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EFFECTS OF TRANSPORT ON FECAL GLUCOCORTICOID LEVELS IN CAPTIVE-BRED COTTON-TOP TAMARINS (*SANGUINUS OEDIPUS*)[1]

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ABSTRACT

The relocation of animals can induce stress when animals are placed in novel environmental conditions. The movement of captive animals among facilities is common, especially for non-human primates used in research. The stress response begins with the activation of the hypothalamic-pituitary-adrenal (HPA) axis which results in the release of glucocorticoid hormones (GC), which at chronic levels could lead to deleterious physiological effects. There is a substantial body of data concerning GC levels and reproduction, and rank and aggression in primates. However, the effect of transport has received much less attention. Fecal samples from eight (four male and four female) captive-bred cotton-top tamarins (*Saguinus oedipus*) were collected at four different time points (two pre-transport and two post-transport). The fecal samples were analyzed using an immunoassay to determine GC levels. A repeated measures analysis of variance (ANOVA) demonstrated that GC levels differed among transport times ($p = 0.009$), but not between sexes ($p = 0.963$). Five of the eight tamarins exhibited an increase in GC levels after transport. Seven of the eight tamarins exhibited a decrease in GC levels from three to six days post-transport to three weeks post-transport. Most values returned to pre-transport levels after three weeks. The results indicate that these tamarins experienced elevated GC levels following transport, but these increases were of short duration. This outcome would suggest that the negative effects of elevated GC levels were also of short duration. [J PA Acad Sci 88(2): 84-88, 2014]

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INTRODUCTION

The movement of animals to different enclosures or facilities can be stressful, but frequently occurs with non-human primates used in research (Kim et al. 2005). The physiological stress response begins with the activation of the hypothalamic-pituitary-adrenal (HPA) axis, which causes the release of glucocorticoids (GC) from the adrenal cortex. At chronic levels, elevated GC may lead to deleterious physiological effects. Hormone profiles can be accurately monitored through the use of fecal steroid assays, offering a noninvasive means of assessing GC hormones in primates and other animals (Ziegler and Wittwer 2005). Feces can be collected without restraint or immobilization, making GC analysis increasingly popular in studies of both captive and free-ranging animals (Whitten et al. 1998). A substantial amount of research conducted by primatologists has compared the influence of reproductive status, and rank and aggression to GC levels in primates (Czoty et al. 2009; Hoffman et al. 2010). However, few studies have investigated the effects of transport on GC levels in primates. The intent of this study was to measure fecal GC levels during pre- and post-transport periods in cotton-top tamarins (*Saguinus oedipus*) transported between captive facilities as an assessment of stress levels.

MATERIALS AND METHODS

Animal husbandry

This study involved eight cotton-top tamarins (four females and four males) obtained from the Department of Psychology at the University of Wisconsin, Madison, WI. The tamarins were born into a captive colony at the University of Wisconsin and were used in non-invasive hormonal,

behavioral and cognitive studies. Animal age varied from three to thirteen years (Table 1). The tamarins were transported to Frostburg State University (FSU), Frostburg, MD on June 25, 2008 for continued involvement in similar non-invasive research. Facilities and animal husbandry methods were similar at both institutions. Animals were housed in the same male-female pairs in 1.60 x 2.36 x 0.93 m cages made of black polyurethane-coated steel mesh on anodized aluminum framing. Cages were spaced 0.46 m apart and opaque fabric sheets prevented physical and visual contact among the pairs. Each of the cages contained natural tree branches, platforms, and a nest box. Cage floors were covered with pine shavings, which were replaced weekly. Cage platforms were sanitized once a week. Temperature was maintained between 25.5-28.3 °C, and full-spectrum overhead fluorescent lighting was set on 12 hour light/dark cycle. Water was given ad libitum and animals were fed three times per day. No physical restraint or handling of the tamarins occurred in fecal sample collection for this study. This study was conducted in accordance with the guidelines laid down by the U.S. Office of Laboratory Welfare and the research was approved by the Institutional Animal Care and Use Committee from each respective University.

Transport

On the night prior to transport to FSU, the tamarins were removed from their cages and placed in medium-sized animal carriers (36.8 x 58.4 x 29.2 cm). Transit time was approximately 12 hours—two and half hours by vehicle to the airport, a flight of six hours, and another three and a half hours by vehicle from the airport to FSU. A tree branch and platform were placed in the carrier so the tamarins could remain elevated. Tamarins were given grapes, New World Monkey Chow (Zupreem, 10504 W 79th St., Shawnee, KS) prior to the flight, and water (in the form of a gelatin) in flight. A sheet covered the cage at most times to reduce the amount of visual stress.

Fecal collections

Samples were collected from each animal between 9:00 and 10:00 AM immediately after defecation. Date, collection time, freeze time, and unusual circumstances (e.g., medication given or any disturbances to their schedule) were recorded during sampling. Samples were frozen at -20º C within two hours of collection. Samples were collected at four different time points. The first (Time 1) was 17-18 days pre-transport. The second (Time 2) was one to two days pre-transport, the third (Time 3) was three to six days post-transport and the final (Time 4) was 9-21 days post-transport. Fecal samples were stored at -80 °C within three weeks of collection and prior to processing. Also, all of the samples were processed within eight months of final sample collection.

Steroid extraction

Steroids were extracted from the scat following Wasser et al. (2000) with modifications. Briefly, samples were lyophilized using a VirTisTM Bench Top K Series Freeze Dryer (SP Industries, Inc., Gardiner, NY). Samples were pulverized thoroughly using a mortar and pestle, then sifted through a screen sieve (#60) to remove vegetative and other large particulate matter (Millspaugh et al. 2002; Washburn and Millspaugh 2002). Five mL of 95% ethanol was added to each 15 mL glass screw cap centrifuge tube (Kimble Glass, Inc., Vineland, NJ) containing 0.1 g of sample. The glass tube was placed in a beaker of boiling water and heated for 20 minutes with additional ethanol added as needed to maintain a volume of 5.0 mL. The extracted sample was centrifuged for 20 minutes at 500 x g and the supernatant fluid removed to a new 15 mL tube. The pellet was resuspended in 5.0 mL of 95% ethanol, vortexed for 1 minute, and centrifuged as before. The supernatant fluids were pooled for further processing. The ethanol was removed and the extracts were dried using a CentriVap Mobile System (Labconco; Kansas City, MO). Dried samples were stored at -20 °C prior to assay.

Assay procedure

The Correlate-EIA™ Corticosterone Enzyme Immunoassay Kit (Assay Designs, Inc.; Ann Arbor, MI) was used to determine the GC levels in the extracted fecal samples. Manufacturer's instructions were followed with the following modification. The extracted samples were dissolved in 2.0 mL of assay buffer and diluted 1:20 before performing the assay. A standard curve for each plate was created using corticosterone concentrations of 20,000, 4,000, 800, 160, and 32 pg/mL. All samples were assayed in duplicate. A SPECTRAmax Microplate Spectrophotometer (Molecular Devices Corporation; Sunnydale, CA) was used to determine absorbance at 405 and 650 nm. If a sample was outside the linear portion of the standard curve, the sample was further diluted an additional 1:10 and re-assayed.

Data analysis

The suitability of the Correlate-EIA™ Corticosterone assay in determining GC levels in cotton-top tamarins was evaluated by initially performing a parallelism assay (Zar 2010). The difference between the slope of a serially diluted kit standard and the slope of a serially diluted pooled sample of five randomly chosen fecal samples was determined using

a Student's t-test. Validation would be demonstrated by failing to detect a significant difference between the two slopes indicating the assay as being appropriate for assessing GC levels in tamarins.

A repeated measures Analysis of Variance was performed to compare average GC levels among the four transport periods by sex, and to assess interactions between these two factors. Tukey HSD pairwise comparisons were examined at a studentized range critical value of 4.02 (0.05, 4, 17) (Zar 2010). STATA (StataCorp LP; College Station, TX) was used for all statistical comparisons. Statistical tests were considered to be significant at an alpha level < 0.05.

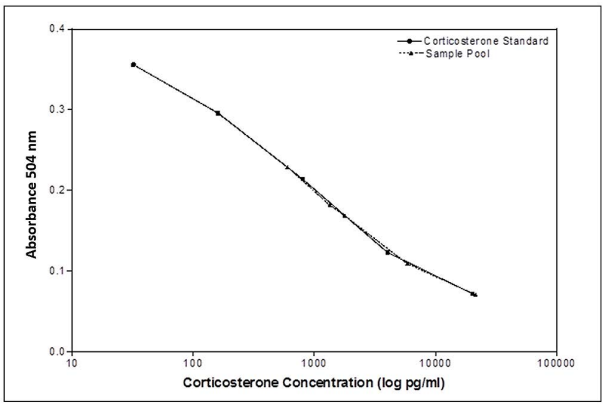

Figure 1. Parallelism between the Correlate-EIA™ Corticosterone Enzyme Immunoassay Kit (Assay Designs, Inc.; Ann Arbor, MI 48108, USA) standard curve and serially diluted pooled random sample conducted at Frostburg State University, 2010.

RESULTS

Kit validation

There was no significant difference between slopes of the serially diluted standard and the pooled sample. Therefore, the Correlate-EIA™ Corticosterone Enzyme Immunoassay Kit was validated for use with cotton-top tamarins (p = 0.350) (Fig. 1).

Transport and GC levels

GC levels differed among transport times (p = 0.009), but not by sex (p = 0.963). Tukey HSD pairwise comparisons demonstrated significant differences between Times 1 and 3, Times 2 and 3, and Times 3 and 4 (all p < 0.05). Five of the eight tamarins had increased GC levels after transport, one had decreased GC levels, and two showed relatively little change (Table 1). Seven of the eight tamarins showed decreased GC levels from Time 3 to Time 4, with most values returning to pre-transport values by Time 4. The mean post-transport level of 952.70 (SE =241.50) ng/g dried feces and was about four times that of Time 1 and Time 2, and about three times that of Time 4.

Table 1. Glucocorticoid (GC) concentrations (ng/g dried feces) for eight cotton-top tamarins transported from the University of Wisconsin-Madison to Frostburg State University on 6/25/08. Times 1 and 2 represent pre-transport GC levels (about 17-18 days and 1-2 days, respectively, prior to transport) and Times 3 and 4 represent post-transport GC levels (about 3-6 days and 9-21 days, respectively, after transport).

Tamarin	Sex	Cage mate	Age	Time 1 GC concentration (ng/g dried scat)	Time 2 GC concentration (ng/g dried scat)	Time 3 GC concentration (ng/g dried scat)	Time 4 GC concentration (ng/g dried scat)
GA	F	QE	13	80.61	232.92	107.64	235.55
QE	M	GA	3	238.63	270.67	778.95	N/A
IY	F	WN	4	74.60	59.02	1072.54	524.76
WN	M	IY	8	107.23	147.22	287.72	237.74
ZT	F	YI	11	10.64	126.59	1955.31	413.15
YI	M	ZT	5	31.96	442.73	1149.85	336.06
WA	M	VO	11	898.05	408.15	443.90	268.94
VO	F	WA	8	185.27	206.62	1825.68	218.94
F mean				265.98	206.71	894.85	360.60
M mean				140.77	266.81	1010.55	264.25
Overall mean				203.37	236.74	952.70	319.31

DISCUSSION

Non-human primates represent a small (estimated at <0.3%) but critical proportion of animals used in laboratory research in the United States (Capitanio et al. 2006). Of the approximately 1,800 cotton-top tamarins in captivity, 64% are housed in research laboratories (Savage et al. 1997). Many such research laboratories obtain animals from domestic breeding sources or other facilities which require shipment, often over large distances. Transport, along with environmental and social change, can constitute a long-term stressor for non-human primates, potentially leading to increases in GC levels. Chronically increased GC levels have been linked to deleterious physiological effects (Pride 2005; Sapolsky 1989).

This study demonstrated that the use of the Correlate-EIA™ Corticosterone Enzyme Immunoassay Kit is an appropriate technique for determining GC levels in cotton-top tamarins. This was demonstrated using a parallelism assay in which there was no difference between the slopes of a serially diluted standard and a serially diluted pooled sample.

Average GC concentrations exhibited an approximately four-fold increase from pre-transport to immediate post-transport levels (Time 3). These results are consistent with studies in other species (e.g., domestic pig (*Sus domesticus*), greyhound (*Canis familiaris*), and mouse (*Mus musculus*) (Kim et al. 2005; Nyberg et al 1988; Leadons and Mullins 1991; Tuli et al. 1995) that have detected higher GC concentrations following transport. Although tamarins showed a post-transport elevation in GC levels, this increase was transient. The immediate post-transport mean (Time 3) was significantly greater than all other times (Time 1, 2, and 4). However, GC levels returned to or near pre-transport level within three weeks. The large increase in GC levels directly after transport is presumed to be a stress response.

GC concentrations varied among individuals. Although the difference in GC levels between two individuals (WN and ZT) was large (Table 1), primates naturally exhibit two to 10 fold variation in GC levels, with daily fluctuations of $\geq$ 2.2 fold, depending on the species (Sapolsky 1992). Individual variation could due to previous exposure to stressors (Sapolsky et al. 2000). Although none of the subject animals had been transported across facilities before, previous experience with such stressors (e.g., movement to a new cage or room or veterinary procedures) may have altered the effects of transport for some individuals. For example, GA was moved to a new cage prior to transport and exhibited lower GC levels following transport than during baseline data collection.

Glucocorticoid levels may have been higher if the animals had not been transported in male/female pairs. Close proximity of a heterosexual partner has been shown to reduce the physiological consequences of novel-cage housing for black tufted-ear marmosets (*Callithrix kuli*) and other New World monkeys (Hennessy et al. 1995; Smith et al. 1998). In the callitrichid primates (marmosets and tamarins), there is a strong, long-term socio-sexual pair bond that exists between the breeding male and female in a social group (Evans 1983; Anzenberger 1993; Schaffner et al. 1995). If this close bond provides a form of support during stressful events, paired tamarins and marmosets may exhibit a reduced stress response. All of the tamarins in this study were transported with an established mate; therefore their physiological stress responses may have been socially mitigated. Regardless, there was clear evidence that transport induced increased GC levels among the majority of tamarins in the study.

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LITERATURE CITED

Anzenberger, G. 1993. Social conflict in two monogamous New World primates: pairs and rivals. In: Mason, W. A., and S. P. Mendoza (eds.). Primate Social Conflict. State University of New York Press, Albany, NY, 291-330.

Capitanio, J.P., R.C. Kyes, and L.A. Fairbanks. 2006. Considerations in the selection and conditioning of Old World monkeys for laboratory research: animals from domestic sources. ILAR J. 47:294-306.

Czoty, P.W., R.W. Gould, and M.A. Nader. 2009. Relationship between social rank and cortisol and testosterone concentrations in male Cynomolgus monkeys (*Macaca fascicularis*). J. Neuroendocrinol. 21:68-76.

Evans, S. 1983. The pair-bond of the common marmoset; *Callithrix jacchus*: an experimental investigation. Anim. Behav. 31:651-658.

Hennessy, M.B., S.P. Mendoza, W.A. Mason, and C.P. Moberg. 1995. Endocrine sensitivity to novelty in squirrel monkeys and titi monkeys: Species difference in characteristic modes of responding to the environment. Physiol. Behav. 57:331-338.

Hoffman, C.L., J.E. Ayala, A. Mas-Rivera, and D. Maestripier. 2010. Effects of reproductive condition and dominance rank on cortisol responsiveness to stress in free-ranging female rhesus macaques. Am J. Primatol. 72:559-565.

Kim, C.Y., J.S. Han, T. Suzuki, and S.S. Han. 2005. Indirect indicator of transport stress in hematological values in newly acquired cynomolgus monkeys. J. Med. Primatol. 34:188-192.

Leadons, D.P., and E. Mullins. 1991. Relationship between kennel size and stress in greyhounds transported short distances by air. Vet. Rec. 129:70.

Millspaugh, J.J., B.E. Washburn, M.A. Milanich, J. Beringer, L.P. Hansen, and T.M. Meyer. 2002. Non-invasive techniques for stress assessment in white-tailed deer. Wildl. Soc. Bull. 30:899-907.

Nyberg, L., K. Lundstrom, I. Edfors-Lilja, and M. Rundgren. 1988. Effects of transport stress on concentrations of cortisol, corticosteroid-binding globulin and glucocorticoid receptors in pigs with different halothane genotypes. J. Anim. Sci. 66:1201-1211.

Pride, E.R. 2005. High faecel glucocorticoid levels predict mortality in ring-tailed lemurs (*Lemur catta*). Biol. Letters 1:60-63.

Sapolsky, R.M. 1989. Hypercortisolism among socially subordinate wild baboons originates at the CNS level. Arch. of Gen. Psychiatry 46:1047-1051.

Sapolsky, R.M. 1992. Stress, the aging brain, and the mechanisms of neuron death. In: Becker, J., D. Crews, and M. Breedlove (eds.). Behavioral Endocrinology. Massachusetts Institute of Technology Press, Cambridge, MA, 287-324.

Sapolsky, R.M., M.L. Romero, and A.U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21:55-89.

Savage, A., S.E. Shideler, L.H. Soto, J. Causado, L.H. Giraldo, B.L. Lasley, and C.T. Snowdon. 1997. Reproductive events of wild cotton-top tamarins (*Saguinus oedipus*) in Columbia. Am. J. Primatol. 43:329-337.

Schaffner, C.M., R.E. Shepard, C.V. Santos, and J.A. French. 1995. Development of heterosexual relationships in Wied's black tufted-ear marmosets (*Callithrix kuhli*). Am. J. Primatol. 36:185-200.

Smith, T.E., B. McGreer-Whitworth, and J.A. French. 1998. Close proximity of the heterosexual partner reduces the physiological and behavioral consequences of novel-cage housing in black tufted-ear marmosets (*Callithrix kuhli*). Horm. Behav. 34:211-222.

Tuli, J.S., J.A. Smith, and D.B. Morton. 1995. Stress measurements in mice after transportation. Lab. Anim. 29:132-138.

Washburn, B.E., and J.J. Millspaugh. 2002. Effects of simulated environmental conditions on glucocorticoid metabolite measurements in white-tailed deer feces. Gen. Comp. Endocrinol. 127:217-222.

Wasser, S.K., J.L. Brown, K. Cooper, C.M. Crockett, U. Bechert, J.J. Millspaugh, S. Larson, and S.L. Monfort. 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gen. Comp. Endocrinol. 120:260-275.

Whitten, P.L., D.K. Brockman, and R.C. Stavisky. 1998. Recent advances in noninvasive techniques to monitor hormone-behavior interactions. Yearb. Phys. Anthropol. 41:1-23.

Zar, J.H. 2010. Biostatistical Analysis, 5th ed. Prentice-Hall, Inc, Upper Saddle River NJ, 960 pp.

Ziegler, T.E., and D.J. Wittwer. 2005. Fecal steroid research in the field and laboratory: improved methods for storage, transport, processing, and analysis. Am. J. Primatol. 67:159-174.