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Research article

A preliminary analysis of the influence of handling method on adrenal activity in zoo African and Asian elephants

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Abstract
As a first step towards investigating the effect of management choice – free contact (FC) or protected contact (PC) – on zoo elephant well-being, this study evaluated serum cortisol concentrations in weekly samples collected over a 2-year period from 112 female elephants (58 African, 54 Asian) managed in either FC (n=58) or PC (n=54) management systems at 48 facilities. Results showed there were no differences in overall or baseline mean concentrations of serum cortisol between the two management systems. A GLM analysis exploring the response of individual baseline cortisol concentration to management (FC vs PC), facility, species, and the interaction of management and facility revealed that the only parameter with significant explanatory power was the facility where the elephants were housed. Thus, it may be more important to evaluate specific facility effects on adrenal activity, such as enclosure conditions, enrichment opportunities, or social interactions, rather than handling technique. Although many zoos are moving to a PC management approach, particularly within the American Zoo and Aquarium Association, from a welfare standpoint there is probably not a one-size-fits-all management strategy that is ideal. Rather, it may be necessary to consider individual elephant coping styles and social needs on a case by case basis before deciding whether FC or PC is most appropriate for management, especially when considering how to address welfare concerns.

Introduction

In recent years, questions have been raised about how different handling methods, specifically free contact (FC) versus protected contact (PC), affect the well-being of elephants in zoos. As defined by the Association of Zoos and Aquariums (AZA) Standards for Elephant Management and Care (2011), FC involves the direct handling of an elephant where the keeper and elephant share the same unrestricted space, whereas PC involves the handling of an elephant while the keeper and elephant do not share the same unrestricted space. Supporters of FC methods believe the positive interactions of hands-on training are an enriching experience, providing mental and physical stimulation for the elephants (Hediger 1955; Schmidt and Markowitz 1977; Molter 1980; Dudley 1986; Koehl 2000). Indeed, a study by Kastelein and Wiepkema (1988) found that training reduced stereotypic swimming in a captive Steller sea lion. Others state that elephants in FC are controlled using negative reinforcement and occasional harsh discipline so that handlers can maintain dominance (Leach 1992; Koontz and Roush 1996). Risk of injury to keepers may also be increased in dominance-based management systems (Chapple and Ridgeway 2001). However, if the relationship between keeper and elephant is good, use of the ankus as a guide only and/or verbal commands often suffice. Strong bonds between elephants and keepers in a FC system are common and viewed as positive experiences for both (Brown et al. 2008). However, there is disagreement about whether elephants in FC situations can be managed using positive reinforcement only (Brown et al. 2008).

Protected contact was first developed in 1989 to increase the safety of keepers and was initially used to handle bulls and aggressive cows (Desmond and Laule 1991). With PC, the handler does not enter the elephant's enclosure so there is no need for them to be dominant. This system relies on operant conditioning and positive reinforcement, and participation of the elephants is voluntary (Desmond and Laule 1991). This system is believed to offer a sense of security and safety for the elephant because it has control over its environment, something that in other captive mammals has been shown to reduce stress related pathologies (e.g. Weiss 1971; Weiss et al. 1981; Broom and Johnson 1993; Holmer 2003). Carlstead and

Shepherdson (2000) discuss a preliminary study that found salivary cortisol in elephants decreased using positive reinforcement in PC as compared to negative reinforcement in FC. However, disadvantages of the PC system include the keeper's inability to intervene during conflicts between herdmates, promote exercise, care for newborns or provide emergency veterinary care if needed.

To assess welfare in relation to environmental conditions in captivity, many studies measure glucocorticoids, a biological indicator of stress. Stress, marked by elevated cortisol, is a vital biological response allowing individuals to react to changes in their environment (Moberg 2000). However, when stress becomes chronic, it can lead to deleterious effects, or distress (Moberg 2000). Distress in captivity can arise when animals do not feel in control of their environment and cannot display coping behaviours such as hiding, huddling, or fleeing (Carlstead and Shepherdson 2000). For example, a study by Carlstead et al. (1993a) found that urinary cortisol concentrations were elevated in leopard cats (*Felis bengalensis*) when housed in a facility where they could hear and smell lions and tigers, two potential predators. That same study showed a decrease in urinary cortisol when leopard cats were moved to another building, and a further reduction occurred when the environment was enriched with hiding places and vegetation. Likewise, Moreira et al. (2007) reported an increase in faecal glucocorticoid concentrations when female tigrinas (*Leopardus tigrinus*) were moved from large enriched enclosures to small barren enclosures, and then decreased when the small enclosures were enriched with nest boxes and vegetation. Additionally, Wielebnowski et al. (2002) found that lack of vertical enclosure space, visual contact with predators, and being on exhibit were correlated with elevated faecal glucocorticoids in clouded leopards (*Neofelis nebulosa*).

To date no standardised studies have investigated the impact of handling methods on adrenal activity of elephants. Therefore, this study took the first step in assessing the impacts of management choice on zoo elephant well being through the evaluation of serum cortisol concentrations in elephants managed in either FC or PC systems across 48 facilities over a 2-year period. The goal of this study was to determine if the type of management system affected overall and baseline cortisol concentrations as an index of stress.

Methods

Animals and management data

This study was approved by the Smithsonian Conservation Biology Institute Animal Care and Use Committee. The type of management system used for elephants was obtained from surveys sent to all facilities in the North American Elephant Studbook as part of a reproductive assessment (Brown et al. 2004; Proctor et al. 2010a). Only non-pregnant females housed at facilities that did not have a change in handling method within the past year were considered for this study. A total of 112 female elephants (58 African, 54 Asian) at 48 facilities, managed in either free-contact (FC, n= 58) or protected-contact (PC, n=54) management systems were evaluated. Twenty-six facilities used FC while 22 facilities used PC.

Sample collection and hormone analysis

Morning blood samples were collected weekly over a 2-year period (104 samples per elephant) from either a caudal vein in the ear or the saphenous vein in the leg. All elephants were trained for blood collection as part of the normal, weekly husbandry routine. After centrifugation, serum was stored at -20° C and evaluated for cortisol using a radioimmunoassay (Cortisol, Coat-A-Count®; Seimens Medical Solutions Diagnostics, Los Angeles, CA) previously validated for elephants (Brown et al. 1995). The lowest kit standard (5.0 ng/mL) was diluted with zero standard to create a 2.5 mL standard that bound at approximately 90% of maximum binding, and was considered the assay sensitivity limit. Samples binding at >90% were assigned a value of 2.5 ng/mL (<2% of samples). Intra-assay and inter-assay coefficients of variation were 9% and 13%, respectively.

Statistical analyses

Baseline cortisol values were determined for each individual by iteratively excluding values that exceeded the mean plus 2 standard deviations (SD) until no values exceeding the mean + 2SD remained. Raw data were log transformed after testing for a normal distribution via Shapiro–Wilk. Raw data differed from a normal distribution before (P = 0.0001), but not after transformation (P = 0.1203). Summary statistics (mean baseline cortisol concentration, SEM) were calculated using raw data values and tested using t-tests, while a generalised linear mode (GLM) was conducted using normally distributed log-transformed values and a Gaussian probability distribution to evaluate the response of individual baseline cortisol to the following explanatory variables: management (FC vs PC), facility, species, and the interaction of management and facility. Reproductive status was not included as a model parameter because only non-pregnant females were included in the study and results from a previous study demonstrated that cortisol was not significantly different between cycling and noncycling female elephants (Proctor et al. 2010b). Additionally, mean baseline cortisol (±SEM) was calculated for each handling method, overall and by species. A two-tailed t-test was used to test for differences in mean cortisol concentrations between FC and PC. Summary statistics and the GLM were conducted using R (version 3.0.1; R Core Team 2013). Differences were considered significant at P<0.05.

Results

Summary statistics including the overall mean, SEM and range of cortisol concentrations with respect to handling method are presented in Table 1. Overall inter-individual variation varied across individual (t = 1.987, P = 0.0428); however, there was no difference in mean cortisol concentration between the two handling methods (t = 0.763, P = 0.447). Data were also analysed for African and Asian elephants separately, with no differences found in mean cortisol concentrations between the two handling methods for

Table 1. Summary of serum cortisol overall mean (± SEM) and mean range for elephants managed using either free-contact or protected-contact handling techniques.

Handling technique	n	Overall mean (ng/mL)	Overall mean range (ng/mL)
Free contact	58	17.22±1.19	4.39–52.89
African	15	15.79±0.88	4.39–26.72
Asian	43	21.05±2.08	4.89–52.89
Protected contact	54	16.1±0.85	5.96–30.05
African	43	16.22±1.00	5.96–30.05
Asian	11	15.61±1.45	8.85–26.04

Table 2. Parameter estimates from the generalised linear model evaluating the response of individual baseline cortisol to management (FC vs PC), facility, species, and the interaction of management and facility. An * denotes statistically significant explanatory variables.

Parameter	Estimate	Standard error	t-value	P
Intercept	3.282	0.336	9.770	<0.001*
Management	-0.307	0.184	-1.667	0.099
Facility	-0.024	0.014	-2.350	0.021*
Species	-0.095	0.104	-0.914	0.363
Management:facility	0.011	0.006	1.703	0.091

Residual deviance: 21.790 on 106 degrees of freedom

either African (t = -0.0321, P = 0.749) or Asian (t = 1.143, P = 0.265) elephants. The boxplots in Figure 1 further indicate no differences in baseline cortisol concentrations for African or Asian elephants managed using either FC or PC handling techniques, or for both species combined (t = 0.6434, P = 0.5215).

The results of the GLM exploring the response of individual baseline cortisol concentration to management (FC vs PC), facility, species, and the interaction of management and facility are presented in Table 2. A residual deviance of 21.790 on 106 degrees of freedom indicated a good fit of the model. The only parameter with significant explanatory power on individual baseline cortisol concentration was the facility where the elephants were housed. All other model parameters, including management method employed (FC or PC), were not significant in explaining variance of individual baseline cortisol.

Discussion

This preliminary study found that choice of management system, FC or PC, did not explain differences in individual mean or baseline cortisol concentrations. Thus, there was no evidence that a hands-off management system was any better or worse in relation to an effect on adrenal activity. Even when facility was accounted for by looking at the interaction of management and facility, management choice remained insignificant. Interestingly, when considered alone, the facility in which the elephants were housed was the only significant explanatory variable. Therefore, it may be more important to focus on the physical structure of the facility, enrichment opportunities or the availability of social interaction, rather than handling technique. Many studies assessing welfare in captivity have found distress to be correlated with environmental conditions, such as enclosure space (Wielebnowski et al. 2002; Moreira et al. 2007; Scarlata et al. 2012; He et al. 2014), availability of diverse structure and enrichment opportunities (Carlstead et al. 1993a; Wielebnowski et al. 2002; Resende at al. 2009; Scarlata et al. 2012; Eguizabal et al. 2013), presence of external stressors (Carlstead et al. 1993a; Wielebnowski et al. 2002; Chosy et al. 2014), and opportunities for social interaction (Novak and Suomi 1988).

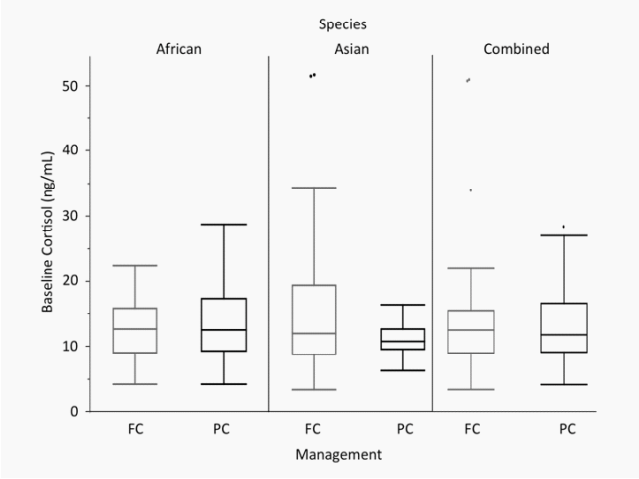

Figure 1. Boxplot showing baseline cortisol concentrations (ng/ml), including mean ±SEM, for elephants managed using either free-contact (FC) or protected-contact (PC) handling techniques. Data are presented for both species of elephants, Asian (FC, n=43; PC, n=11) and African (FC, n=15; PC, n=43), as well as a combined species comparison of FC (n=58) vs PC (n=54).

Another consideration is the elephant–keeper relationship, which may be a more important influence on the well-being of captive elephants than a particular handling technique. Indeed, it has been documented in both domestic and laboratory animals that the quality of caretaking is negatively correlated with glucocorticoid concentrations (Carlstead et al. 1993b; Pedersen 1994; Pedersen et al. 1998; Hemsworth and Barnett 2000) and an important factor for animal well-being (Mellen et al. 1998; Hemsworth and Barnett 2000). Mellen (1991) further found that a positive human–animal relationship was positively correlated with reproductive success in non-domestic cats. In a study by Wielebnowski et al. (2002), it was the amount of time a few primary keepers spent with individual clouded leopards, rather than the total number of keepers, that had a negative relationship with faecal glucocorticoid concentrations. A recent survey of 130 zoo professionals (Hosey and Melfi 2012) found that most indicated they had a bond with at least one of their animals, and identified benefits for both the animal (i.e. more calm or less stressed, enjoyed contact with keepers, seemed more content) and themselves (e.g. sense of well-being and work enjoyment, animal easier to handle and treat). Therefore, factors such as high-quality, consistent husbandry, low keeper turnover, and human animal bonding may do more to ameliorate the effects of captivity than using one particular handling technique over another. The finding by Gore et al. (2006) that 24% of elephant attacks were directed against keepers who were new, or former keepers that were visiting suggests the quality of the human–animal relationship is an important consideration, albeit a complex one (Waiblinger et al. 2006; Carlstead et al. 2009).

Although baseline cortisol values were used in this study due to the focus on the effects of long-term management conditions, there was a high degree of inter-individual variation in cortisol secretory patterns, possibly reflecting natural differences in coping responses within a population (Koolhaas et al. 1999). Typically, two coping phenotypes exit: proactive and reactive (Koolhaas et al. 1999). Proactive individuals are bolder, more defensive, and aggressive – they are less flexible to changing environments (Koolhass et al. 1999). Reactive individuals are more reserved, less aggressive, and tend to be more flexible (Koolhaas et al. 1999). Physiologically, proactive individuals have higher sympathetic reactivity, while reactive individuals have higher adrenal and parasympathetic reactivity (Koolhaas et al. 1999). The theory behind PC increasing captive elephant welfare is based on the premise that the animals will be less stressed with increased control over their environment. However, Carlstead and Shepherdson (2000) identified four additional environmental enrichment techniques that can assist in increasing captive well-being besides increasing an animal's control over its environment: 1) presenting cognitive challenges; 2) rewarding exploration with new and useful information; 3) meeting specific behavioural

needs such as stalking, hiding, or foraging; and 4) stimulating social interaction. For highly social and intelligent animals like elephants, stimulating social interactions and providing cognitive challenges may be equally important forms of environmental enrichment. Thus, the appropriate handling technique may well depend on how each elephant responds to different husbandry and management approaches, as has been suggested in the management of non-human primates, which also are highly social and intelligent animals (Novak and Suomi 1988). And while it may not be practical, optimal welfare may require taking into consideration what system (PC or FC) works best on an individual animal basis.

In conclusion, overall and baseline cortisol concentrations were not different between the two elephant handling methods: FC and PC. Caution is advised, however, in over-interpreting cortisol data because animals can become habituated to chronic stress, and increases in cortisol indicative of adrenal hypersensitivity may only occur in response to novel stressors (Moberg 2000). Also, chronic psychosocial stress can lead to attenuated cortisol responses to even acute stressors (Kristenson et al. 2004) due to the down-regulation of cortisol receptors in the hypothalamus and pituitary, inhibiting negative feedback mechanisms (Mendoza et al. 2000; Ganong 2003). Thus, before drawing the conclusion that neither FC nor PC has an impact on captive elephant well-being, additional evaluations should be conducted, such as analyses of catecholamines (Pervanidou 2008; Wang 1997), the neutrophil:lymphocyte (N:L) ratio (Davis et al. 2008), prolactin status (Sobrinho 2003), stereotypic behaviours (Friend and Parker 1999; Krawczel et al. 2005), and health status (Clubb and Mason 2002). Administering an ACTH challenge to measure acute cortisol responses in each individual might help determine if there are differences in individual adrenal functionality between management systems (Kirschbaum and Hellhammer 1989; Epel et al. 2000). Studies are needed to further explore the impact of additional environmental enrichment techniques on elephant well-being, such as social bonding opportunities, cognitive challenges, and allowing for natural foraging behaviours.

Since this study was initiated, the AZA has established a new policy that states 'elephant care providers at AZA facilities shall not share the same unrestricted space with elephants, except for the specific purposes of required health and welfare procedures, transport, research, active breeding and calf management programs, and medical treatments and testing' (http://www.animalpeoplenews.org/anp/2011/10/18/american-zoo-association-to-require-protected-contact-elephant-care/). The new policy will become part of the AZA accreditation standards for elephant management and care, and be in place by the end of 2014. However, non-AZA facilities are not bound by this policy and can continue to manage elephants in FC if desired. There is probably not a one-size-fits-all approach that can be applied to all elephants in the captive population. Rather, it may be more important from a welfare standpoint to consider the quality of keeper–animal interactions and individual elephant coping styles before deciding on a particular handling technique for an individual elephant.

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