American Journal of Primatology 70:1145–1151 (2008)

RESEARCH ARTICLE

Effectiveness of Saliva Collection and Enzyme-Immunoassay for the Quantification of Cortisol in Socially Housed Baboons

BRANDON L. PEARSON[1], PETER G. JUDGE[2]*, AND DEEANN M. REEDER[3]
[1]*Animal Behavior Program, Bucknell University, Lewisburg, Pennsylvania*
[2]*Psychology Department, Animal Behavior Program, Bucknell University, Lewisburg, Pennsylvania*
[3]*Biology Department, Bucknell University, Lewisburg, Pennsylvania*

Circulating cortisol levels are often used to assess the biological stress response in captive primates. Some methods commonly used to collect blood samples may alter the stress response. As such, noninvasive means to analyze cortisol levels are increasingly being developed. We adapted an existing collection method to simultaneously obtain saliva from multiple socially living hamadryas baboons (*Papio hamadryas hamadryas*) and validated an enzyme-immunoassay kit to quantify cortisol within the saliva samples. Over a period of 12 months, saliva samples were regularly collected from approximately half of the 18-member colony, representing younger monkeys who were more willing to participate. The assay met the four criteria typically used to assess the effectiveness of a new analytical technique: parallelism, precision, accuracy, and sensitivity. Cortisol levels were also proportional to those expected given published plasma levels of cortisol in baboons. Further, salivary cortisol levels increased in individuals following significant stress-related events, such as removal from the group, indicating biological validation. The technique provided a reliable and effective means to assess a physiological indicator of stress in a social group without initiating a stress response owing to handling or sedation, and provided a real-time assessment of cortisol levels and reactivity. Am. J. Primatol. 70:1145–1151, 2008.

Key words: hamadryas baboon; HPA axis; saliva assay; cortisol; pole method

INTRODUCTION

Stress, whether triggered by physical, immunological, or psychosocial stimuli, is associated with the activation of the hypothalamic–pituitary–adrenal (HPA) axis to mobilize fuels for adaptive behavioral and physiological responses [McEwan, 2000]. Under stress, primates release the glucocorticoid hormone cortisol from the adrenal cortex into the systemic circulation, which binds to intracellular steroid receptors to produce transcriptional changes that function to restore homeostasis [Sapolsky et al., 2000]. Acute activation of the HPA axis response is adaptive [Reeder & Kramer, 2005], but long-term perturbations of the HPA axis are associated with psychological [Gold & Chrousos, 2002; Tronche et al., 1999] and physical pathology [Koolhaas et al., 1999]. Accordingly, assays are frequently used to study glucocorticoid (cortisol or corticosterone) levels.

The most common method for assessing baseline and stress-induced HPA activity in humans and nonhuman animals is by assaying circulating levels of glucocorticoids in blood plasma [Cohen et al., 1995]. To assay cortisol in nonhuman primates, an animal is typically captured, restrained, and blood is extracted intravenously for assay. A problem with this method is that increases in cortisol are detectable in the bloodstream within a few minutes of a stressful event, and the procedure itself is stressful [Balcombe et al., 2004; Reeder & Kramer, 2005]. If samples are not collected immediately, then one is not investigating an animal's baseline level of stress, but its reactivity to capture or restraint. Even if samples are taken quickly, administration of some anesthetizing agents to aid in blood collection can alter glucocorticoid levels [Bentson et al., 2003] and animals may respond to the sight of technicians preparing for the procedures. One solution is to train animals to provide a limb for voluntary sampling [e.g. Prescott & Buchanan-Smith, 2003], but this

Contract grant sponsors: Biology and Psychology Departments; Animal Behavior Program; Bucknell University Graduate Fellowship.

*Correspondence to: Peter G. Judge, Psychology Department, Animal Behavior Program, Bucknell University, Lewisburg, PA 17837. E-mail: pjudge@bucknell.edu

Received 17 May 2008; revised 16 August 2008; revision accepted 17 August 2008

DOI 10.1002/ajp 20613
Published online 11 September 2008 in Wiley InterScience (www.interscience.wiley.com).

method is likely impractical for primates during colony housing.

An alternative to plasma sampling is noninvasive assessment of cortisol levels in feces, urine, and hair [Davenport et al., 2006; Queyras & Carosi, 2004]. Cortisol is metabolized soon after it is released and its metabolites can be assayed in feces and urine. However, metabolites within excreta are subject to bacterial degradation, which can reduce the accuracy of results [Negrão et al., 2004]. In addition, the cortisol in these media represent average levels over extended periods, which may be of practical value, but do not allow investigators to attribute hormone levels to particular events or stressors [Lutz et al., 2000]. Further, using these media, it is not feasible to collect a controlled number of samples across short time intervals [Lutz et al., 2000].

Assessing glucocorticoid hormones via salivary cortisol may be the best alternative to plasma sampling. Glucocorticoid hormones are lipophilic, so they passively diffuse across membranes [Riad-Fahmy et al., 1982]. As such, free cortisol diffuses into saliva from the parotid gland at a consistent 10–15% fraction of circulating levels [Katz & Shannon, 1969]. An advantage to measuring cortisol in saliva is that, unlike in blood or urine samples, only the unbound and putatively metabolically available cortisol diffuses into the saliva. The carrier proteins that can accompany cortisol in circulation do not diffuse into saliva and affect interpretation [Kirshbaum & Hellhammer, 1989] perhaps rendering saliva a better index of cortisol response than blood samples [Vining et al., 1983]. Another advantage to assaying cortisol through saliva is that peak cortisol responses are not detectable in saliva until approximately 20–30 min after the onset of a stressor [Kirschbaum & Hellhammer, 1989], so the possible effects of researcher presence (e.g. distress or fear) can be alleviated if saliva samples are collected promptly [Lutz et al., 2000]. Finally, as subjects may voluntarily participate, saliva samples can be obtained with no pain or discomfort [Queyras & Carosi, 2004], and be collected while an animal remains in its social group. Thus, noninvasive measurement of glucocorticoids in saliva may be an ideal means to assess ongoing levels of stress.

Despite the potential utility of assessing cortisol levels via saliva, the procedure is not prevalent in primate facilities. One hindrance is that plasma cortisol levels are widely publicized and plasma assays have been validated for a wide range of species. However, saliva assays are being validated for an increasing number of primate species, which may promote more widespread use of the technique. For example, salivary cortisol assays have been validated in macaques [*Macaca mulatta*, Boyce et al., 1995; Lutz et al., 2000], marmosets [*Callithrix jacchus,* Cross et al., 2004], squirrel monkeys [*Saimiri sciureus*, Fuchs et al., 1997; Tiefenbacher et al., 2003], gorillas [*Gorilla gorilla gorilla*, Kuhar et al., 2005], and orangutans [*Pongo pygmaeus*, Elder & Menzel, 2001].

The purpose of this study was to explore the utility of collecting saliva samples from a social group of hamadryas baboons and to validate the salivary cortisol assay for this new species. We created a modified version of the pole-method for collecting saliva [Lutz et al., 2000] in which a plastic tube containing a protruding rope was held up to the caging and animals approached and voluntarily chewed on the rope to deposit saliva. Saliva was then assayed for cortisol using a commercially available enzyme-immunoassay kit (EIA) and subjected to procedures necessary to validate an immunoassay for a novel application. Validation of the assay might promote more widespread use of noninvasive saliva sampling to assess cortisol levels among various primate genera. In addition, using a valid cortisol assay from voluntary saliva samples obtained from a social group would allow us to correlate ongoing behavioral events in the colony to a physiological indicator of stress that was unperturbed by the methods used to obtain it.

METHODS

Animals and Housing

A captive group of 18 socially housed hamadryas baboons ranging in age from less than 1 year to 17 years was sampled. All but one subject were descended from a colony established in 1968 at Bucknell University's Animal Behavior Laboratory. The exception was an adult male who was introduced in 1996 and was the harem leader of the group at the time of the study. The group contained one adult male, five adult females, two subadult males, two subadult females, and eight juveniles.

Subjects were housed in an outdoor enclosure measuring $9 \times 11 \times 4.5$ m with an adjacent $9 \times 6 \times 2.25$ m concrete, temperature-controlled indoor structure made up of three interconnected compartments. The outdoor enclosure contained gravel and boulder substrate with various perches and multiple enrichment devices. Commercial primate diet and fresh water were continuously available and supplemented with an assortment of fruits, nuts, and/or vegetables. The baboons typically had access to both indoor and outdoor areas, but were restricted to the indoor compartments each year when the temperature dropped below 7°C.

Sample Collection and Analysis

Saliva samples were collected from a hand-held, structurally modified pole apparatus modeled after Lutz et al. [2000]. The device consisted of a polyvinyl chloride (PVC) tube housing a clamped, removable piece of flavored, $\frac{1}{2}$'' (1.27 cm) diameter white cotton

rope. The body of the apparatus consisted of a threaded canister in which an approximate 8 cm length of rope was clamped during sample collection. An assembled apparatus measured approximately 50 cm in length and 6.5 cm in diameter (Fig. 1). Rope was soaked in concentrated, uncolored Ice Kool lemonade KoolAid© (Kraft Foods, Northfield, IL) drink crystal solution (one part crystals to two parts tap water) for 30 min and thoroughly dried in a refrigerator before being used in sample collection. Clear flavoring on a white rope was employed to prevent distortion of the spectrophotometer values needed to read the results of the assay kit. Clear flavoring also allowed us to more easily notice if blood was deposited on the white rope owing to overly vigorous chewing. We checked each sample for signs of blood contamination because the elevated concentration of cortisol in blood compared with that diffused into saliva [Boyce et al., 1995] would drastically distort the assay. Several steps were taken during validation (see below) to determine if the chemical composition of the rope or Kool-Aid© affected cortisol levels [Gordon et al., 2005; Shirtcliff et al., 2001].

During a typical sample collection session, one to ten people aligned themselves along the side of the enclosure while holding one or two apparatuses each. Up to 12 apparatuses were used during a sampling session. Assistants would distribute themselves evenly in front of the enclosure, and the baboons quickly learned to approach and sit or stand in front of an assistant. After the animals distributed themselves evenly along the fence, the devices were simultaneously presented to the monkeys through the caging. Subjects were allowed to chew the rope and deposit saliva for 1–3 min at which time the devices were withdrawn. The entire collection process lasted less than 10 min. Each apparatus was numbered to assure accurate identification of samples. Once the poles were withdrawn, the time of day, subject ID, apparatus number, and sample collector's initials were noted on a datasheet. Samples were discarded if more than one animal contacted the rope or if blood was visible.

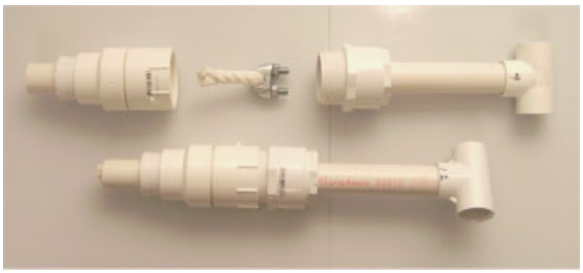

Fig. 1. Modified pole apparatus with a threaded canister to house a clamped section of rope. When assembled, flavored rope protruded out of the end approximately 1–2 cm to allow baboons to chew the rope.

Saliva-saturated rope was cut away and immediately centrifuged in customized vials to extract the saliva. The centrifuge tubes included a perforated cryogenic vial plug, which was placed within a 15 mL conical vial to allow the accumulation of saliva at the bottom during centrifugation while impeding the rope. After spinning for 5 min, up to three aliquots of at least 20 μL each were pipetted into separate 1.2 mL cryogenic vials and frozen at −20°C until assayed. Typically, saliva samples were collected two to three times per week, but always between 13:00–15:00 hr to control for expected daily cortisol fluctuation [Reeder & Kramer, 2005]. The afternoon is generally the trough (nadir) of the daily cortisol cycle, where the most amount of individual variation can be detected in circulation [Queyras & Carosi, 2004]. All procedures were approved by Bucknell's Institutional Animal Care and Use Committee according to requirements within the Guide for the Care and Use of Laboratory Animals [National Research Council, 1996].

Cortisol Assay

Assays were conducted in the Laboratory of Comparative Ecophysiology at Bucknell University with a commercially available EIA kit (Cortisol Express, Cayman Chemical© Ann Arbor, MI). Absorbance values were determined at 415 nm using a plate reader (BioRad Model 550© Hercules, CA). Samples were assayed in duplicate or triplicate yielding 35 or 23 samples per plate respectively as a function of the number of wells available in the precoated micro-plates.

The assay was validated using standard techniques [e.g. Buchanan & Goldsmith, 2004; Reeder & Widmaier, 2008;]. First, parallelism was established by comparing the slope of three serially diluted samples with the standard curve. Secondly, precision was evaluated by testing for consistent values throughout assays. Initially, during the preliminary validation steps, one sample was repeatedly assayed within one plate and the coefficient of variation (CV) was calculated to assess intra-assay variation. Duplication of samples within each plate also tested for intra-assay variation. Additionally, a pooled saliva sample was repeatedly assayed in every plate and the CV was calculated for inter-assay variation. Any assays or specific samples with greater than 10% variation were reanalyzed or excluded. Less than 10% variation for precision tests is commonly considered acceptable when conducting immunoassays [Murray et al., 1993] although, in some circumstances, up to 25% variation is deemed acceptable [Findlay et al., 2000]. Thirdly, accuracy was assessed by spiking previously assayed saliva samples with cortisol standards of known concentration and comparing observed to expected levels.

Lastly, the sensitivity of the assay was determined by calculating the least detectable dose.

RESULTS

Saliva Sample Collection

Saliva sampling was conducted during 102 sessions from Fall 2006 to the Summer of 2007. The Mean ± SE number of samples collected per session was 5.81 ± 0.22. When animals elected to use an apparatus, it proved to be an effective method for collecting saliva samples. Centrifuged rope produced a Mean ± SE of 299.79 ± 9.82 μL of saliva per sample, which is well above the 2–10 μL necessary to perform an assay. However, individuals varied considerably in their willingness to provide samples and exhibited a great deal of individual variation in the average amount of saliva collected per sample (Table I). The five adults rarely provided samples whereas subadults and juveniles 8-years old and younger did so regularly.

Assay Validation

Parallelism. Cortisol concentrations across standard dilutions from three preliminary samples were parallel to each other and the standard curve indicated that the antibodies were binding the hormone in the saliva sample at a similar rate as those in the standard (Fig. 2).

Precision. The Mean ± Standard Error (SE) intra-assay CV across 24 plates was 6.62 ± 0.35%. A large (2 mL) preliminary sample was aliquoted and frozen to be assayed in every plate throughout all analyses. The Mean ± SE inter-assay CV calculated from this pooled sample in 24 plates was 5.57 ± 0.49%. These relatively low percentages indicate consistent results between reaction plates independent of any temporal confounds such as sample storage or experimenter proficiency.

Accuracy. Three different saliva samples were each spiked with three known concentrations of pure cortisol. Samples were assayed and observed values were compared with expected values calculated from the original sample's value plus the amount of cortisol added. Accuracy was determined to be 89.54 ± 4.38%.

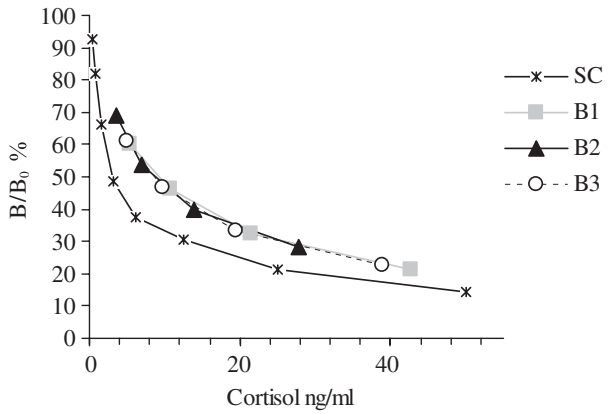

Fig. 2. Log transformed plot of analytical absorbance readings (B/B_0%) between the serially diluted kit standard curve (SC) and three baboon saliva samples (B1, B2, B3). Values indicated that cortisol levels were accurate across various concentrations and that other molecules within baboon saliva were not causing interference.

TABLE I. Baboon Demographics, Sampling Success, and Cortisol Values

Identification code	Sex	Age	Samples collected	Samples analyzed	Mean sample volume (μL)	Mean ± SE, cortisol (ng/mL)	Cortisol range
As	M	1	22	5	288.33	20.82 ± 2.98	13.88–28.86
Ca	F	1	21	4	181.67	15.06 ± 4.12	10.29–27.37
Rh	F	2	34	34	302.21	20.47 ± 3.26	4.59–77.20
Ac	F	3	25	0	272.71	–	–
Cr	M	3	52	52	420.77	19.46 ± 1.73	3.44–64.20
Ch	M	4	59	58	240.34	15.91 ± 1.58	5.39–64.92
Ka	F	4	48	46	259.48	21.42 ± 3.67	3.41–138.41
Rb	F	4	62	59	445.23	15.48 ± 1.51	2.12–53.80
Kr	M	5	55	48	339.91	13.59 ± 0.95	6.05–36.62
Ro	M	5	64	58	248.63	15.93 ± 1.76	4.33–73.66
Kb	M	6	71	64	243.20	17.36 ± 2.21	3.95–136.30
Cl	M	7	56	54	272.82	26.74 ± 3.67	5.19–138.32
Ks	F	7	11	0	405.45	–	–
Al	F	8	1	0	305.00	–	–
Ct	F	11	0	0	–	–	–
Am	F	14	0	0	–	–	–
Rt	F	16	1	0	45.00	–	–
Dg	M	17	7	1	168.57	36.67	–
Sum or mean ± SE			595	483		18.39 ± 1.15	2.12–138.41

Differences between the numbers of samples collected and the number analyzed were owing to blood contamination, insufficient volume, or inconsistent participation.

Sensitivity. Averaging values across all assays through the duration of the study at 95% binding indicates that the lower limit of detection for this assay was 0.26 ng/mL. The range (20–80% binding) of reliable values at a 1:100 dilution was 1.60–43.19 ng/mL. Samples that exceeded the upper limit of 43.19 ng/mL (80% binding) were diluted further to a ratio 1:200 or 1:500 and re-assayed. Less than 10% of all samples required additional dilution beyond 1:100.

Variation in Cortisol Levels

Mean cortisol levels were fairly variable between subjects ranging from 13.59–26.74 ng/mL (Table I) with some animals showing twice the average level of others. Levels also varied considerably within subjects. Among the subjects with over 40 samples analyzed, the range of levels was as little as 30 ng/mL for Kr (6.05–36.62) and as high as 135 ng/mL for Ka (3.41–38.41). Three animals (Ka, Kb, and Cl) showed markedly high upper-range cortisol values relative to the other animals and these appeared to be correlated with stress-inducing events within the colony.

The high value for 4-year-old nulliparous female Ka in mid November 2006 (Fig. 3a) coincided with sexual receptivity and conception as she was sexually swollen at the time and was actively mating. She gave birth in late May of 2007. As gestation lengths for hamadryas baboons have been estimated at approximately 170 days [Swedell & Leigh, 2006], conception likely occurred shortly after this spike in cortisol. The second case of an extremely high cortisol value was for 6-year-old subadult male Kb in late April 2007 (Fig. 3b). The spike occurred shortly after he was removed from the group and restricted to one of the three indoor quarter compartments. He was removed owing to his excessive aggression against a juvenile female during his attempts to form his own harem while challenging the alpha male harem leader (Dg). The 2-year-old female (Rh) that received excessive neck bites from subadult male Kb was also removed from the group at the time for treatment of injuries. Her cortisol levels were elevated during the time she was being targeted with aggression in mid March and her levels continued to rise once she was removed from the group and housed singly in early April (Fig. 3c). The third case of a high upper range was 7-year-old subadult male Cl (Fig. 3d). At the beginning of the study, Cl defeated the alpha male harem leader (Dg) and began to herd the alpha male's females away from him. When the females resisted, Cl escalated his aggression against them and we removed him from the group, housing him in one of the three adjacent indoor compartments within visual and vocal contact of the rest of the group. His first cortisol spike occurred immediately after he was removed from the group in June of 2006 and housed singly. Cl was singly housed for the remainder of the study and he exhibited the highest average cortisol level of 26.74 ng/mL (Table I). Cl's highest cortisol

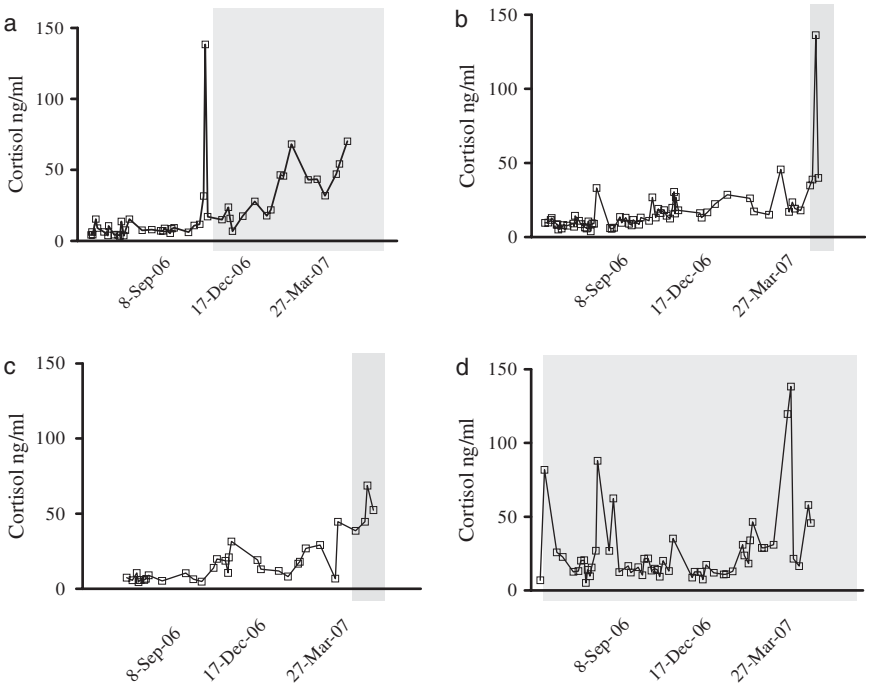

Fig. 3. Longitudinal cortisol levels from four individuals exhibiting conspicuous peaks. One peak (**a**) occurred in a 4-year-old female (Ka) immediately prior to conception and gestation (shaded area). Peaks occurred in Kb (**b**), Rh (**c**), and Cl (**d**) immediately after they were removed from the group and housed singly for husbandry reasons (shaded area). See text for further explanation.

level occurred in April of 2007 at a time when he was witnessing Kb challenging the alpha male and injuring the juvenile female.

DISCUSSION

Saliva collection from group housed baboons using a pole apparatus provided a reliable means for assessing cortisol levels in socially living juvenile baboons. The technique did have limitations as we were unable to sample the adults. We would expect that, if we continued sampling, juveniles that provided samples in this study would continue to do so as adults. The harem-based social system of hamadryas baboons may have played a role in the lack of sampling of adults. Adult females risk punishment from their harem male for straying too far from his side [Kummer, 1968] and because their male harem leader was largely disinterested in providing samples, the females may have elected to stay with him rather than move away to the collection area.

The salivary cortisol assay performed well on the four standard tests required for validation of an assay for a new species [Buchanan & Goldsmith, 2004]. Assay results exhibited parallelism, precision, accuracy, and sensitivity with all CV calculated at less than 10%. It is possible that compounds within the rope or the flavoring may have interfered slightly with the immunoassay, but we found no evidence for this. Likewise, in macaques Lutz et al. [2000] found no significant effects of drink crystals on salivary cortisol levels.

A fifth criterion for validation of an assay for a new species is biological validation, which is an indicator that the assay accurately reflects the physiology of an organism. Biological validation could be demonstrated in several ways including showing that cortisol increases in response to stress-inducing events. The salivary cortisol levels we obtained were comparable to published plasma cortisol levels within this genus at the 10–15% fraction secreted in saliva [Negrão et al., 2004; Queyras & Carosi, 2004]. For example, in restrained and sedated male savannah baboons (*Papio cynocephalus/anubis*), Bentson et al. [2003] reported plasma concentrations of 232 ± 8 ng/mL, which were approximately 10–15 times higher than the salivary cortisol levels we obtained. Similarly, among restrained adult male hamadryas baboons, Taranov and Goncharov [1981] reported average plasma cortisol levels ranging from 150 to 500 ng/mL depending upon the time of day and degree of habituation to restraint.

Acute increases in salivary cortisol levels were observed in three animals immediately after they were removed from the group and housed individually. Two animals experienced sharp rises in cortisol during a stressful period when the alpha male's position was being challenged by a subadult male. One of these animals was being targeted with continued aggression at the time. In addition, one female showed a spike in cortisol levels about the time of conception. The increase may have been owing to hormonal changes in cortisol related to reproductive physiology that occur about the time of sexual receptivity [e.g. Ziegler et al., 1995] or she may have been reacting adversely to the subadult males' competition for copulation. Taken together, we consider the colony events associated with the cortisol changes observed as additional validation of the sampling technique and assay.

We conclude that the technique was a valid and appropriate means by which to assess an objective biological indicator of stress. Other researchers may be able to adapt this apparatus to quantify steroid levels in group housed primates without significantly influencing stress responses or distress associated with handling, restraint, or venipuncture.

ACKNOWLEDGMENTS

Jenny Bohrman, Adam Conrad, Callista Costopolous, Mary Gavitt, Daniel Hanley, Erin Henry, Lauren Kelly, Allison Kueck, Lauri Kurdziel, Nicole Rold, Carol Sadowsky, Jess Scott, Lindsey Shamberger, Lindsey Smart, Mallory St. Pierre, and Risa Wright provided sampling assistance and technical support. Brandon Pearson was supported by a Bucknell University Graduate Fellowship. The primate colony is supported by Bucknell University. All procedures were approved by Bucknell's Institutional Animal Care and Use Committee according to requirements within the Guide for the Care and Use of Laboratory Animals [National Research Council, 1996].

REFERENCES

Balcombe JP, Barnard ND, Sandusky C. 2004. Laboratory routines cause animal stress. Contemp Top Lab Anim Sci 43:42–51.

Bentson KL, Capitanio JP, Mendoza SP. 2003. Cortisol responses to immobilization with Telazol or ketamine in baboons (*Papio cynocephalus/anubis*) and rhesus macaques (*Macaca mulatta*). J Med Primatol 32:148–160.

Boyce WT, Champoux M, Suomi SJ, Gunnar MR. 1995. Salivary cortisol in nursery-reared rhesus monkeys: reactivity to peer interactions and altered circadian activity. Dev Psychobiol 28:257–267.

Buchanan KL, Goldsmith AR. 2004. Noninvasive endocrine data for behavioural studies: the importance of validation. Anim Behav 67:183–185.

Cohen S, Kessler RC, Gordon LU. 1995. Measuring stress: a guide for health and social scientists. New York: Oxford University Press. 256p.

Cross N, Pines MK, Rogers LJ. 2004. Saliva sampling to assess cortisol levels in unrestrained common marmosets and the effect of behavioral stress. Am J Primatol 62:107–114.

Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. 2006. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. Gen Comp Endocrinol 147:255–261.

Elder CM, Menzel CR. 2001. Dissociation of cortisol and behavioral indicators of stress in an orangutan (*Pongo pygmaeus*) during a computerized task. Primates 42: 345–357.

Findlay J, Smith W, Lee D, Nordblum G, Das I, DeSilva B, Khan M, Bowsher R. 2000. Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective. J Pharm Biomed Anal 21:1249–1273.

Fuchs E, Kirschbaum C, Benisch D, Bieser A. 1997. Salivary cortisol: a non-invasive measure of hypothalamo–pituitary–adrenocortical activity in the squirrel monkey, *Saimiri sciureus*. Lab Anim 31:306–311.

Gold PW, Chrousos GP. 2002. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs. low CRH/NE states. Mol Psychiatry 7:254–275.

Gordon MK, Peloso E, Auker A, Dozier M. 2005. Effect of flavored beverage crystals on salivary cortisol enzyme-immunoreactive assay measurements. Dev Psychobiol 47:189–195.

Katz FH, Shannon IL. 1969. Parotid fluid cortisol and cortisone. J Clin Invest 48:848–855.

Kirschbaum C, Hellhammer DH. 1989. Salivary cortisol in psychobiological research: an overview. Neuropsychobiology 22:150–169.

Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, De Jong IC, Ruis MA, Blokhuis HJ. 1999. Coping styles in animals: current status in behavior and stress physiology. Neurosci Biobehav Rev 23:925–935.

Kuhar CW, Bettinger TL, Laudenslager ML. 2005. Salivary cortisol and behaviour in an all-male group of western lowland gorillas (*Gorilla g. gorilla*). Anim Welf 14:187–193.

Kummer H. 1968. Social organization of hamadryas baboons. A field study. New York: Karger. 190p.

Lutz CK, Tiefenbacher S, Jorgensen MJ, Meyer JS, Novak MA. 2000. Techniques for collecting saliva from awake, unrestrained, adult monkeys for cortisol assay. Am J Primatol 52:93–99.

McEwan BS. 2000. The neurobiology of stress: from serendipity to clinical relevance. Brain Res 886:172–189.

Murray W, Peter AT, Teclaw RF. 1993. The clinical relevance of assay validation. Compend Contin Educ Pract Vet 15:1665–1675.

National Research Council. 1996. Guide for the care and use of laboratory animals. Washington, DC: National Academy Press.

Negrão JA, Porcionato MA, De Passille AM, Rushen J. 2004. Cortisol in saliva and plasma of cattle after ACTH administration and milking. J Dairy Sci 87:1713–1718.

Prescott MJ, Buchanan-Smith HM. 2003. Training nonhuman primates using positive reinforcement techniques. J Appl Anim Welf Sci 6:157–161.

Queyras A, Carosi M. 2004. Non-invasive techniques for analysing hormonal indicators of stress. Ann Ist Super Sanita 40:211–221.

Reeder DM, Kramer KM. 2005. Stress in free-ranging mammals: integrating physiology, ecology, and natural history. J Mammal 86:225–235.

Reeder DM, Widmaier EP. In press. Hormone analysis. In: Kunz TH, Parsons S, editors. Ecological and behavioral methods for the study of bats, 2nd ed. Baltimore, MD: The Johns Hopkins University Press.

Riad-Fahmy D, Read GF, Walker RF, Griffiths K. 1982. Steroids in saliva for assessing endocrine function. Endocr Rev 3:367–395.

Sapolsky RM, Romero LM, Munck AU. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 21:55–89.

Shirtcliff EA, Granger DG, Schwartz E, Curran MJ. 2001. Use of salivary biomarkers in biobehavioral research: cotton-based sample collection methods can interfere with salivary immunoassay results. Psychoneuroendocrinology 26:165–173.

Swedell L, Leigh SR. 2006. Reproduction and fitness in baboons: behavioral, ecological, and life-history perspectives. New York, NY: Springer. 322p.

Taranov AG, Goncharov NP. 1981. Circadian rhythms of corticosteroids and their precursors in *Papio hamadryas* depending on initial functional state of the hypophyseo-adrenocortical system. Bull Exp Biol Med 91:215–217.

Tiefenbacher S, Lee B, Meyer JS, Spealman RD. 2003. Noninvasive technique for the repeated sampling of salivary free cortisol in awake, unrestrained squirrel monkeys. Am J Primatol 60:69–75.

Tronche F, Kellendonk C, Kretz O, Gass P, Anlag K, Orban PC, Bock R, Klein R, Schütz G. 1999. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nat Genet 23:99–103.

Vining RF, McGinley RA, Maksvytis JJ, Ho KY. 1983. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. Ann Clin Biochem 20:329–335.

Ziegler TE, Scheffler G, Snowdon CT. 1995. The relationship of cortisol levels to social environment and reproductive functioning in female cotton-top tamarins, *Saguinus oedipus*. Horm Behav 29:407–424.