JZAR JOURNAL OF ZOO AND AQUARIUM RESEARCH
www.jzar.org

Research article

An examination of salivary cortisol concentrations and behaviour in three African elephants *Loxodonta africana* at Zoo Atlanta

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Keywords: captive elephant, non-invasive, stereotypic behaviour, welfare

Article history:
Received: 11 Nov 2022
Accepted: 21 Mar 2024
Published online: 30 Apr 2024

Abstract
Salivary cortisol assay is an effective method to quantify free cortisol levels, track diurnal patterns and measure acute hypothalamus-pituitary-adrenal activation in response to acute stressors. This study examined salivary cortisol and behaviour in three African elephants *Loxodonta africana*. Salivary cortisol was within normal ranges for this species, declined across the day and responded to a mild social stressor. The relationship between salivary cortisol and stereotypic swaying in two of the elephants was also examined. Swaying was significantly associated with decreased cortisol values in one of the two elephants, indicating this stereotypy may function to reduce arousal, but also emphasising the complicated relationship between physiology and behaviour. This is the first study to demonstrate that swaying reduces salivary cortisol concentrations in some elephants. The opportunities this finding presents for future research and the complex relationship between physiology and behaviour are discussed.

Introduction

Accredited zoological institutions increasingly emphasise the importance of welfare assessment and enhancement (Maple and Perdue 2013). Animal welfare needs can vary widely by species as well as by individual, making this task exceedingly challenging. Thus, welfare must be examined as an objective quality measurable through various indicators (Broom 2001; Veasey 2017), including physiological measures, presence of species-typical behaviour and/or absence of stereotypic behaviours (Bettinger and Laudenslager 1998; Brown et al. 2008; Kirschbaum and Hellhammer 2000; Mason and Veasey 2010a;Veasey 2019). Given that each measure has inherent limitations, many researchers suggest using "multiple, complementary, well-chosen indices" (Mason and Veasey 2010b, p. 251).

Numerous studies have been conducted on a wide array of species using one or a combination of these indicators to assess welfare (e.g. Clark et al. 2012; Izzo et al. 2011; Warwick et al. 2013). Although these indicators are often used individually, it is generally recommended that they not be used as sole indicators because of the complexity of the relationships among behaviour, physiology and other welfare measures. For example, there is not a straightforward relationship between stereotypy and welfare and it does not appear that greater stereotypy rates always indicate worse welfare (Mason 1991a). The behavioural and physiological responses of animals can be viewed as an attempt to cope with current and future welfare challenges (Hill and Broom 2009). Stereotypies frequently become independent of the initial eliciting situation, which obscures the connection between stereotypy performance and welfare. However, animals that perform stereotypies should be considered at risk for welfare decrements (Swaisgood and Shepherdson 2005), mainly because of the consequences

associated with stereotypic behaviour, such as excessive wear to foot pads or skin irritation from overgrooming.

Perceived physical and psychological demands faced by animals, often labelled stress, lead to a rapid and specific reaction of the hypothalamus-pituitary-adrenal (HPA) axis (Kirschbaum and Hellhammer 2000). This reaction results in the release of cortisol, a hormone produced by the HPA axis. The production of cortisol in reaction to environmental challenges has resulted in the use of cortisol as a physiological marker of wellbeing (Bettinger and Laudenslager 1998). However, there are some complications to using cortisol as a marker of stress. Namely, activation of the HPA axis can be triggered by a variety of demands, including beneficial ones such as enrichment or training (Hambrecht et al. 2021; Williams et al. 2018). The relationship of cortisol to welfare is also complex because chronic stress can depress basal cortisol (Brown et al. 2008) and cortisol can return to basal levels quickly after a stressor (Crockett et al. 1993), especially for individuals able to cope with challenges behaviourally. Cortisol responses can vary greatly by individual and cortisol responses to environmental demands may reflect coping (Carlstead et al. 1992; Williams et al. 2018). In fact, stereotypies have been shown to reduce arousal and signs of stress in correlational studies (Bettinger et al. 1997; Briefer Freymond et al. 2020; Mason 1991a, b; Wiepkema et al. 1987) and prevention of stereotypies has been associated with increased corticosteroid levels (Dantzer and Mormede 1983; Kennes et al. 1988). Therefore, it is crucial to examine the many inter-relationships of the multiple indicators of welfare and not rely solely on one measure.

The current study explores elephant welfare using behavioural and physiological measures, specifically examining the relationship between salivary cortisol and stereotypic swaying. Among other topics, previous studies of elephant behaviour have investigated species-typical and stereotypic behaviours, finding a wide range of behavioural rates with the majority of time often spent feeding (Greco et al. 2016). Measures of the percentage of time spent feeding in a zoo setting have ranged from as low as 25% (Clubb and Mason 2002) to 75% or greater (Finch et al. 2020; Wilson et al. 2001) which is equivalent to rates in wild elephants (Wyatt and Eltringham 1974), demonstrating that there is considerable variation among individuals in human care. Overall, research on captive elephants has elucidated that species-typical behaviours relate to food consumption, social interactions and cognitive challenges (Veasey 2019). Stereotypic behaviour in zoo elephants has been documented repeatedly (e.g. Greco et al. 2017; Mason and Veasey 2010a; Rees 2009; Wilson et al. 2004). The predominant stereotypic behaviour displayed by elephants is swaying—a repetitive sideways body movement in which the elephant shifts weight from one side to another. Repetitive swaying may result from previous or current intensive housing systems, such as chaining as a form of restraint (Kurt and Garaï 2001) to prevent overnight aggression. Swaying in elephants may also be related to arousal from unsatisfied appetitive behaviour or anticipation of a variety of predictable events (Kurt and Garaï 2001; Rees 2004; Wilson et al. 2004).

However, others have suggested that stereotypies are associated with cases of under-stimulation, predominantly in restricted or barren environments, and a need for greater stimulation to attain optimal arousal levels (Broom 1983; Mason 1991a). Wemelsfelder (1984) noted that it is often difficult to ascertain if a behaviour arises from boredom or frustration but it is important to examine physiology to determine deviations from homeostasis and adaptation to the environment. Therefore, additional research is required to understand how stereotypic behaviours influence the welfare of elephants and how elephant management affects both stereotypic behaviour and welfare.

Cortisol has been validated and used as an indicator of welfare in elephants (Grand et al. 2012). For example, salivary cortisol morning values have been positively correlated with personality ratings of fearfulness in African elephants (Grand et al. 2012). Previous studies of cortisol or its metabolites in elephants have found increases above baseline after an introduction to new conspecifics (salivary: Dathe et al. 1992; urinary: Schmid et al. 2001), relocation (faecal: Laws et al. 2007), a change in chaining procedures (salivary: Dathe et al. 1992) and the opening of a new exhibit to the public (salivary: Menargues et al. 2008). However, no increases in cortisol were found following flooring renovations (serum: Boyle et al. 2015) or with increases in self-directed behaviour after potentially stressful events, such as close-contact tourist interactions (faecal: Manning et al. 2022). Several of these studies examining cortisol or its metabolites (faecal: Laws et al. 2007; urinary: Schmid et al. 2001) also observed behavioural changes following changes such as reintroduction, which when combined with cortisol increases likely indicated decreased welfare.

Wilson et al. (2004) examined the association between behavioural indicators of stress and serum cortisol in the same three elephants that were included in the current study. Swaying was more prevalent before shifting into the barn at night and gaining access to the evening meal. Other research has found similar trends (Kurt and Garai 2001; Rees 2004), indicating that swaying may be related to arousal from frustrated appetitive behaviour or anticipation of a variety of predictable events. It has been suggested that stereotypic behaviours such as swaying may function to reduce mean cortisol values (Bettinger et al. 1997; Manning et al. 2022), suggesting that frequent swaying and lower serum cortisol values should be associated. There were individual differences in mean serum cortisol values found by Wilson et al. (2004) but those values did not correspond with the amount of stereotypic swaying.

The main goal of the current case study was to establish individualised baseline values of salivary cortisol in three African elephants *Loxodonta africana* and use that information to examine the relationship between cortisol and behaviour and to determine how swaying relates to welfare. This study sought to determine how swaying affects salivary cortisol concentrations to provide a better understanding of the function of swaying and its relation to elephant welfare. It has been hypothesised that stereotypic swaying would lead to a decrease in arousal as measured through cortisol (Wemelsfelder 1984). Salivary cortisol was measured because it has the following advantages over other measures of cortisol: less invasive (Kobelt et al. 2003), allows tracking of diurnal patterns and allows measurement of acute stress reactions (Kuhar et al. 2005), which can be detrimental to welfare (Brown et al. 2008). Changes in salivary cortisol values over the day were examined, including assessing how a mild social stressor, namely being put in a stall with a dominant conspecific for fifteen minutes, affects salivary cortisol. Behavioural data were collected to quantify swaying in these individuals and confirm the anecdotal circular dominance used for the mild stressor.

Methods

Subjects and housing

The subjects of this study were three wild-born, adult female African elephants *Loxodonta africana*: North American Elephant Studbook numbers 227 (born ~1983, hereafter Kelly), 220 (~1982, hereafter Dottie) and 221 (~1982, hereafter Tara), who resided at Zoo Atlanta beginning in 1986. Data were collected March 2007–July 2008; thus, all elephants were young adults between approximately 24 and 26 during the study. These elephants were also the subjects of Wilson et al. (2004). They were chained nightly until 1989 when the practice was terminated. For a full description

of their housing, see Brockett et al. (1999). Zoo staff were present from approximately 0700 to 1800 and elephants were in their naturalistic habitat for public viewing from approximately 0800 to 1730 every day. The outdoor portion was 1,373 m^2 in size and consisted of a pool, shade structure, mud wallow and logs for scratching and tusking. Additionally, the exhibit included a barn, which was divided into an area accessible to visitors of 336 m^2 and another room of 164 m^2 which housed the elephant restraint device, and an outdoor paddock of 541 m^2 . Elephants were sometimes brought into the barn during the day for habitat cleaning, demonstrations or training but were mostly in the outdoor portion. The Institutional Animal Care and Use Committee (IACUC) of Georgia Institute of Technology and Zoo Atlanta personnel approved this study.

General behavioural data

Continuous focal animal sampling (Altmann 1974) was used to collect behavioural data (see Supplementary Information for ethogram) using Observer® software on a personal digital assistant. A total of 30 hours of data were collected per elephant. Fifteen hours of behavioural data were collected in 30-minute sessions balanced across 0815–0945 (AM), 1130–1300 (noon) and 1600–1730 (PM), with 10 observations per time period. These periods of day were matched to those included in Wilson et al. (2004). Additional behavioural data were collected outside of those times periods for an additional 15 morning and 15 afternoon 30-minute observations per elephant. The ethogram used was adapted from Wilson et al. (2004). It included solitary, stereotypic, affiliative social and agonistic social behaviours.

Salivary cortisol

Salivary cortisol was collected in a baseline period and during a variety of behavioural and husbandry events to establish a range of cortisol measures for each elephant, separately from the behavioural observations. All samples were taken between 0715 and 1745—AM samples were collected between 0715 and 1150 and PM samples between 1200 and 1745. During the baseline phase, saliva samples were taken every other hour from 0730 to 1730 for three separate days. A tandem serum sample was also taken for three of the saliva samples to allow for correlation with saliva samples to biologically validate the salivary assay test.

Thereafter, other than the social stressor described below, saliva samples were taken based on planned husbandry events, such as training (maintenance and novel, 12 samples for each training type per elephant although one from Dottie's novel training was not of sufficient volume) or enrichment (routinely provisioned food puzzles, 18 per elephant). A maximum of two samples was collected per elephant per day. A total of 202 samples were collected: 106 from Kelly (69 AM and 37 PM), 68 from Dottie (49 AM and 19 PM) and 96 from Tara (62 AM and 34 PM). Saliva samples were collected using a standard Salivette® swab placed caudally in the mouth using haemostats. The elephants had been previously taught to open their mouths on cue and this training was modified for ease of sampling. All samples were collected quickly, generally within one to two minutes, but always within five minutes from initiation of the collection procedure to minimise cortisol changes associated with keeper interactions and the resulting HPA activation.

Mild social stressor

To examine salivary cortisol responses to a mild social stressor, each elephant was placed in a stall (164 m^2, Brockett et al. 1999) with its dominant conspecific for fifteen minutes. For these individuals dominance was circular, based on anecdotal observations by elephant keepers and managers: Tara was subordinate to Kelly, Kelly was subordinate to Dottie and Dottie was subordinate to Tara. Thus, the pairings for the social stressor sessions were Kelly and Tara, Dottie and Kelly and Tara and Dottie. These challenges took place in the morning, beginning at 1045–1050. Samples were collected at the beginning and end of the 15-minute mild social stressor condition as well as 15 and 30 minutes after release, for a total of four samples. Individuals were observed for signs of aggression and testing would have been discontinued if aggression had occurred.

Swaying samples

Only two elephants exhibited stereotypic swaying behaviour during this study: Kelly and Tara. Kelly's swaying bouts were more frequent and of longer duration than those of Tara, who often walked away after only a few minutes of swaying. For Kelly, swaying bouts were interrupted, meaning that she was called over by care staff, and five samples each were collected at 1, 5, 10, 15 and 30 minutes into the bout, for a total of 25 separate interrupted bouts. Tara swayed when she was alone in her stall or alone in the yard, but these bouts were infrequent and short, so collection continued until five samples each were collected at 1, 5 and 10 minutes into a swaying bout, for a total of 15 separate interrupted bouts.

Sample processing

Saliva samples were refrigerated after collection, centrifuged at 2000 g for two minutes (Lamey and Nolan 1994) and then stored in O-ring sealed tubes at −20°C until they were shipped on dry ice for processing. Cortisol was measured by a commercial salivary cortisol enzyme immunoassay with a detection limit of 0.003 µg/dl (Salimetrics Assay 1-3002) according to the manufacturer's directions. Any values below detection were reported as 0.003 based on the test sensitivity (n=25). Intra-assay CV for elephant saliva was 1.25%. Serial dilution of elephant saliva indicated acceptable parallelism given that the standard curve and the homogeneity of regression value was not significant, demonstrating that the slopes of the two regression lines are not different ($F=0.501$, $P=0.485$, $n=16$), with serial dilutions spiked to create concentrations between 0.3 and 3.0 µg/dl demonstrating an average spiking recovery of 92.6±6.3%.

Data analysis

Behavioural data were not normally distributed, thus nonparametric analyses were used. Mann-Whitney U tests were performed to determine any behavioural differences between elephants. Behaviours tested included swaying, consuming and time spent proximate. Means and medians were used to determine how frequently the elephants swayed. Additionally, Mann-Whitney U tests examined the relationships between elephants to validate anecdotal reports of circular dominance as confirmation for the mild social stressor.

The relationship between serum and salivary cortisol was analysed using a Spearman correlation. Cortisol differences between elephants were analysed using Mann Whitney U tests. For the remaining analyses, each elephant was analysed individually to reduce the influence of individual variation (Palme et al. 2000). Individual analysis is especially important for measuring stress responses because responses can be very individualistic (Owen et al. 2004). Cortisol trends across the day (from 0715 and 1745) were also analysed using a Spearman correlation. Cortisol concentrations during the mild social stressor were compared to the confidence interval for each elephant. A Spearman correlation was used to analyse the association between swaying duration and salivary cortisol levels. Specifically, cortisol values were correlated with the time into sway bout when interrupted for sample collection as an ordinal variable. All analyses were performed using SPSS (multiple versions).

Table 1. Summary table of the methodology including type of data and purposes.

Type of data	Purpose(s)	Details
Behavioral	Quantify swaying, confirm anecdotal circular dominance	45 hours per elephant
Serum cortisol	Validate biological assay	9 samples per elephant
Salivary cortisol	Establish individual baseline, examine relationship between cortisol and stereotypic behavior, examine diurnal patterns, examine impact of mild stressor	106 from Kelly, 96 from Tara, and 68 from Dottie

Results

General behavioural data

Thirty hours of behavioural data per elephant were collected to quantify time budgets and social relationships. The occurrences of antagonistic and affiliative behaviours confirmed the anecdotal reports of circular dominance and of a more affiliative relationship between Tara and Dottie than Kelly with the others (Table 2). Proximity levels confirmed this relationship between Dottie and Tara. Almost no contact was observed but mean percentage time spent proximate was higher for Dottie (M=48.48%, mdn=48.84%) and Tara (M=45.05%, mdn=47.30%; same as Dottie: U=1672, P=0.502) than Kelly (M=32.94%, mdn=31.05%; versus Dottie: U=1144.5, P=0.001; versus Tara: U=1231.5, P=0.003).

No significant relationships were found between stereotypic swaying and time (Kelly: r_s=−0.053, P=0.688; Tara: r_s=0.113, P=0.391) or temperature (Kelly: r_s=−0.131, P=0.319; Tara: r_s=0.190, P=0.145). Kelly spent significantly more time swaying than Tara (M=24.77% versus 2.25%, mdn=13.92% versus 0%, U=600, P<0.001, Figure 1). Subsequently, Kelly spent less time consuming food compared to the other elephants (M=52.96%, mdn=49.32% versus Tara M=73.42%, mdn=81.02%, U=1290, P=0.007; Dottie M=69.59%, mdn=73.71%, U=1161.5, P=0.001). Tara only swayed once during the behavioural observations, whereas Kelly swayed in 41 separate observations. Based on the behavioural observations, keeper reports and swaying observed during salivary cortisol collections, Tara only swayed at the gate that the elephants went through to shift into the barn. Kelly swayed in various locations. The shift gate was her most frequent location but she also swayed farther back from the gate if other elephants were blocking it and also in other yard and barn locations. Kelly also spent significantly more time at the shift gate (M=20.19%, mdn=0%, SD=19.02%) than Tara (M=6.68%, mdn=0%, SD=14.97%; U=864.5, P<0.001).

General salivary cortisol

Tandem salivary and serum cortisol samples were taken during a baseline phase for validation purposes. Baseline serum (M=1.824 µg/dl, SD=1.127) and salivary cortisol (M=0.04352 µg/dl, SD=0.01996) values were significantly correlated (ρ=0.792, P<0.001, n=25), therefore saliva was a valid measure of cortisol in this study. After baseline validation, additional samples were taken during swaying bouts, a social stressor and various husbandry events, together called non-baseline. Total mean salivary cortisol including baseline and non-baseline was highest for Kelly

Table 2. Counts of antagonistic and affiliative behaviors recorded per elephant dyad. All incidents are per the 60 30-min long observation sessions, 30 AM and 30 PM, per elephant. Dominant relationships are in bold. The first elephant in the dyad is the initiator and the second is the recipient.

	Strike	Drive	Displace	Trunk Touching
Kelly-Tara	3	0	24	3
Kelly-Dottie	0	0	0	0
Dottie-Kelly	3	10	53	1
Dottie-Tara	0	1	2	4
Tara-Kelly	0	0	1	1
Tara-Dottie	0	2	11	12

Journal of Zoo and Aquarium Research 12(2) 2024
http://doi/org/10.19227/jzar.v12i2.726

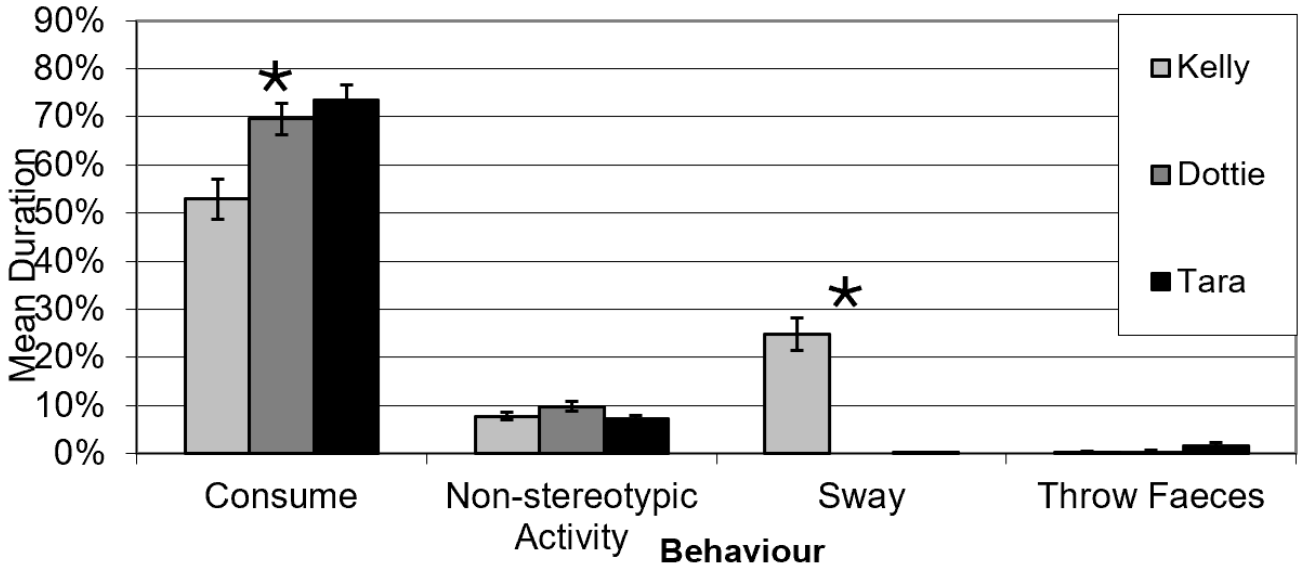

Figure 1. Mean percentage (+/- SEM) of selected behaviors by elephant. All percentages are based on 60 30-min long observations per elephant. Significant differences between Kelly and the other elephants are indicated by *.

(M=0.0557 µg/dl; CI_{95}=0.0456, 0.0659; versus Tara: U=2992.5, P<0.001; versus Dottie U=2700, P=0.005), next highest for Dottie (M=0.0313 µg/dl; CI_{95}=0.0272, 0.0353; versus Tara U=2433.5, P=0.006) and lowest for Tara (M=0.0239; CI_{95}=0.0207, 0.0272) (Table 2). Total salivary cortisol, baseline plus non-baseline, decreased throughout the day for all elephants (Kelly: r^2=0.079, P=0.003; Dottie: r^2=0.156, P=0.001; Tara: r^2=0.122, P<0.001; Figure 2).

Mild social stressor

Mild social stressor samples were collected at the beginning and end of the 15-minute confinement in the stall with their dominant conspecific as well as 15 and 30 minutes after release, for a total of four samples per elephant. For all elephants, these samples exhibited a peak in salivary cortisol 15 minutes after release. This peak began to decline by 30 minutes after release (Figure 3). The peak values were outside of the confidence intervals of the overall

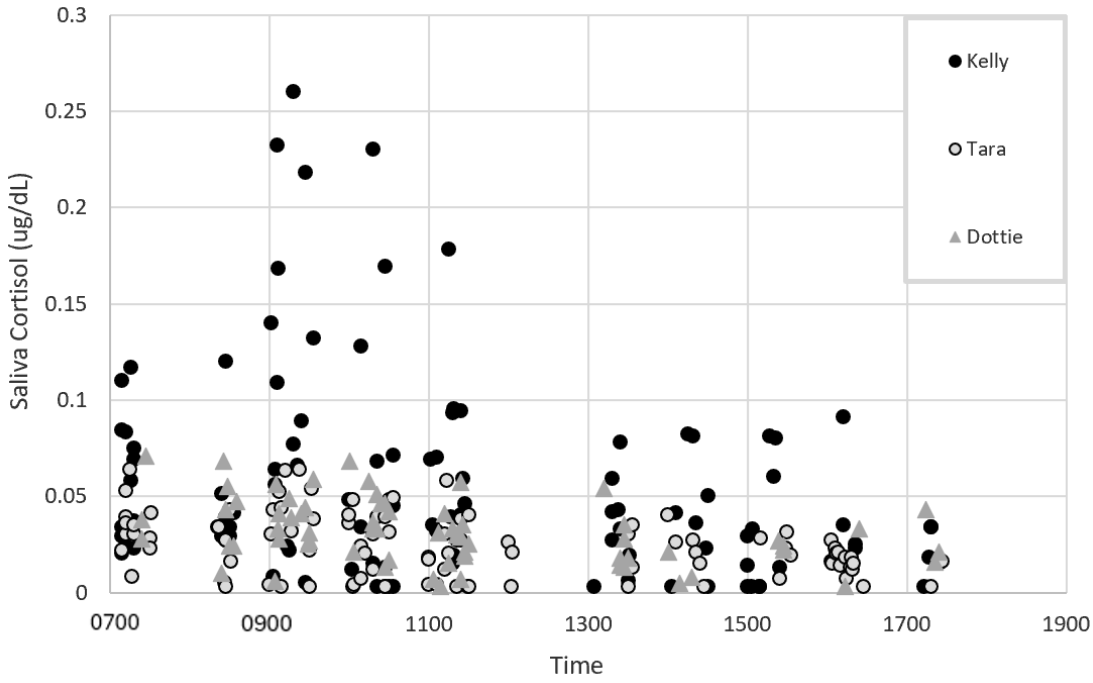

Figure 2. All salivary cortisol samples, baseline plus non-baseline, throughout the day for all elephants.

Table 3. Means and standard deviations of total cortisol, baseline, and non-baseline (µg/dl) and cortisol after swaying bouts by length of bout for each elephant.

Elephant	Condition	Mean Cortisol	Standard Deviation	n
Kelly				
	All Samples	0.0557	0.0527	106
	Baseline	0.054	0.0249	18
	Non-baseline	0.0561	0.0568	88
	Sway 1 min	0.0468	0.0303	5
	Sway 5 min	0.0438	0.0219	5
	Sway 10 min	0.0196	0.02	5
	Sway 15 min	0.031	0.0252	5
	Sway 30 min	0.017	0.0126	5
Dottie				
	All Samples	0.0313	0.0167	68
	Baseline	0.034	0.0169	17
	Non-baseline	0.0303	0.0167	51
Tara				
	All Samples	0.0239	0.0162	96
	Baseline	0.03	0.0097	17
	Non-baseline	0.0226	0.017	79
	Sway 1 min	0.0204	0.00702	5
	Sway 5 min	0.0152	0.00521	5
	Sway 10 min	0.0166	0.00945	5

means for both Kelly (peak=0.178 µg/dl) and Tara (peak=0.058 µg/dl) but not Dottie (peak=0.033 µg/dl), who was placed with an elephant (Tara) with whom she had a closer relationship as evidenced by the behavioural data (e.g. trunk touches and decreased strikes, drives and displacements).

Salivary cortisol and swaying

Since Dottie did not exhibit swaying behaviour, no swaying samples were collected from her and no correlation was calculated. For Tara, the sway cortisol samples were collected at only three time points during a bout and all in the afternoon for a total of 15 samples (Table 2). There was not a significant association between time into sway bout when interrupted and salivary cortisol (r_s=−0.180, P=0.260). For Kelly, sway cortisol samples were collected at five time points during a bout and a total of 25 samples (5 morning and 20 afternoon) were collected. She did exhibit a significant association between length of sway bout when interrupted and salivary cortisol (r_s=−0.404, P=0.022), which increased after controlling for time of day (AM versus PM; r_s=−0.427, P=0.019).

Discussion

The salivary cortisol values of all elephants in this study were within the range of or lower than those found by previous work (e.g. Casares et al. 2016; Grand et al. 2012; Hambrecht et al. 2021) and tended to decrease across the day as seen in other studies (Brown et al. 2010; Casares et al. 2016; Grand et al. 2012; White et al. 2019). Short-term confinement with a dominant conspecific led to a short-term spike in cortisol. Therefore, the elephants in the current study did not have blunted diurnal rhythms, blunted cortisol responses or cortisol levels outside of generally reported levels. Thus cortisol measures did not indicate welfare concerns.

The primary focus of this study was to examine the factors influencing captive elephant behaviour and wellbeing. Specifically, this study investigated whether stereotypic swaying in elephants was related to increased or decreased activation of the adrenal axis within a subject, as measured by salivary cortisol. To the authors' knowledge, this is the first evidence that in some elephants, stereotypic swaying reduces arousal as measured with salivary cortisol. However, swaying and cortisol were found to have a complex relationship. Swaying location in the exhibit and the fact that it was significantly associated with decreased cortisol values for only one out of two elephants who swayed suggest that it may be associated with times of elevated stimulation, but there may be variation in function across individuals.

Kelly spent more time stereotypically swaying than the mean percentage time found for African elephants in Greco et al. (2016) and when she was previously measured (Wilson et al. 2004), but not as much time as elephants in other studies (Elzanowski and

Sergiel 2006). For Kelly, swaying occurred in multiple locations and appeared to be more obligatory, to the point that it impacted the amount of time she spent consuming food, which was also seen in Schiffmann and Clauss (2019). On the other hand, Tara spent less time swaying, swayed in only one location and tended to perform a more active swaying motion with pauses and more varied interbout behaviour (data not reported). Kelly had the highest cortisol concentrations in the baseline and non-baseline conditions of all the subjects. Over the course of her swaying bouts cortisol decreased to levels below those of the other two elephants in the baseline and non-baseline conditions. Tara had the lowest cortisol concentrations in the baseline and non-baseline conditions of all the subjects. Over the course of her shorter swaying bouts, cortisol decreased to the lowest levels measured in this study. Therefore, evidence exists for a potential coping effect for Tara but because of her low starting cortisol level and short swaying sessions, it was not possible to achieve the power needed to find a statistically significant decline in cortisol. Tara's swaying bouts may have been shorter because her cortisol levels were lower to start and decreased substantially within five minutes of swaying, whereas it took ten minutes of swaying for Kelly's cortisol to decrease substantially. Thus, for both subjects stereotypic swaying may have been a behavioural coping mechanism (Hill and Broom 2009) that was reinforcing (Mason 1991b) and/or fulfilled a need (Ödberg 1987) to reduce arousal. Evidence for these claims comes from other species where it has been found that stereotypies reduce arousal and signs of stress in correlational studies (Mason 1991a, b). Battery hens have been found to have normal cortisol levels once they become accustomed to their cages and stereotypic behaviour becomes habitual (Beuving 1980). Furthermore, prevention of jumping stereotypies in bank voles leads to increased corticosteroid levels (Kennes et al. 1988).

It is important to note that wellbeing must be examined at the individual level and it is difficult to obtain a valid marker of distress that can be applied across multiple animals. As discussed in Bayazit (2009) the complexity of stress reactions and the individualised nature of how multiple systems react to stressors leads stress to be difficult to investigate and requires an individualised approach. Various measures may also have conflicting interpretations for welfare (Mason and Mendl 1993). For example, Hambrecht et al. (2021) found a cortisol increase when elephants were presented with a novel object, suggesting that behavioural context may be crucial for interpreting physiological reactions. Although the findings of the current case study are limited in generalisability, the evidence presented here indicates that stereotypical swaying may serve to reduce cortisol in elephants and this has implications for the management of elephants in captivity. High rates of stereotypic behaviour exhibited daily by an individual may suggest a welfare problem because the animal is displaying a need to minimise adverse stimulation (Mason 1991b). Consequently, managers may want to decrease stereotypic behaviour, but reducing this behaviour should address the adverse stimulation itself and not simply prevent expression of stereotypic behaviour, which may be helping the animal cope. Rees (2009) suggests increased foraging opportunities and unpredictability may decrease stereotypic behaviour in elephants and enhance activity budgets. Other studies suggest increased time spent interacting with keepers (Brown et al. 2019; Carlstead et al. 2019; Greco et al. 2016) or conspecifics (Brown et al. 2019; Readyhough et al. 2022; Schiffmann and Clauss 2019) or other welfare enhancing husbandry changes (Brown et al. 2019; Finch et al. 2020) can also decrease swaying behaviour in elephants. Time spent with keepers and conspecifics along with environmental enrichment was associated with lower faecal glucocorticoid metabolite concentrations (Brown et al. 2019). However, certain changes or social situations may cause frustration or lack of control and may increase stereotypic behaviours (Greco et al. 2017). Future studies should assess cortisol and behavioural differences among a larger number of elephants with differing swaying patterns and rates. This work would contribute to understanding the difference found here between an individual for whom swaying seems obligatory with greater potential coping utility and one for whom swaying is much less frequent and of shorter duration.

There are other aspects of using cortisol to assess welfare that require caution. Cortisol is secreted in a pulsing fashion and it shows both circadian and annual variation (Coe and Levine 1995), especially in diurnal species (Janssens et al. 1995). Cortisol has been found to peak in the morning and decline through the day in Asian (Bechert et al. 2021; Bettinger and Laudenslager 1998; Plangsangmas et al. 2020) and African (Bechert et al. 2021; Casares et al. 2016; Hambrecht et al. 2019) elephants, which was confirmed in this study. Overall, given these and the previously mentioned challenges to using cortisol or behaviour to determine welfare, it has been suggested that cortisol measures be combined with other indicators of welfare (Williams et al. 2018) to more fully analyse the welfare of an individual animal and guide management decisions.

Acknowledgments

The authors thank all the Zoo Atlanta staff and others involved in this project, particularly Adam Stone, who collected saliva samples for the validation procedures and Patrick Benitez who performed the cortisol assays and validation study. This study was funded by a Psi Chi Dissertation Grant and a Graduate Development Fund from the Psychology Department at Georgia Institute of Technology. Support was also provided by the Bailey Fund and the Smithgall Endowment though the Center for Conservation and Behavior at Georgia Institute of Technology and the Behavioral Immunology and Endocrinology laboratory (BIEL), University of Colorado Anschutz Medical Campus, Aurora Colorado.

We would like to acknowledge the invaluable contributions of our co-authors, Dr. Terry Maple and Dr. Mark Laudenslager, to this work. Their expertise and support significantly influenced the quality of this manuscript. Although they are no longer with us, their passion for research and commitment to improving animal wellbeing continue to inspire us.

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