesia. The remaining 40 subjects were born in captivity and ranged between 2 and 9 years of age; maximum lifespan in captivity is at least 15 years (Kunz and Jones 2000). All subjects were reproductively mature and theoretically reproductively capable at the beginning of the study. All subjects were housed in same-sex groups and no females were pregnant during the study. The experiments described below were performed in July of either 2002 or 2003; both glucocorticoid and testosterone levels are seasonally low in July, with breeding occurring in each fall in captivity (Reeder et al. 2005). In this colony, nearly identical seasonal glucocorticoid and testsoterone profiles have been found in bats maintained in same-sex vs. breeding groups (Reeder et al. 2005). Male bats in this study weighed 1308.2 ± 47.3 g (mean ± SE); females weighed 1039.5 ± 21.0 g. Each bat was easily identified for blood sampling by a combination of markers (bleached symbols in their fur and colored and numbered thumb bands). Animals were fed a mixture of fresh fruits, vegetables, and monkey chow (Purina) daily at 1500 hours. There was at least one food bowl per subject and bowls were spaced sufficiently apart to minimize competition. Water was available ad libitum.

Sampling protocol and hormone assays

For assessing circadian rhythms, four different groups of animals (each with six males and six females) were sampled at each of four time points: 0300, 0900, 1500, and 2100 hours in July 2002. Each animal was only sampled once, for a total of 48 subjects. At each time point, bats were quickly hand captured and brought to a centrally located staging area outside of their pen. Bats were manually restrained and blood samples (1 cm^3) were collected without anesthesia within three min of capture via venipuncture from either a small wing vein or a vein in their forearm. Rapid collection of blood samples was necessary to avoid measuring the response to handling and sampling itself (Widmaier and Kunz 1993; Widmaier et al. 1994). Blood was placed into EDTA-containing microtubes in an ice-water bath and plasma was stored at −20°C until assay.

To investigate the hormonal responses to stress, a total of three blood samples were collected from each subject: (1) a baseline sample immediately after capture in the home cage, (2) a stress sample, collected after a set period of time in a restraint device, and (3) a recovery sample, collected 30 min after releasing the animals from the restraint device. The baseline sample was

obtained as described above in the circadian study. After collection of the baseline sample, animals were placed into a restraint device as previously described for *P. hypomelanus* (Widmaier and Kunz 1993) consisting of a soft mesh tube with wood plugs at the ends (16 cm in diameter × 57 cm tall). Restraint tubes were then hung with bats inside of them (head down) in a larger cage within a temperature-controlled environment. Restraint was applied for 15, 30, 45 or 60 min to six males per duration of restraint. A second sample was obtained at the end of restraint, and the animals were returned to their home cages. Thirty minutes after their return to the home cage, animals were again quickly hand captured and a third blood sample was collected. The data for one male in the 60 min restraint group were discarded due to greatly elevated glucocorticoid levels apparently resulting from a recent wound. This study was performed in July 2002 between 0700 and 1000 hours.

To control for the possibility that brief capture and handling elicits a HPA response, animals were briefly handled and returned to their home cage and a blood sample was collected 30 min later, using the same procedures described above. A total of eight males and seven females were used. Samples were collected in July 2003 at 1400 hours.

Lastly, to test whether restraint stress altered basal HPA activity on the following day, eight males and eight females were subjected to restraint stress for 30 min, starting at 1400 hours. This was followed by the collection of additional blood samples (0.4 ml) at 0700 hours the following morning. Samples were collected in July 2003.

Cortisol and corticosterone were assayed in duplicate, separately and without extraction in a volume of 10 and 5 µl plasma, respectively, using a radioimmunoassay (RIA) kit from MP Biomedicals (Irvine, CA), as previously described and validated for this and other species in the genus *Pteropus* (Widmaier and Kunz 1993; Widmaier et al. 1994; Reeder et al. 2004a). Negligible cross-reactivity (<0.05%) of the corticosterone assay for cortisol and vice versa (3%) is reported for these kits by the manufacturer and was validated in our laboratory for *Pteropus* plasma as previously described for plasma of *Myotis lucifugus* (Reeder et al. 2004b). Testosterone was measured in 15 µl of plasma by RIA (MP Biomedicals) as previously described and validated (Reeder et al. 2004a). Plasma ACTH was measured in 20 µl of plasma by immunoradiometric assay (Raff and Findling 1989) as previously described for *P. hypomelanus* (Widmaier et al. 1994), using human $ACTH_{(1–39)}$ standard, ^{125}I-labeled monoclonal antibody against human $ACTH_{(1–17)}$, and polyclonal antibody affinity purified against $ACTH_{(34–39)}$ and coupled to biotin. Parallelism for ACTH was demonstrated by diluting nine different bat samples (1:4, 1:10, and 1:40). Intra-assay and inter-assay CV's were 6 and 8%, respectively, at concentrations present in bat plasma samples. Least detectable doses were as follows: ACTH, 2.0 pg/ml; cortisol, 6.6 ng/ml; corticosterone, 0.094 ng/ml; testosterone, 0.15 ng/ml.

Statistical analyses

For an analysis of circadian rhythms, cortisol and corticosterone were evaluated with a 2 (sex) × 4 (time) ANOVA (data were collected cross-sectionally, not longitudinally), and differences in testosterone between time points were tested with ANOVA. For the restraint stress experiment, repeated measures ANOVA were used within each of the four time series to detect differences in hormone levels with respect to time (e.g., for each time series, the three repeated hormone measures for baseline, stress, and recovery from stress were included in the analysis). Significant effects were explored a priori with *t* tests (see Results below); *P* values were corrected for multiple comparisons using the Bonferroni method with layering and corrected *P* values are indicated by "pc". Either ANOVA or *t* tests were used to assess differences between animals in each series in the hormone level reached immediately following restraint (e.g., levels reached after 15 vs. 30 vs. 45 vs. 60 min of restraint), and in the hormone level reached at time 0, time 45, and time 60, when animals in more than one group were sampled at the same time (e.g., at time 45, depending upon what time series an animal was assigned to, some animals had their third (recovery) bleed drawn and others had their second (stress) bleed drawn).

Results

Plasma cortisol levels were several-fold greater than corticosterone at all timepoints sampled during a 24 h period in *P. vampyrus* (Fig. 1). There was significant diurnal variation in circulating levels of both glucocorticoids, with a trough at 0900 hours and a peak at 2100 hours (Fig. 1; cortisol ($F_{(3,40)}=6.122$, $P=0.002$); corticosterone ($F_{(3,40)}=4.719$, $P=0.007$)). No sex differences were detected at any time point (not shown). Testosterone levels in males tended to decline during the

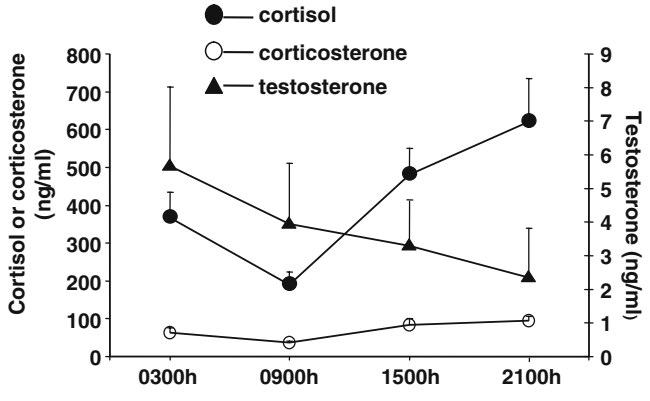

Fig. 1 Circadian fluctuations in glucocorticoid hormones (both cortisol and corticosterone) and testosterone in *Pteropus vampyrus*. Sample size for each timepoint is 12 (6 male, 6 female, no sex differences). All data were collected cross-sectionally. Values are mean ± SE

day and early evening, but the change was not significant.

Restraint stress resulted in a significant increase in plasma levels of ACTH, cortisol, and corticosterone in all four timed experiments (Fig. 2). Testosterone levels tended to decrease following restraint, but this was significant only in the 15 min restraint group (Fig. 2; 15 min restraint: $F_{(2,10)}=4.356$, $P=0.044$). Baseline levels (time 0) of each hormone were not significantly different between the animals in each of the four tests. After a peak at 15 min, ACTH levels plateaued during continued restraint, then declined at a similar rate in all experimental groups once restraint was terminated and the animals were allowed to recover. Significant differences were found within each time series for ACTH (15 min of restraint, 30 min of recovery: $F_{(2,10)}=9.997$, $P=0.004$; 30 min of restraint, 30 min of recovery: $F_{(2,10)}=4.996$, $P=0.031$; 45 min of restraint, 30 min of recovery: $F_{(2,10)}=5.680$, $P=0.022$; 60 min of restraint, 30 min recovery: $F_{(2,8)}=4.582$, $P=0.047$). The peak in ACTH at 15 min of restraint was not significantly different from the levels of ACTH reached in response to stress after other periods of restraint. However, this time series (15 min of restraint) was the only one in which, after Bonferroni correction, a significant increase in ACTH between time 0 (baseline) and the restraint bleed (in this case 15 min) and a significant decrease (to levels not distinguishable from baseline) during the recovery period (30 min after the restraint bleed) was detected (T0–T15: $t=-3.614$, $df=5$, pc = 0.03; T15–T45: $t=4.967$, $df=5$, pc = 0.012; T0–T45: $t=-2.055$, $df=5$, $P=0.10$).

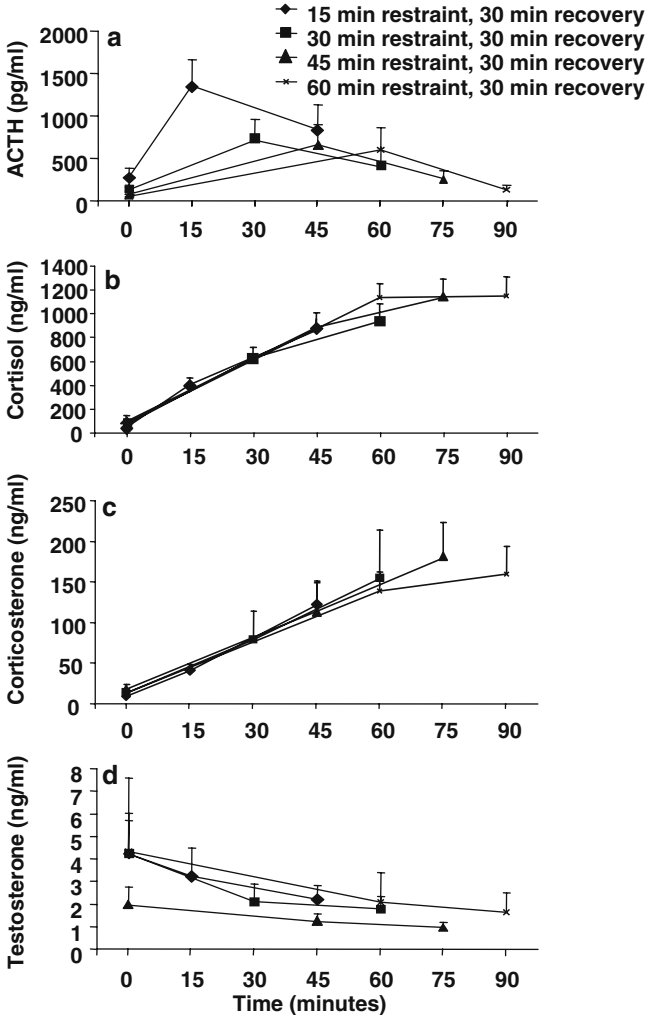

Fig. 2 Hormone levels in response to restraint stress of either 15, 30, 45, or 60 min, followed by a 30 min recovery period in *Pteropus vampyrus*. **a** Adrenocorticotropic hormone (ACTH); **b** Cortisol; **c** Corticosterone; **d** Testosterone. Each of the four time series has $N=6$ (males). Values are mean ± SE. See text for description of significant differences

Despite the decline in ACTH, plasma levels of both glucocorticoids continued to increase or remained at a significantly elevated plateau during the 30-min recovery period following restraint (independent of the duration of the restraint period). For cortisol, significant increases were found within each time series (15 min of restraint, 30 min of recovery: $F_{(2,10)}=62.867$, $P<0.0005$; 30 min of restraint, 30 min of recovery: $F_{(2,10)}=28.107$, $P<0.0005$; 45 min of restraint, 30 min of recovery: $F_{(2,10)}=48.340$, $P<0.0005$; 60 min of restraint, 30 min recovery: $F_{(2,8)}=43.466$, $P<0.0005$). A significant elevation in cortisol levels in response to restraint was seen within each time series (15 min of restraint: $t=-6.317$, $df=5$, $P=0.001$; 30 min: $t=-6.463$, $df=5$, pc = 0.002; 45 min: $t=-8.061$, $df=5$, pc < 0.001; 60 min: $t=-9.134$, $df=4$, pc = 0.002). When cortisol levels reached in response to restraint were compared cross-sectionally across the time series, there was a significant increase over time, with the greatest levels reached in those animals restrained for 60 min ($F_{(3,19)}=9.584$, $P<0.0005$). For those animals restrained for 15, 30, and 45 min, cortisol levels continued to increase significantly during the 30 min 'recovery' period (recovery after 15 min of restraint: $t=-7.772$, $df=5$, pc = 0.002; 30 min: $t=-3.288$, $df=5$, $P=0.022$; 45 min: $t=-3.029$, $df=5$, $P=0.029$). For animals restrained for 60 min, cortisol levels after the 30 min 'recovery' period did not significantly differ from those after restraint, suggesting a final plateau in the stress response. The corticosterone profile is nearly identical to that of cortisol; significant increases were found within each time series (15 min of restraint, 30 min of recovery: $F_{(2,10)}=13.362$, $P=0.001$; 30 min of restraint, 30 min of recovery: $F_{(2,10)}=5.737$, $P=0.022$; 45 min of restraint, 30 min of recovery: $F_{(2,10)}=12.253$, $P=0.002$; 60 min of restraint, 30 min recovery: $F_{(2,8)}=15.611$, $P=0.002$). An elevation in corticosterone levels in response to restraint was seen within each time series, and was significant for all but the 30 min of restraint time point (15 min of restraint: $t=-4.141$, $df=5$, pc = 0.018; 30 min: $t=-2.012$, $df=5$, $P=0.1$; 45 min: $t=-3.012$, $df=5$, $P=0.03$; 60 min: $t=-4.870$, $df=4$, pc = 0.016).

As with cortisol, when corticosterone levels reached in response to restraint were compared cross-sectionally

across the time series, there was a marked increase over time, with the greatest levels reached in those animals restrained for 60 min, but these differences were not significant. Similarly, for those animals restrained for 15, 30, and 45 min, corticosterone levels continued to increase during the 30 min 'recovery' period, but this was only significant after Bonferroni correction for the recovery after 15 min of restraint ($t = -3.199$, $df = 5$, $P = 0.024$). The same final plateau in hormone levels seen 90 min after the initiation of stress (the 30 min 'recovery' sample following 60 min of restraint) found in cortisol was also found in corticosterone.

In contrast to the response to restraint stress seen above, capture and brief handling (~5 s) such as might occur during routine animal husbandry failed to elicit an HPA response (not shown). In animals of both sexes, total glucocorticoid levels measured 30 min after capture and brief handling were not significantly different from baseline levels collected at the same time (1400 hours) several days earlier (males, baseline total glucocorticoids = 639 ± 89 ng/ml vs. post-handling = 707 ± 92 ng/ml; females, 428 ± 100 ng/ml vs. 549 ± 66 ng/ml). In addition, no alterations in total glucocorticoid levels were observed the morning following exposure to restraint stress in *P. vampyrus* (morning baseline total glucocorticoids when exposed to restraint stress the previous day vs. when not exposed to a previous-day stressor; see Fig. 3).

Discussion

We report that the major circulating glucocorticoid in *P. vampyrus* is cortisol, with circulating levels of corticosterone accounting for approximately 15% of total glucocorticoids throughout the day, thus confirming and extending our earlier observations (Widmaier and Kunz 1993). While similar to many mammals, including *P. pumulis* (Widmaier et al. 1994), this ratio is nonetheless lower than that found in *P. hypomelanus*, in whom circulating corticosterone constitutes up to or more than one-third of total glucocorticoids in stressed and non-stressed conditions (Widmaier and Kunz 1993; Widmaier et al. 1994; Reeder et al. 2004a). Although we have not determined the basis of this difference, it could reflect a differential expression of adrenocortical CYP17α, the enzyme required for shifting cholesterol metabolism towards cortisol and androgens.

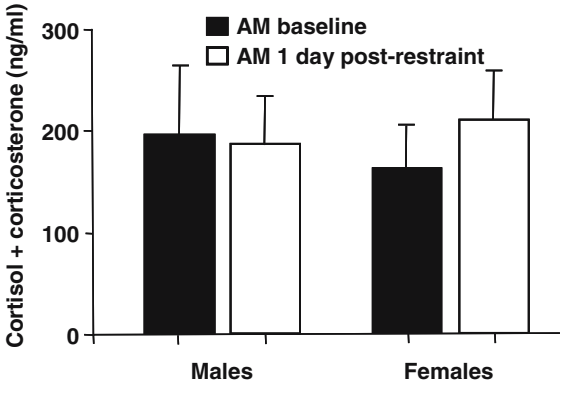

Fig. 3 Total glucocorticoid levels (cortisol + corticosterone; mean ± SE) in both males ($N = 8$) and females ($N = 7$) measured at 0700 hours when exposed to restraint stress the previous day (AM 1 day post-restraint) versus when not exposed to a previous-day stressor (AM baseline). Exposure to restraint stress did not alter basal glucocorticoid levels the following day

Male and female *P. vampyrus* exhibited an intact glucocorticoid diurnal rhythm with no sex differences (at least in July, when the sex differences in baseline glucocorticoid levels found at some other times of the year do not occur; Reeder et al. 2005). The pattern of the daily change in plasma glucocorticoids is that which would be expected for free-ranging pteropodid bats, with a peak in glucocorticoid levels at dusk at the beginning of their active period. The association of peaks in circulating glucocorticoids with an animal's active period is characteristic of most mammals (e.g, rat, Dallman et al. 2000; human, Selmaoui and Touitou 2003). Plasma levels of glucocorticoids at the diurnal trough and peak were considerably greater than those typically reported in laboratory rodents and other mammals (reviewed in Fenske 1991). This was not likely the result of the blood sampling procedure itself, since samples were obtained within the timeframe generally considered to be too soon to detect stress-induced increases in circulating glucocorticoids (Romero 2002). We also do not believe that the high glucocorticoid levels signify a condition of chronic stress in our captive animals for several reasons. First, at least two other related species- *P. pumulis* and *P. hypomelanus*- have similarly high glucocorticoid levels (Widmaier and Kunz 1993; Widmaier et al. 1994; Reeder et al. 2004a; Reeder et al. 2005), unlike other non-pteropodid captive or wild-caught bats whose glucocorticoid levels are very low (Widmaier and Kunz 1993; Widmaier et al. 1994; Reeder et al. 2004b). Second, the bats in this study have been bred and raised or housed in captivity with regular veterinary care for as long as 15 years, thus have had considerable time to become acclimated to their environment. Finally, the animals have thrived in captivity as evidenced by overall good health and reproductive success. It is possible that the relatively high glucocorticoid levels in *Pteropus* may be explained by high circulating levels of corticosteroid-binding globulin or by low affinity glucocorticoid receptors, but we do not have evidence to support either of these possibilities at this time. These results suggest, therefore, that the long-term captive environment of *P. vampyrus* is not chronically stressful and that future studies on the effects of changes in social environment on the pituitary–adrenocortical axis are valid and feasible.

In contrast to glucocorticoids, no significant diurnal rhythm was detected in testosterone in male animals. This may reflect the fact that these samples were collected in July, which is a period of relatively low measurable testosterone in this species (in another study,

testosterone measured at 2 PM in July averaged 4.51 ± 0.52 ng/ml; testosterone in September at the peak of the breeding season measured 24.33 ± 2.58 ng/ml; Reeder et al. 2005). Thus, not enough variation in testosterone levels was present to detect a significant circadian rhythm if one actually existed.

Restraint has been widely employed to non-invasively elicit a stress response in mammals (e.g., in rats, Dallman et al. 2004; silver fox, *Vulpes vulpes*, Moe and Bakken 1997), including *P. hypomelanus* (Widmaier and Kunz 1993). Our results for both ACTH and glucocorticoids demonstrated that restraint initiated a rapid pituitary–adrenocortical response that was not further affected by increasing amounts of time in the restraint device. Further tests confirmed that the mere act of handling alone was insufficient to initiate this response (at least for glucocorticoids; ACTH was not measured in those samples). The decrease in ACTH that occurred after removal from the restraint stress is similar to that observed in the rat (Garcia et al. 2000), and that observed in the squirrel monkey, which, like the Malayan flying fox, has greatly elevated baseline and stress–responsive glucocorticoid levels (Lyons et al. 2004). This descrease could be due to a decrease in activity of the stimulatory stress pathways, glucocorticoid negative feedback, or a combination of the two. By contrast, plasma glucocorticoids were significantly increased 15 min after the initiation of the stressor, then continued to increase during the 30-min recovery period. A plateau in the glucocorticoid stress response occurred after 60 min of restraint, and may represent the upper limit of plasma glucocorticoids attainable in this species. As described in other mammals (Buckley and Ramachandran 1981; Keller-Wood et al. 1983), the magnitude and duration of the adrenocortical response to ACTH is a function of both ACTH concentration and duration of exposure to ACTH, which most likely explains why glucocorticoid levels in *P. vampyrus* continued to rise or remain at a plateau even when ACTH levels were decreasing during the recovery period.

The stress-induced glucocorticoid levels were among the highest observed in mammals, less than *P. hypomelanus* (some individuals of which exceed 3,000 ng/ml) but more than twice as high as recorded for *P. pumulis* (Widmaier et al. 1994). It is unknown how stress-induced glucocorticoid levels increase to such an extraordinary degree in these species, but as described above for basal steroid levels, it may be a characteristic of all Pteropodidae. Other bats, such as the insectivorous little brown myotis (*M. lucifugus*) have basal and stress-induced glucocorticoid levels that are similar to those of laboratory animals and other mammals (basal 100–600 ng/ml; stress-induced 500–1,000 ng/ml; Widmaier et al. 1994; Reeder et al. 2004b). Exactly when during the evolutionary history of Chiroptera adrenocortical physiology diverged resulting in the ability of some species to achieve very high levels of circulating steroids without apparent health consequences (e.g., immune suppression, increased catabolism) is unknown.

We expected to observe a stress-induced decrease in testosterone in response to restraint in *P. vampyrus*, but this was only observed at one timepoint. As with the circadian study, this may be due to sampling in July at a low point in the seasonal rhythm. In support of this, we have recently demonstrated stress-induced suppression of testosterone in this species at other times of year (Reeder et al. 2005).

Laboratory rats have the ability to compensate for exposures to stress by decreasing their glucocorticoid levels the following day (Dallman et al. 2000), thereby keeping the total daily output of glucocorticoids stable and avoiding the deleterious effects of repeated stressors. Similarly, captive European starlings (*Sturnus vulgaris*) exposed to chronic, non-invasive stress gradually reduce their circulating glucocorticoid concentrations (Rich and Romero 2005). However, when baseline samples were collected from *P. vampyrus* on the morning after an afternoon restraint stress experiment, there was no significant difference in morning glucocorticoids following a stressor the previous day compared to morning glucocorticoids that were not preceded by stress. Thus, acute restraint stress does not confound subsequent measurements of basal adrenocortical activity in this species.

With their documented seasonality, high levels of glucocorticoid hormones, and range of social behaviors, *Pteropus* are excellent models for exploring social influences on physiology, as well as the general physiology of the HPA and HPG axes. *P. vampyrus*, along with *P. hypomelanus* (Reeder et al. 2004a; Reeder et al. 2005), with their exceptionally high glucocorticoid levels may prove to be valuable models for glucocorticoid resistance. Whether or not *Pteropus* are maximizing free glucocorticoid levels to counteract end-organ resistance (as is the case with squirrel monkeys; Scammell 2000) and if so, what mechanisms are involved should be pursued.

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