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Welfare assessment of captive Asian elephants (Elephas maximus) and Indian rhinoceros (Rhinoceros unicornis) using salivary cortisol measurement

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Abstract

The measurement of salivary cortisol allows non-invasive assessment of welfare in captive animals. We utilised this technique to test the effect of zoo opening on six Asian elephants and two Indian rhinoceros at the Terra Natura Zoological Park, Alicante, Spain, during pre-opening, opening and post-opening periods. Salivary cortisol concentrations were found to be significantly higher during the opening period than during pre- and post-opening periods for both species. This method could prove a useful tool in monitoring the success of decisions taken to improve the welfare of captive animals.

Keywords: *animal welfare, Asian elephant, Indian rhinoceros, management, salivary cortisol, stress*

Introduction

One of the most important objectives of zoological parks is maintaining the psychological and physiological well-being of animals in their care. Changes in the animals' life such as acute environmental or social upheaval (eg introduction to novel environments and new group formation) can be extremely stressful (Soltis *et al* 2003). Although no widely-held definition exists, stress generally refers to a variety of responses to internal or external stimuli (stressors) that modify the homeostasis of an individual. These stimuli can be physical, physiological, behavioural or psychological factors (Dantzer & Mormede 1983; Stratakin & Chrousos 1995). Immediately following exposure to a stressor, activation of the hypothalamic-pituitary-adrenal axis occurs and glucocorticoids are released from the adrenal cortex (Munck *et al* 1984). Thus, corticosteroids and glucocorticoids are secreted in response to stress and these can provide information regarding the physiological well-being of animals (Whitten *et al* 1998).

Plasma cortisol has been used to evaluate animal stress responses to capture and translocation (Morton *et al* 1995; Denhard *et al* 2001), disturbances within their environment (Zoldag *et al* 1983; Powell *et al* 2006) or psychogenic stress (Haemisch 1990) but capture and handling, along with blood sample collection via venipuncture, can be stressors themselves, leading to increased plasma cortisol concentration (Cook *et al* 1973; Dawson & Howe 1983; Sabatino *et al* 1991; Whitten *et al* 1998; Schoech *et al* 1999; Romero & Romero 2002).

Non-invasive glucocorticoid collection techniques allow accurate monitoring of stress without the bias of capture-induced or disturbance-induced increases in cortisol levels (Hamilton & Weeks 1985; Harper & Austad 2000; Millspaugh *et al* 2001; Washburn & Millspaugh 2002). Measurement of glucocorticoids via analysis of faecal samples has been used to monitor stress responses in different species (Wasser *et al* 2000; Von der Ohe & Servheen 2002; Shepherdson *et al* 2004; McKenzie & Deane 2005; Lane 2006). Another such method is saliva collection which can be used with habituated or laboratory-housed animals. The use of salivary cortisol levels to monitor stress has been validated in humans (see Kirchbaum & Hellhammer 1994 for a review) and other species such as dogs (*Canis familiaris*) (Vincent & Michell 1992; Beerda *et al* 1996), rhesus monkeys (*Macaca mulatta*) (Boyce *et al* 1995), squirrel monkeys (*Saimiri sciureus*) (Fuchs *et al* 1997), and tree shrews (*Tupaia belangeri*) (Ohl *et al* 1999). This technique has also been used in Indian rhinoceros (*Rhinoceros unicornis*) (Kuckelkorn & Dathe 1990) and in Asian elephants (*Elephas maximus*) (Dathe *et al* 1992). Studies in pigs (*Sus scrofa*) have revealed that salivary cortisol is related to free plasma cortisol and represents approximately 10% of the concentration in plasma (Haeckel 1989; Parrot *et al* 1989). Saliva samples are easy to collect and store and sampling can be performed frequently, which is of particular importance as it allows acute stress to be monitored by measuring short-term changes in cortisol levels (Queyras & Carossi 2004).

In this study we used salivary cortisol to assess the degree to which zoo opening affected stress levels of Asian elephants and Indian rhinoceros and also sought to assess the viability of this method in terms of monitoring animal welfare in captivity and providing a tool for testing the success of management decisions designed specifically to improve or maintain welfare.

Materials and methods

Location

The study was conducted at Terra Natura Zoological Park, Alicante, Spain. Salivary cortisol concentrations were recorded from Asian elephants and Indian rhinoceros for three separate timeperiods: (1) pre-opening, ie during the month prior to opening (15th February–14th March 2005), at which time animals were confined to their cages and internal courtyards; (2) opening, ie when the zoo opened its doors for the very first time and throughout the month that followed (15th March–14th April 2005), during which time the animals spent their days in dry meadows and had direct visual contact with not only zoo visitors but also the final remnants of the construction work which had yet to be completed and (3) post-opening, ie the following month (15th April–14th May 2005), during which time animals were found in their prairies and were in direct visual contact with visitors. By this point the construction work, which had consisted of excavation, meadow and artificial lake construction, tree planting, power line, water and sewage line installation and the construction of ancillary buildings (eg stables, offices, restaurants, shops, etc), was completed.

Animals

Six adult female Asian elephants, ranging from 23 to 45 years of age and two juvenile female Indian rhinoceros aged three and five years, were studied. Three of the elephants (Tania, Jasmin and Motki) arrived at Terra Natura Zoological Park in August 2004 from Austria (Gänserndorf Safari Park) whilst the others (Petita, Baby and Kaisoso) arrived in December 2004, having previously resided at the Vergel Safari Park (Vergel, Alicante, Spain). The rhinoceros arrived in March 2003: Nisha from Stuttgart Zoo and Shiwa from Munich Zoo. At night, each rhinoceros was housed in an individual cage measuring 4 × 5 × 2.50 m (length × width × height) and during the day (0900 to 1800h), they ranged freely in a 2,000 m² meadow. One of the elephants (Baby) was housed individually in a cage measuring 5 × 6 × 3.50 m during the night due to conflict with other elephants, while the rest of the herd was housed in two cages measuring 5 × 8 × 3.50 m; one with a group of two individuals (Kaisoso and Petita) and the other with a group of three (Tania, Modki and Jasmin). During the day time was spent in three different 3,000 m² dry meadows. The opening period represented the first time these animals had encountered the meadows as they had been confined to the internal courtyards since arriving. Animals were fed on a diet of oats, branches, fruit and vegetables with *ad libitum* water. All the animals were trained and managed using free-contact by keepers with whom they became familiar. This made it possible to use saliva sampling as a non-invasive method for assessing animal welfare.

Sample collection

Saliva samples were taken on a daily basis at 0730h during February, March, April and May 2005. Samples were always collected at this time to avoid variations in saliva cortisol concentration, ie significantly large variation in concentrations can be seen during early morning, within relatively short time intervals. Samples were collected using the Salivette® kit (Sarstedt, AG & Co, Nümbrecht, Germany) and centrifuged at 2,000 rpm for two minutes at 15°C. The eluted saliva was stored at –20°C until assaying.

Hormone analysis

Salivary cortisol was measured in duplicate, using a modification of the solid-phase RIA (Coat-A-Count®, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) and the tubes were counted on a Packard Cobra Auto Gamma Counter (Auto-Gamma® 5000 series, Cobra 5005, Packard Instruments Company, Meridien, CT, USA). A minimum of 0.4 ml of saliva was required for the duplicate assay. Modifications were made in the form of increasing the volume of the sample to 200 μl, in the standard curve, through 1:10 dilutions of the standards and increasing the incubation period to 24 h to increase assay sensitivity (López-Mondejar *et al* 2006).

Assay validation

The saliva cortisol assays of the Asian elephants and Indian Rhinoceros were validated through demonstration of the correlation between serial dilutions of pooled saliva and the standard curve (Meyer *et al* 2004). Analytical sensitivity, or minimum detection limit, was calculated by interpolation of the mean minus two standard deviations of ten replicates of the zero calibrator. Assay precision was assessed by calculating intra- and inter-assay coefficients of variation (CVs) of the percentage bound of the internal controls. Specificity was extracted from the information supplied by the manufacturer's kit.

Statistical analysis

Data were analysed by repeated measures analysis of variance (ANOVA), using the cortisol concentration data obtained from each individual in order to test for differences between individual elephants and periods. The same procedure was carried out for rhinoceros. The Huynh and Feldt correction was used in Mauchly's Test of Sphericity in elephant analysis. The Bonferroni multiple comparison test was used to test for pairwise differences in mean cortisol values among pre-opening, opening and post-opening periods, for both species. Statistical analyses were conducted using SPSS, version 13.0.

Results

Assay validation

The dilution curve for the pooled Indian rhinoceros saliva correlated positively with that of the Asian elephants ($r = 0.997$, df = 6, $P < 0.001$). The dilution curve for the pooled saliva correlated positively with the standard curve for the rhinoceros ($r = 0.962$, df = 6, $P < 0.001$), and the elephants ($r = 0.946$, df = 6, $P < 0.001$).

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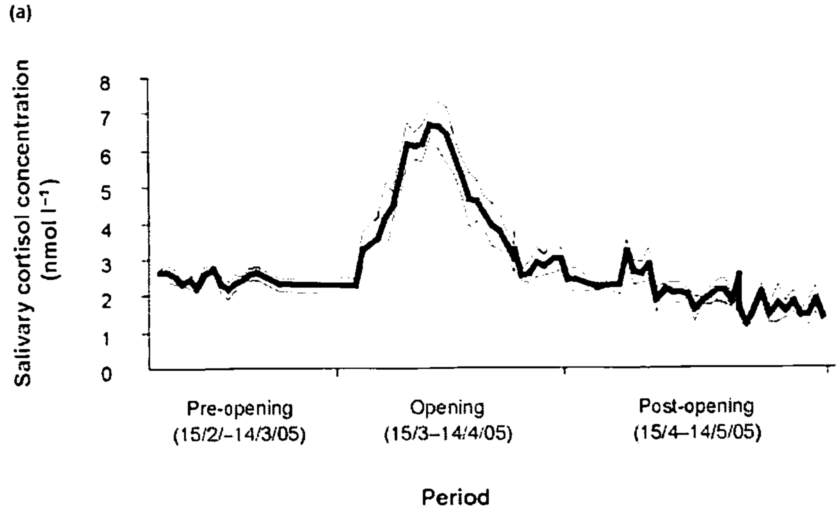
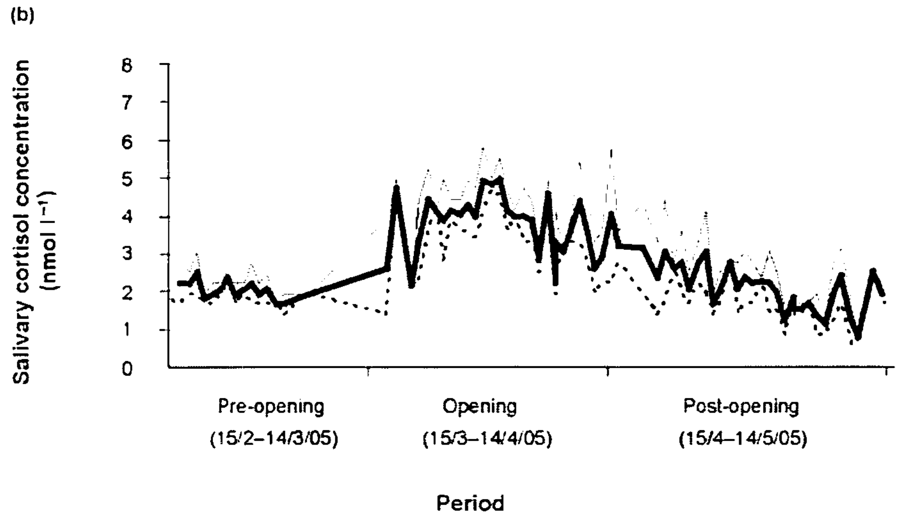
Figure 1

Mean salivary cortisol concentration during pre-opening, opening and post-opening period in (a) Asian elephants.



Mean salivary cortisol concentration during pre-opening, opening and post-opening period in (b) Indian rhinoceros.



Sensitivity was 0.82 nmol l^{-1} of cortisol, intra- and inter-assay coefficients of variation were 8% (n = 10) for Asian elephants and 5.8% (n = 10) for Indian rhinoceros. The cortisol antibody crossreactivity was: 100% with cortisol, 11.4% with 11-deoxycortisol, 2.3% with prednisone, 1% with corticosterone and cortisone, < 1% with aldosterone, 11-deoxycorticosterone, dexamethasone, oestriol, oestrone, flumethasone, methotrexate, pregnenolone, progesterone, tetrahydrocortisol and triamcinolone.

Hormone analysis

The mean concentration of salivary cortisol in both elephants and rhinoceros was increased during the opening period, compared to pre- and post-opening periods (Figure 1). Table 1 shows that for both species the lowest concentration of salivary cortisol was obtained during the post-opening period.

Repeated measures ANOVA revealed significant changes in the cortisol concentration in Asian elephants ($F_{9.203, 377.341}$ = 5.964, $P < 0.001$) and Indian rhinoceros ($F_{2, 82}$ = 4.694, P = 0.012) between the pre-opening, opening and post-opening periods. Bonferroni multiple comparison tests (Figure 2) revealed that cortisol concentrations during the opening period were significantly higher than during pre- and post-opening periods for both species, but no significant differences were seen between pre- and post-opening periods' cortisol concentration for both species.

Discussion

Glucocorticoids reflect physical and behavioural stress situations and their concentration in blood plasma is quantitatively related to the degree of stress (Dathe *et al* 1992). Saliva sampling is a preferred, non-invasive means of assessing free

Table 1 Means of salivary cortisol of all Asian elephant and Indian rhinoceros individuals during the zoo pre-opening, opening and post-opening periods.

Species	Animal	Period	Mean (nmol l^{-1})	SD	n
Elephas maximus	Baby	Pre-opening	2.49	0.39	27
		Opening	4.59	1.78	29
		Post-opening	1.97	0.87	29
		Total	3.03	1.64	85
	Jasmin	Pre-opening	2.36	0.50	27
		Opening	3.75	1.57	29
		Post-opening	1.78	0.64	29
		Total	2.64	1.32	85
	Kaisoso	Pre-opening	2.68	0.65	27
		Opening	4.95	1.90	29
		Post-opening	1.73	0.92	29
		Total	3.13	1.87	85
	Modki	Pre-opening	2.33	0.37	27
		Opening	4.17	2.15	29
		Post-opening	1.85	0.62	29
		Total	2.80	1.65	85
	Petita	Pre-opening	2.71	0.57	27
		Opening	4.35	1.76	29
		Post-opening	2.08	0.68	29
		Total	3.06	1.49	85
	Tania	Pre-opening	2.17	0.39	27
		Opening	3.32	1.38	29
		Post-opening	2.26	0.80	29
		Total	2.59	1.08	85
	Total	Pre-opening	2.46	0.49	189
		Opening	4.26	1.77	196
		Post-opening	1.96	0.73	210
		Total	2.87	1.51	595
Rhinoceros unicornis	Nisha	Pre-opening	2.15	0.59	27
		Opening	4.18	1.02	29
		Post-opening	2.36	0.99	29
		Total	2.91	1.28	85
	Shiwa	Pre-opening	2.24	0.59	27
		Opening	3.47	0.89	29
		Post-opening	1.71	0.45	29
		Total	2.48	1.00	85
	Total	Pre-opening	2.22	0.49	54
		Opening	3.69	0.80	56
		Post-opening	1.89	0.56	60
		Total	2.59	1.00	170

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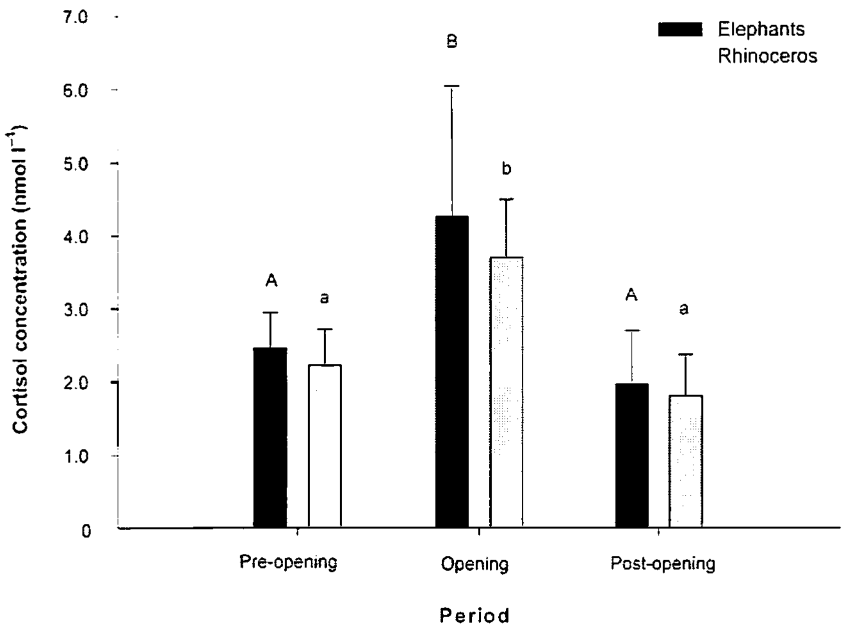
Figure 2

Vertical bars indicate means of cortisol concentration during pre-opening, opening and post-opening period. Bars with the same letter do not differ significantly.



cortisol concentration (Boyce *et al* 1995; Tiefenbacher *et al* 2003) because, depending on the collection method used, it is less stressful to animals than collecting blood samples (Cross *et al* 2004). Salivary cortisol concentration can be used for assessing plasma cortisol concentration since it accurately reflects the concentration of the biologically-active free fraction of the hormone in plasma (Haeckel 1989) and represents approximately 10% of the concentration in plasma (Haeckel 1989; Parrot *et al* 1989).

Our aim in this study was to assess a potential tool for indicating the welfare of animals during zoo openings. This was carried out through measurement of salivary cortisol concentrations in Asian elephants and Indian rhinoceros. This non-invasive method of examining stress responses in these species was selected because these animals were familiarised with their keepers and trained and managed using free-contact.

One question that needed to be addressed was the amount of cortisol concentration that could be attributed to stress-induction in the animal. The term 'stress' is generally considered to be a reference to the negative consequences of an animal's failure to cope with factors in its environment (Wielebnowski *et al* 2002) and increases in cortisol concentration are normally established as a negative experience (Norcross & Newman 1999; Cross *et al* 2004; Dehnhard 2007). That said, not all stressors have negative impacts on animal health (Moodie & Chamove 1990) and, in certain circumstances, stress is a necessity in ensuring survival and allowing adaptation to environmental change (Wielebnowski *et al* 2002).

So-called 'negative stress' can be identified when animals appear to incur biological costs such as impaired reproduction (Swaisgood 2007) or immune function (Siegel *et al* 1987; Herbert & Cohen 1993) from responding to stressors (Moberg 2000). Due to the limitations imposed by a study only lasting three months, we were unable to confirm whether or not the cortisol concentrations obtained in these three periods could have biological costs. Thus, in order to determine which concentration represented 'negative stress', it was necessary to assess the normal range of cortisol concentration for these species; not only to establish a baseline range of salivary cortisol to detect deviations which we could attribute to stressful situations, but to also monitor their evolution in individuals and in groups (Dathe *et al* 1992). Furthermore, in mammals, the production of basal cortisol generally peaks at the beginning of daily activity, which can be explained by a circadian rhythmicity that has evolved to help the organism predict daily environmental changes, such as food seeking and consumption (Moore-Ede & Sulzman 1977). To ensure results were not influenced by this circadian rhythmicity, samples were always collected at the same time (0730h).

Once the normal cortisol concentration range of both species had been established, we observed that during the opening period, salivary cortisol concentrations of both species increased significantly, compared to pre- and post-opening periods. We considered these higher values to be 'negative stress' and they may have been attributable to the continuation of building work, causing increased disturbance during this period. Some studies have documented negative responses of giant pandas (*Ailuropoda melanoleuca*) to construction noise (Powell *et al* 2006). In addition, during this opening period, the animals were introduced into their dry meadows for the first time whereas previously they had been confined to their cages and internal courtyards. Furthermore, during the opening period, animals had their first direct visual contact with visitors that year. Several studies have shown correlations between increased cortisol concentration in animals and zoo visitors (Glatston *et al* 1984; Thompson 1989; Carlstead *et al* 1999) as well as with changes in animals' enclosures (Ohl *et al* 1999; Cross *et al* 2004).

After the opening period, salivary cortisol values decreased, due to possible habituation to their new meadows and visitors, during the month following the opening. Furthermore, Bonferroni multiple comparison tests reveal that cortisol levels were slightly lower during the post-opening period than during the pre-opening period. This suggests animal installation conditions improved after the zoo opening.

Moreover, we found high inter-individual variation in salivary cortisol concentrations which reflects an individual's ability to cope with the captive situation (Zayan 1991; Carlstead & Shepherdson 2000). An intra-specific metabolic variation has been noted in other species, such as cattle and other ruminants (Palme *et al* 1999, 2000; Morrow *et al* 2002), elephants (Stead *et al* 2000), rhinoceros (Brown *et al* 2001), clouded leopard (Wielebnowski *et al* 2002) and other mammals and avian species (Wasser *et al* 2000). In spite of these inter-individual differences, it was observed in all individuals that higher cortisol concentration values were found in the opening period; this time being particularly stressful for all animals concerned.

Some studies have documented positive responses to environmental enrichment (Wells & Hepper 2000; Wells 2004, 2005; Graham *et al* 2005; Mallapur *et al* 2005; Wells *et al* 2007) and cortisol concentration could be used to test the success of these enrichment techniques (Schapiro *et al* 1993; Carlstead & Shepherdson 2000; de Groot *et al* 2000; Ernst *et al* 2006). We propose that this non-invasive method of measuring salivary cortisol in Asian elephants and Indian rhinoceros could be a useful tool for zoo management and an objective measure to test environmental enrichment techniques and improve animal welfare. This method could be equally applicable to other captive animals which are trained for this technique and have direct contact with keepers.

Conclusions

The findings of this study indicate that measurement of salivary cortisol concentrations could be a useful tool for assessing animal welfare and for testing the suitability of the captive environment.

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