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ORIGINAL PAPER

Response to long-distance relocation in Asian elephants (*Elephas maximus*): monitoring adrenocortical activity via serum, urine, and feces

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Abstract Understanding how elephants respond to potentially stressful events, such as relocation, is important for making informed management decisions. This study followed the relocation of eight Asian elephants from the Cocos (Keeling) Islands to mainland Australia. The first goal of this study was to examine patterns of adrenocortical activity as reflected in three different substrates: serum, urine, and feces. We found that the three substrates yielded very different signals of adrenocortical activity. Fecal glucocorticoid metabolites (FGM) increased as predicted post-transport, but urinary glucocorticoid metabolites (UGM) were actually lower following transport. Serum cortisol levels did not change significantly. We suggest that the differences in FGM and UGM may reflect changes in steroid biosynthesis, resulting in different primary glucocorticoids being produced at different stages of the stress response. Additional studies are needed to more thoroughly understand the signals of adrenocortical activity yielded by different substrates. The second goal was to examine individual variation in patterns of adrenal response. There was a positive correlation between baseline FGM value and duration of post-transfer increase in FGM concentration. Furthermore, an individual's adrenocortical response to relocation was correlated with behavioral traits of elephants. Elephants that were described by keepers as being "curious" exhibited a more prolonged increase in FGM post-transfer, and "reclusive" elephants had a greater increase in FGM values. Future research should investigate the importance of these personality types for the management and welfare of elephants.

Keywords Cortisol · Glucocorticoids · Individual variation · Noninvasive · Transfer · Transport

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K. V. Fanson · T. Keeley
Wildlife Reproductive Centre, Taronga Western Plains Zoo, Dubbo, NSW 2830, Australia

K. V. Fanson (✉)
School of Life and Environmental Sciences, Deakin University, Geelong, VIC 3216, Australia
e-mail: kerryfanson@gmail.com

M. Lynch
Veterinary Department, Melbourne Zoo, Melbourne, VIC 3052, Australia

L. Vogelnest · G. Miller
Taronga Wildlife Hospital & Elephant Division, Taronga Zoo, Sydney, NSW 2088, Australia

Introduction

Elephants have long captured the human imagination, which makes them both a popular and a controversial species for zoos. Assessing their physiological and psychological welfare poses a significant challenge (Whitham and Wielebnowski 2009; Mason and Veasey 2010). While there are numerous studies on elephant behavior and reproduction (e.g., Hildebrandt et al. 2006; Szdzuy et al. 2006; Brown et al. 2007; Freeman et al. 2010a), there are remarkably few studies on the stress physiology of captive elephants. To be clear, there are several studies that have validated noninvasive techniques and provided initial descriptions of adrenocortical activity in elephants (Brown et al. 1995; Stead et al. 2000; Ganswindt et al. 2003; Dehnhard 2007; Brown et al. 2010; Menargues et al. 2012). However, there are few

studies that allow us to predict how elephants will respond to certain management practices (Dathe et al. 1992; Schmid et al. 2001; Laws et al. 2007; Menargues et al. 2008). Therefore, much remains to be learned about how captive management practices contribute to elephant well-being.

Transport or relocation of captive animals is a common management practice. It is often necessary to move animals between institutions for breeding purposes or for their own well-being (e.g., better facilities, social dynamics, etc.). However, relocations are inherently stressful events which are associated with both acute and long-term stressors (Dickens et al. 2010). Two previous studies have monitored adrenocortical activity in Asian elephants during relocation, but the sample size and/or duration of these studies were minimal (Schmid et al. 2001; Laws et al. 2007). Laws et al. (2007) monitored fecal glucocorticoid metabolites in a single male for 10 days before and after transfer to another institution and detected a significant increase following the relocation. Schmid et al. (2001) monitored urinary glucocorticoid metabolites in two relocated females for 6 months, but did not detect a significant change.

This study seeks to provide a more comprehensive description of adrenocortical activity in translocated Asian elephants (*Elephas maximus*) by increasing both sample size and study duration. We monitored long-term (1 year) patterns of adrenocortical activity in eight Asian elephants relocated from the Cocos (Keeling) Islands (CKI) to mainland Australia. Furthermore, we monitored adrenocortical activity via three different substrates (serum, urine, and feces), thereby providing a valuable comparison of the different endocrine signals produced by different substrates.

The development of noninvasive hormone monitoring has revolutionized our ability to assess an animal's physiological condition and has provided researchers with an increasing number of techniques for monitoring hormone expression. It is generally assumed that once techniques and assays are properly validated, different hormone monitoring techniques are more or less interchangeable (i.e., different substrates should yield qualitatively similar patterns of underlying changes in physiology). However, there are factors that may alter the signal measured via different validated hormone monitoring techniques and consequently lead to different conclusions. First, hormones are secreted in a pulsatile manner. Serum samples only provide a "snapshot" of these pulses, whereas feces and urine provide a "pooled" estimate of hormone expression (Palme 2005; Touma and Palme 2005). Therefore, serum, urine, and feces may yield different signals because they summarize adrenocortical activity over different lengths of time. Second, collection of serum samples may be stressful for animals, thereby increasing circulating glucocorticoid levels and confounding assessments. Finally, circulating hormones are metabolized by the liver and by bacteria when they are excreted in urine or feces (Möstl and Palme 2002; Goymann 2005; Touma and Palme 2005; Sheriff et al. 2011). Consequently, the signal of adrenocortical activity detected in excreta may be influenced by route of excretion, steroid metabolism, or affinity of the assay for particular metabolites. In order to ensure accurate interpretation of results, it is critical to understand the signaling properties and inferential space of different hormone monitoring techniques.

The specific goals of this study were to: (1) evaluate the adrenocortical response of Asian elephants to long-distance relocation, (2) compare patterns of hormone expression obtained from different substrates (serum, urine, and feces), and (3) examine the relationship between individual patterns of adrenocortical activity and elephant temperament, as assessed by keepers. As such, this study seeks to provide useful information about elephant management, as well as the signaling properties of different hormone monitoring techniques.

Methods

Study animals

Eight Asian elephants (one male, seven females) were relocated from Thailand to Australia to establish a conservation breeding program in Australia. Elephants ranged in age from 5 to 14 years old when sampling was initiated on CKI, and all of the females exhibited regular estrous cycles (as determined by serum progesterone profiles). Ethics approval was granted for all procedures in this study.

Prior to relocation, the elephants were held in a pre-export quarantine facility in Thailand for 18 months. The facility was a large single paddock with shelter and bathing areas. The animals could mix freely during daylight hours but were tethered separately at night. Behavioral training sessions were conducted daily during this period and included a minimum of 6 months of conditioning to their transport crates. All individuals became accustomed to entering and being confined in the crates before the first transfer was initiated.

The relocation included a 3-month quarantine period on CKI, an Australian territory located in the Indian Ocean. The elephants were transported to CKI on 30 July 2006 via plane. Animals were lightly sedated when necessary by a qualified wildlife veterinarian using a combination of xylazine and butorphanol or acepromazine delivered intramuscularly. Elephants were closely monitored by veterinarians and experienced elephant keepers throughout the transport process. Housing and husbandry for the elephants on CKI was similar to the pre-export quarantine facility. All animals could mix freely during daylight hours in a large (13,000 m^2) paddock, but were tethered at night. Behavioral

training continued on a daily basis, as did conditioning to their transport crates in preparation for the second leg of their journey to mainland Australia.

The elephants were transported to mainland Australia via plane on the first and fourth of November 2006. During transport, elephants were lightly sedated and closely monitored as described above. In Australia, one male and four females went to Taronga Zoo (Sydney, New South Wales). All five animals were housed together for the first 2 years post-transfer. The remaining three females were transported to Melbourne Zoo (Melbourne, Victoria). Melbourne Zoo already had one male and one female who had been at the zoo for ~30 years. Two days after their arrival, the three females from Thailand were introduced to the resident female. The male was housed separately. Daily routines were similar to Thailand and CKI and included behavioral training, washing, and then free time in the paddock. Elephants were no longer tethered during the night at either institution.

Sample collection

Serum, urine, and fecal samples were collected one to two times per week for 1 year. Sampling started after the elephants arrived on CKI (8 August, 18 August, and 15 September 2006 for serum, feces, and urine, respectively). Sample collection continued for 9 months after the elephants arrived in Australia. Due to import restrictions and other logistical issues, we were unable to obtain samples from Thailand prior to the move. Elephants were trained for blood collection from ear veins, and samples were collected late in the morning. Urine and fecal samples were collected opportunistically in the morning when elephants were observed urinating or defecating. As much as possible, samples were collected at the same time of day. All samples were immediately stored at −20 °C and shipped overnight on ice to the Wildlife Reproductive Centre at Taronga Western Plains Zoo (Dubbo, New South Wales) for analysis.

Hormone analysis

Samples were analyzed via enzyme immunoassay (EIA), and all assays followed similar protocols (see below for substrate-specific details). Briefly, 96-well microtiter plates (Nunc MaxiSorp) were coated with 50 μl antibody solution and incubated overnight at 4 °C. Plates were washed to remove unbound antibody. Immediately after being washed, 50 μl of standard, control, or sample and 50 μl of horseradish peroxidase (HRP) conjugate were added to each well. After incubating for 2 h at room temperature, plates were washed and 100 μl of substrate solution (1.6 mM hydrogen peroxide, 0.4 mM azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) in 0.05 M citrate buffer, pH 4.0) was added to each well. The plates were read when the optical density of the maximum binding wells was >0.6 using a 405-nm measuring filter and 630-nm reference filter using an optical density plate reader (Dynex MRX Revelation, Dynex Technologies, Chantilly, VA). For each substrate, assays were biochemically validated for Asian elephants by demonstrating parallelism between a serially diluted sample pool and the standard curve. To monitor precision and reproducibility, low (70 % binding) and high (30 % binding) control samples were run on each plate. All samples were assayed in duplicate.

Serum Serum cortisol was quantified using a single-antibody cortisol EIA. The antibody (cortisol R4866) and corresponding HRP conjugate were obtained from C. Munro (University of California, Davis, CA). The cortisol antibody had the following cross-reactivities: cortisol 100 %, prednisolone 9.9 %, prednisone 6.3 %, cortisone 5 %, and <1 % with corticosterone, desoxycorticosterone, 21-desoxycortisone, testosterone, androstenedione, androsterone, and 11-desoxycortisol. Assay sensitivity was 0.08 ng/ml. Intra-assay coefficients of variation were 6.3 and 3.4 % (n=17) for low and high controls, respectively. The inter-assay coefficients of variation were 10.3 and 5.6 % (n=30), respectively.

Urine Urinary glucocorticoid metabolites (UGM) were quantified using the cortisol EIA described above. This assay has previously been validated for monitoring adrenocortical activity in Asian elephants via urine (Brown et al. 2010; also see Online Resource 1). Following Brown et al. (2010), urine samples were not extracted. To control for variation in urine concentration, UGM concentrations are expressed as nanograms per milliliter creatinine. Creatinine concentration was quantified using a modified Jaffe reaction (Narayanan and Appleton 1980; Monfort et al. 1990), which is based on a reaction in which creatinine turns orange in the presence of alkaline picrate. Briefly, 100 μl of standard, control, or diluted urine sample was added to a 96-well microtiter plate (Nunc MaxiSorp). Then, 50 μl each of NaOH (0.75 M) and picric acid (0.04 M) was added to each well. After 15 min, the plates were read at 405 nm using an optical density plate reader (Dynex MRX Revelation, Dynex Technologies, Chantilly, VA).

Feces Fecal samples were oven dried at ~60 °C overnight to control for variability in water content. Samples were then pounded and sifted to remove debris. Fecal steroid metabolites were extracted by adding 5 ml of 80 % methanol to 0.2 ±0.01 g of dried fecal material in glass scintillation vials. Vials were placed on a rotator overnight and then centrifuged for 15 min at 2,000 rpm. Supernatants were decanted to a clean vial and stored at −20 °C.

Fecal glucocorticoid metabolites (FGM) were quantified using a single-antibody corticosterone EIA which was previously validated for FGM in Asian elephants (Watson et al. 2013). A subset of samples was also analyzed using the cortisol EIA described above to assess assay performance. Although the two assays yielded similar profiles, the corticosterone EIA was more sensitive, demonstrated better parallelism with the standard curve, and yielded a stronger signal following relocation. The antibody (corticosterone CJM06) and corresponding HRP conjugate were obtained from C. Munro (University of California, Davis, CA). The corticosterone antibody had the following cross-reactivities: corticosterone 100 %, desoxycorticosterone 14 %, progesterone 2.7 %, and <0.1 % for tetrahydrocorticosterone, cortisol, cortisone, testosterone, and estradiol-17β. Assay sensitivity was 0.098 ng/ml. Intra-assay coefficients of variation were 12.1 and 4.2 % (n=16) for low and high controls, respectively. The inter-assay coefficients of variation were 13.4 and 5.9 % (n=25), respectively. Data are expressed as nanograms per gram dry fecal weight.

Keeper survey

Questionnaires were distributed to elephant keepers to assess the behavioral profile or temperament of individual elephants. Recent research in animal welfare has highlighted the value of subjective or qualitative assessments conducted by people who are familiar with the animal subjects (Whitham and Wielebnowski 2009). The insight provided by experienced keepers offers a unique dimension to this study.

The survey consisted of 13 questions/scenarios for which keepers were asked to score elephant behavior on a nine-point scale. Bipolar adjective pairs were used to anchor opposite ends of the scale (see Table 1; McCrae and Costa 1985; Rouff et al. 2005). The questions and adjective pairs were based on previous studies (Freeman et al. 2004; Whitham and Wielebnowski 2009; J. Whitham, personal communication). The survey also solicited information about the social relationships among elephants and individual responses to relocation. Between three and four elephant keepers at each institution (Melbourne Zoo and Taronga Zoo) were asked to independently complete the questionnaire for all elephants at their institution. All participants had at least 3 years of experience working with the elephants, and three keepers had traveled with the elephants from Thailand to Australia. Surveys were completed in 2011–2012 (5 years after the elephants arrived in Australia).

Data analysis

Data were analyzed using SAS v9.2 (Cary, NC, USA). Data were log transformed to meet assumptions of normality and homoscedasticity.

Average response to relocation We first examined the average response of all eight elephants to relocation from CKI to Australia. To examine changes in hormone concentrations over time, we calculated average glucocorticoid (GC) values over 4-week intervals for each individual and substrate. To provide a reference value (time 0) for monitoring changes in hormone levels, we averaged hormone values for the final 2 months on CKI. Although it is possible that the move from Thailand to CKI may have induced an adrenocortical response, we argue that this is still a useful reference point to evaluate value changes following transport to mainland Australia. By excluding the first 1.5 months on CKI, we avoid acute responses to transport. Furthermore, hormone values on CKI were similar to hormone levels at the end of the study. We ran a repeated-measures ANOVA to test whether 4-week mean GC values changed over time. A Dunnett's test was used to identify which time intervals were significantly different from time 0 (CKI). The model also included age and institution (Melbourne Zoo or Taronga Zoo). Individual was included as a random effect. Each substrate was run on a separate ANOVA.

Correlation between substrates It is difficult to correlate GC concentrations from different substrates on a point-by-point basis. Each substrate represents a different window of adrenocortical activity, and samples were not collected every day. Serum provides an immediate snapshot of circulating cortisol titers, urine provides a summary of the previous few hours, and feces provides a summary of adrenocortical activity 12–24 h prior to when the sample was collected (Wasser et al. 1996; Brown et al. 2010). Therefore, to compare the patterns of adrenocortical activity reflected in different substrates, we examined correlations at two levels.

First, we examined the correlation among substrates for each individual using averages across 2-week time intervals. We used a shorter time interval for this analysis to provide better resolution for estimating the relationship among substrates. Peak values (defined below) were excluded, since our interest was in general trends. Relationships were tested using Spearman rank correlations.

Second, we tested whether individual levels of adrenocortical activity ranked consistently across substrates (i.e., if an elephant had low FGM, did they also have low UGM and serum cortisol values). Again, we used the Spearman rank correlation and excluded peak values.

Individual variation in response to relocation The final objective of this study was to examine individual variation in adrenocortical activity and the relationship to elephant temperament/personality. To characterize individual patterns of hormone expression, we calculated baseline values for each individual for all three substrates. This was calculated through an iterative process excluding all points greater than

the mean+2.5 SD (Wielebnowski et al. 2002). For FGM, which was the only substrate for which post-transfer increases were consistently detected, we calculated an additional two summary statistics: (1) proportional increase in FGM post-transport and (2) duration of increase in FGM following relocation. Proportional increase in FGM was calculated by dividing the mean peak by the baseline. Mean peak was the average of all points exceeding the baseline+2.5 SD. To assess the duration of the post-transfer stress response, FGM concentrations were averaged over 4-week time intervals, similar to above. ANOVAs were conducted for each individual and pairwise comparisons were used to determine how long FGM concentrations remained significantly elevated above time 0 (CKI). Thus there were five hormone parameters for each elephant: baseline serum cortisol, baseline UGM, baseline FGM, magnitude FGM increase, and duration FGM increase.

To assess individual variation in elephant temperament, keepers were asked to score each elephant on 13 pairs of adjectives representing opposite ends of a continuum. Inter-rater reliability was assessed using Kendall's coefficient of concordance (W), which compares the ranking of subjects across raters (Wielebnowski et al. 2002; Freeman et al. 2010b). Since there were different groups of raters for each institution, W scores were assessed separately for each institution and then averaged (Table 1). The ten traits with W scores >0.5 were then used to examine the relationship between endocrinology and behavior.

Spearman rank correlations were used to examine the relationship between the five hormone variables and ten behavior traits described above. For the behavior variables, the average keeper score was used. Due to the small sample size, it was not possible to use multivariate statistics to examine these relationships.

Results

Average response to relocation

At the group level, "time since transfer" had a significant effect on mean GC values for all three substrates (Table 2 and Fig. 1). Neither age nor institution had a significant effect on measured GC concentrations for any of the substrates. Reported statistical results are based on 4-week GC averages, but results were qualitatively similar for shorter time intervals.

Since "time" had a significant effect on measured GC concentrations, we used the Dunnett's test to determine exactly which time intervals were different from time 0 (CKI; Fig. 1). Qualitative results were the same if we used the final time interval (i.e., 7 months after the elephants arrived in Australia) for the Dunnett's test. Although time had a significant effect on serum cortisol in the global model, the Dunnett's test indicated that none of the individual time intervals were significantly different from time 0. For FGM, concentrations remained significantly elevated for seven intervals (28 weeks) post-transport. Conversely, UGM were significantly *lower* for three intervals (12 weeks) post-transport. These trends were also apparent in individual plots of the raw data (Fig. 2; also see Online Resource 2).

Correlation between substrates

Within individuals, UGM and FGM were significantly negatively correlated for five of the eight elephants (range: $r=-0.47$ to -0.71, $P=0.01$ to 0.04). The three elephants for whom the relationship was not significant exhibited the weakest response to relocation. Serum cortisol did not exhibit a strong relationship with the other substrates in general. It was significantly positively correlated with UGM for only one individual ($r=0.51$, $P=0.03$) and negatively correlated with FGM for another individual ($r=-0.55$, $P=0.03$).

We did not find evidence that elephants were consistently ranked in the same order across substrates. If an elephant had high baseline serum cortisol relative to other elephants, they did not necessarily have high baseline UGM or FGM values. Spearman correlations for all three comparisons were not significant (serum/feces: $r=0.52$, $P=0.18$; urine/feces: $r=-0.02$, $P=0.96$; serum/urine: $r=-0.69$, $P=0.06$). Although the correlation between serum and urine is marginally significant, that result is driven by one elephant. The female with the highest baseline serum cortisol also had the lowest UGM. If she is excluded, the correlation is much weaker (serum/urine: $r=-0.54$, $P=0.22$).

Individual variation in response to relocation

Individual elephants exhibited variability in their adrenocortical response to relocation. Based on longitudinal profiles, baseline FGM values, and duration of increase in FGM, there appear to be two distinct patterns of adrenocortical activity among the female elephants (Fig. 3). Three females had higher baseline FGM values and more prolonged elevation of FGM post-transfer, while the remaining four females had lower baseline FGM values and shorter duration of stress response. Indeed, there was a significant positive correlation between baseline FGM and duration of increase in FGM (Spearman rho $\rho=0.81$, $P=0.014$). After the relocation, FGM concentrations remained elevated longer in individuals that had higher mean FGM concentrations. The one male fell in the middle of the distribution.

Several behavior traits were significantly correlated with at least one measure of adrenocortical activity (Table 1). Elephants that were scored as being curious had more prolonged increases in FGM following the relocation than timid elephants ($\rho=-0.72$, $P=0.046$; Fig. 4). Elephants that

Table 1 List of adjective pairs on keeper survey of elephant behavior/temperament. Keepers were asked to rank each individual on a scale of 1–9. Traits are listed in order of concordance among raters (Kendall's "*W*"). Traits that were significantly correlated with endocrine variables are indicated in the final column along with the directionality of the relationship (positive "+" or negative "−")

Situation/context	Adjective pair		*W* score	Endocrine correlation
	1	9		
Response to new objects or situations	Curious/inquisitive	Timid/cautious	0.94	Duration FGM increase (−)
Willingness to spend time with other elephants	Sociable	Reclusive	0.87	Magnitude FGM increase (+)
Speed at learning new tasks or associating certain events	Fast to learn/intelligent	Slow to learn	0.74	Baseline serum (+), baseline UGM (−)
Interaction with keeper	Affectionate	Aggressive	0.74	
Social placement	Subordinate	Dominant	0.73	
Response when presented with a challenge	Persistent	Easily discouraged	0.70	
Interaction with other elephants	Affectionate	Aggressive	0.65	Baseline UGM (−)
Frequency of stereotypic behavior	Never	Frequently	0.59	
Response to changes in the environment	High strung/excitable	Laid back/calm	0.58	
General activity level	Inactive/Lazy	Active/energetic	0.56	
Willingness to obey keeper commands	Obedient	Defiant	0.43[a]	
Reaction to disturbances	Relaxed	Skittish/nervous	0.36	
Readiness to investigate new things	Shy	Bold	0.18[a]	

[a] For these traits, low *W* scores reflect low variation among elephants and not low agreement among raters

were more reclusive exhibited a greater increase in FGM concentrations than more sociable elephants (ρ=0.71, P=0.047). Baseline serum cortisol values were lower in elephants that were fast at learning new (ρ=0.79, P=0.021). In contrast, baseline UGM values were *higher* in elephants that were fast at learning new tasks (ρ=−0.81, P=0.015). Baseline UGM were also higher in elephants that exhibited more affectionate/affiliative social interactions (ρ=−0.80, P=0.017).

Discussion

Relocation is a common management practice among both wild and captive animal populations. These events are associated with several potential stressors, including acute stressors (e.g., transport, exams, handling) and more long-term or chronic stressors (e.g., disruption of social dynamics and acclimation to a new environment). The goal of this study was to examine the cumulative physiological effects of these events. We found that patterns of adrenocortical activity in Asian elephants changed significantly following relocation. While the change in FGM was in the expected direction, the change in UGM was actually in the opposite direction. We did not detect a significant change in serum cortisol, partially because serum samples could not be collected immediately following the transfer for logistical reasons.

Similar to our findings, Laws et al. (2007) observed a threefold increase in FGM in a single male Asian elephant moved between institutions. However, monitoring only continued for 10 days post-transfer. Similar increases in FGM

Table 2 Effect of time since transfer, elephant age, and Australian institution (Melbourne Zoo or Taronga Zoo) on the levels of adrenocortical activity as measured in serum, urine (UGM), and feces (FGM)

	Serum cortisol	UGM	FGM
Time since transfer	$F_{10,\ 293}$=2.82	$F_{10,\ 406}$=7.75	$F_{10,\ 602}$=15.39
	P=0.002	P<0.001	P<0.001
Age	$F_{1,\ 293}$=0.33	$F_{1,\ 406}$=0.01	$F_{1,\ 602}$=0.14
	P=0.56	P=0.90	P=0.71
Institution	$F_{1,\ 293}$=1.57	$F_{1,\ 406}$=1.20	$F_{1,\ 602}$<0.01
	P=0.21	P=0.27	P=0.98

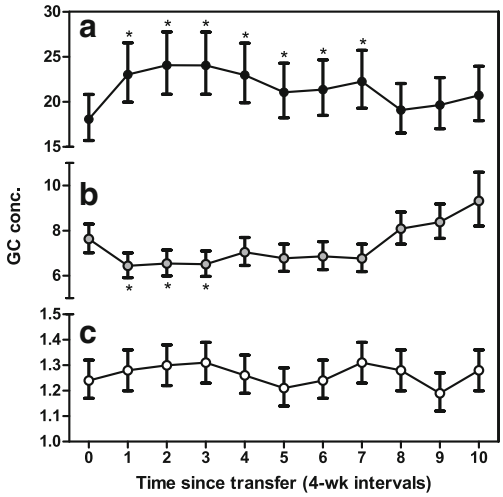

Fig. 1 Effect of relocation on average GC concentrations in three different substrates. **a** FGM (in nanograms per gram), **b** UGM (in nanograms per milliliter creatinine), and **c** serum cortisol (in nanograms per milliliter). Data represent back-transformed LS means±SE. Time 0 is an average of the final 2 months on CKI. Each subsequent *point* represents 4-week intervals. *Asterisks* indicate points that are significantly different from time 0

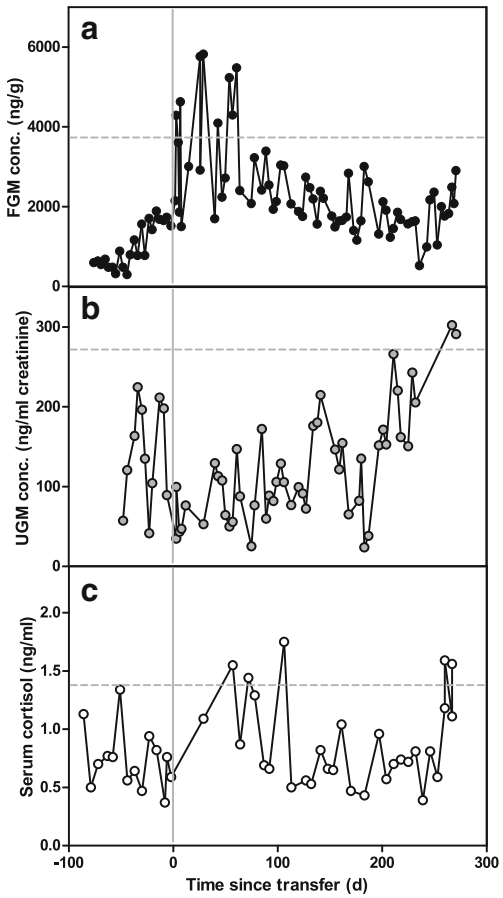

Fig. 2 Representative examples of longitudinal hormone profiles for one female: **a** FGM, **b** UGM, and **c** serum cortisol. *Vertical reference line* indicates when the elephant was transported from CKI to Australia. *Horizontal reference line* indicates cut-off point for baseline and peak samples

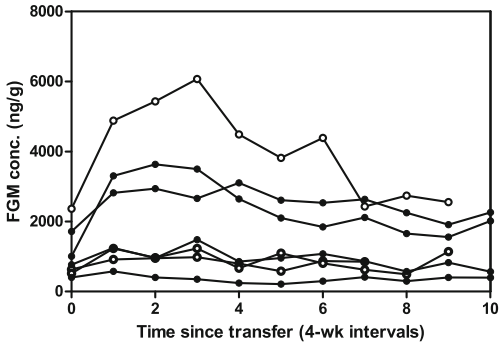

Fig. 3 Variation among female elephants in FGM concentration following relocation (*points* represent 4-week averages). *Open circles* indicate females housed at Melbourne Zoo and *closed circles* indicate females housed at Taronga Zoo

following translocation have also been observed in wild African elephants (Millspaugh et al. 2007; Viljoen et al. 2008), but FGM returned to baseline levels much sooner. In both cases, multiple animals were translocated from one area to another, with similar habitat characteristics. The extended period of elevated FGM detected in our study may possibly be a result of a change in housing conditions and social group dynamics. Captive elephants may also adapt and acclimatize to novel situations and changes in a different way than their wild counterparts. Interestingly, Schmid et al. (2001) did not find an increase in UGM in Asian elephants transferred to a new institution, which corresponds with our findings. They did, however, observe a transient increase in UGM in one of the two resident elephants that were monitored following the social introduction.

The three substrates (serum, feces, and urine) yielded very different signals of adrenocortical activity following the relocation. FGM increased and 95 % of the peaks in FGM values (defined as baseline+2.5 SD) occurred during

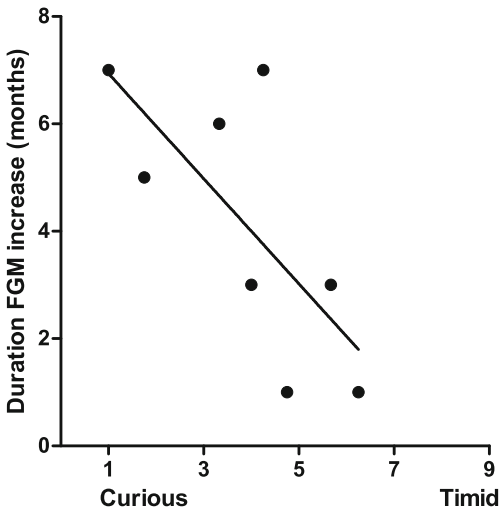

Fig. 4 Correlation between the duration of time that an individual's FGM concentrations remained significantly elevated post-transfer and average keeper score on a nine-point "curious–timid" continuum

the first 3 months post-transport. In contrast, UGM appeared to be suppressed and there was a high occurrence of minimum UGM values within the first 15 days post-transport. Both UGM and FGM have been biologically validated for Asian elephants. UGM show an increase following ACTH challenge (Brown et al. 1995) and parturition (Online Resource 1) and also exhibit expected circadian fluctuations (Brown et al. 2010). FGM increase following relocation (Laws et al. 2007, this study) and nearby construction (Watson et al. 2013).

If both UGM and FGM are valid measures of adrenocortical activity, why do they show opposite patterns in response to relocation? One possible explanation is that adrenal production of GCs may change depending on the individual's physiological state. The adrenal gland synthesizes several GC molecules, including cortisol, corticosterone, 11-deoxycortisol, and 11-deoxycorticosterone (Weiss et al. 1979; Koren et al. 2012). It is widely assumed that each species has one dominant GC (either cortisol or corticosterone) and that the non-dominant GCs are negligible. However, several studies have shown that the expression of GC molecules is independently regulated (Hancock 2010; Koren et al. 2012). Adrenal biosynthesis (and thus the dominant GC produced) changes in certain contexts. For example, in both domestic rabbits and short-beaked echidnas, exposure to a prolonged stressor causes a switch in the dominant adrenal product from corticosterone to cortisol (Kass et al. 1954; Weiss et al. 1979; Llano et al. 1982). This trend is similar (though less extreme) for tree shrews (Collins et al. 1984). Thus, the dominant GC under baseline and acute stress situations may be different compared to chronic stress situations. To the best of our knowledge, patterns of adrenal biosynthesis have not been examined in either Asian or African elephants.

This change in adrenal products could alter UGM and FGM signals because steroid molecules can be metabolized and excreted differently (Ziegler et al. 1989; Palme et al. 1996). If the "acute stress" GC is excreted in both urine and feces but the "chronic stress" GC is predominantly excreted in feces, that could explain why FGM and UGM are positively correlated during routine adrenal activity (e.g., circadian rhythm) and acute stress situations (e.g., ACTH injection), but negatively correlated in chronic stress situations (e.g., relocation). Given these findings, it may be possible to use the ratio between FGM and UGM as an index of the magnitude or severity of the stress response in elephants. Our sampling was not frequent enough to consistently calculate ratios, so we cannot address this question with our dataset. However, the ability to differentiate between "good" and "bad" stress could be a very useful tool for monitoring elephant well-being and merits further investigation. Future studies should continue to investigate the signals of adrenocortical activity yielded by different substrates and seek to understand why these signals may or may not correspond.

There is a rapidly growing body of evidence that individuals exhibit consistent individual differences in patterns of adrenocortical activity (Cook et al. 2011; Rensel and Schoech 2011; Narayan et al. 2013). Furthermore, these differences are often correlated with individual behavioral traits or personality types (Koolhaas et al. 1999; Overli et al. 2005; Carere et al. 2010; Koolhaas et al. 2010). Indeed, we found that an individual's physiological response to relocation was correlated with two behavioral traits. First, elephants that were scored as being more sociable exhibited a lower increase in FGM. This finding suggests that the stress of relocation may be minimized by selecting strong social units. Indeed, there is extensive evidence from psychological studies that stress responses can be modulated by social interactions (for review see Ozbay et al. 2007). Second, elephants that were more curious or inquisitive actually had longer post-transport increases in FGM than more timid or cautious elephants. This result is somewhat counter-intuitive given the number of studies that report bold/curious individuals typically have lower stress responses (Koolhaas et al. 1999; Overli et al. 2007; Carere et al. 2010; Koolhaas et al. 2010).

In addition to characteristics of the stress response, we also considered how baseline patterns of adrenal activity were correlated with behavioral traits. Elephants that were more aggressive towards conspecifics had lower levels of baseline UGM. This agrees with a recent study in African elephants, which found that aggressive elephants had lower levels of both serum and salivary cortisol (Grand et al. 2012). We also found that cognitive ability was correlated with adrenocortical activity, though the nature of the relationship depended on the substrate. Elephants that were fast learners had lower baseline serum cortisol values, but higher baseline UGM values. Other research suggests that animals with lower levels of adrenocortical activity perform better at cognitive tasks (for review see Overli et al. 2007). While our study provides an exploratory analysis of the relationship between behavior and hormone expression in Asian elephants, we suggest that a much larger study is needed to properly assess these relationships.

In conclusion, this study revealed that different substrates can yield drastically different signals of adrenocortical activity. Although serum, urine, and feces have all been validated as biologically relevant indicators of adrenocortical activity in Asian elephants, these three substrates produced much different results in our study, which may be due to changes in adrenal biosynthesis. For Asian elephants, we suggest that FGM provide the most useful measure of broad-scale changes in adrenocortical activity. FGM provide a broader summary of adrenocortical activity than either serum or urine, which helps increase the signal-to-noise ratio (Palme et al. 2003). In addition, FGM are less sensitive to pulsatile patterns of secretion and circadian fluctuations than the other substrates (Wasser et al. 2000; Brown et al.

2010; Sheriff et al. 2011). These results highlight the importance of understanding the signaling properties of different hormone monitoring techniques.

We also found that elephants exhibited two distinct patterns of adrenocortical activity (FGM) following relocation. Furthermore, differences in adrenocortical activity were associated with differences in elephant behavior/temperament. Our findings suggest that it may be important to consider individual personalities of elephants when making management decisions. However, it is important to note that these differences in adrenocortical activity do not necessarily reflect differences in the well-being of the elephants. Across taxa, animals have different ways of coping with stressors, and at this stage, we cannot say whether one coping style is "better." Further research is needed to understand how these individual differences in physiology and behavior affect an individual's well-being or whether we should tailor management practices to an elephant's personality.

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