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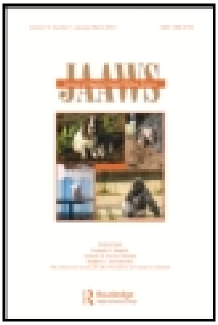

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Behavioral and Hormonal Consequences of Transporting Giant Pandas From China to the United States

Rebecca J. Snyder [a], Bonnie M. Perdue [a b], David M. Powell [c], Debra L. Forthman [b], Mollie A. Bloomsmith [b] & Terry L. Maple [b]

[a] Zoo Atlanta, Atlanta, Georgia

[b] Center for Conservation and Behavior, School of Psychology, Georgia Institute of Technology

[c] Smithsonian Conservation Biology Institute, National Zoological Park, Washington, DC

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ARTICLES

Behavioral and Hormonal Consequences of Transporting Giant Pandas From China to the United States

Rebecca J. Snyder,[1] Bonnie M. Perdue,[1,2] David M. Powell,[3] Debra L. Forthman,[2] Mollie A. Bloomsmith,[2] and Terry L. Maple[2]
[1]*Zoo Atlanta, Atlanta, Georgia*
[2]*Center for Conservation and Behavior, School of Psychology, Georgia Institute of Technology*
[3]*Smithsonian Conservation Biology Institute, National Zoological Park, Washington, DC*

Zoological institutions strive to ensure the welfare of nonhuman animals in captivity. Part of this effort involves reducing the level of distress experienced by an animal to the greatest extent possible. However, some necessary zoo management practices such as transportation induce stress responses. An extensive literature exists concerning the animal welfare implications of road transportation for farm and laboratory animals. There has, however, been little focus on the effects of air transportation on wild animals in captivity. Because many endangered species are transported by air for breeding purposes, it is especially important to study the effects of stress on these species. This study investigated the behavioral and hormonal consequences of transporting 4 giant pandas (2 male–female pairs) by air from China to the United States. An autoregressive test revealed that urinary

Correspondence should be sent to Bonnie M. Perdue, School of Psychology, Georgia Institute of Technology, 654 Cherry Street, Atlanta, GA 30332. Email: bonnie.perdue@gatech.edu

cortisol measures were highest for 2 subjects, Lun Lun and Tian Tian, during the flight than during the remainder of the 30-day period posttransport ($p < .01$). No long-term behavioral changes or problems emerged as a result of the transport. The study found that more research is needed to develop a complete understanding of transportation stress and welfare in captive wildlife.

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Zoological institutions strive to ensure the welfare of nonhuman animals in captivity. However, defining and measuring good welfare is challenging and is typically based on the absence of poor welfare indicators (Swaisgood, 2007). For example, a lack of stereotypic behavior and baseline stress hormone levels are common measures of "good" welfare. This conclusion is often made because indicators of poor welfare, such as stereotypies and increased levels of cortisol, are more easily identified and can be generally related to stress (Swaisgood, 2007). Stress is considered compromising to welfare because long-term stressors cause significant changes in behavior and physiology (Morgan & Tromberg, 2007) and may impair immunological and reproductive function.

Stress response in captive animals can be defined as "the behavioral and physiological adjustments that an organism undergoes to avoid or adapt to a perceived threat that challenges internal homeostasis" (Moberg, 2000, as cited in Swaisgood, 2007, p. 141). Animals have evolved physiological and behavioral mechanisms to respond to environmental demands and stressors (Morgan & Tromberg, 2007). According to Moberg (1985), three types of biological responses are available to an animal trying to maintain homeostasis while experiencing a threatening stimulus or stressor: behavioral, autonomic, and neuroendocrine. Many studies have measured aspects of all three categories, such as activity levels, heart rate, catecholamines, and cortisol. It is important to use multiple measures in assessing stress because individuals might exhibit similar neuroendocrine responses, yet different behavioral responses, to the same stressor.

For zoo researchers, a relevant advance in endocrinology is the development of techniques that measure physiological responses to stress noninvasively (Wasser et al., 2000). Corticosteroid levels, an indicator of hypothalamic-pituitary-adrenal activation, can be measured in feces or urine. Behavior is another useful indicator of stress for zoo researchers, not only because it is easy to measure but also because it gives an accurate assessment of when an animal perceives changes in the environment (Swaisgood, 2007).

It is an oversimplification to view stress as negative. In fact, an acute stress response can be adaptive for an animal. It helps an animal maintain homeostasis and adapt to environmental stressors (Morgan & Tromberg, 2007). However, chronic or unpredictable stressors may also induce changes in an animal's biological function that result in immunological or reproductive suppression (Broom & Johnson, 1993; Carlstead, 1996; Moberg, 1985; Morgan & Tromberg,

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2007). Moberg (1985) argued that these consequences of stress should be used to assess risks to well being. Zoos should monitor potential stressors to ensure that they do not result in a long-term or chronic stress response that compromises animal welfare and suppresses reproduction, particularly in endangered species who are involved in captive breeding programs.

In a review of sources of stress in captive settings, Morgan and Tromberg (2007) identified a variety of potential stressors such as sound, lighting, odor, temperature, restricted movement, lack of retreat space, visitors, abnormal social grouping, and lack of control. One area not discussed in this review, and similarly overlooked in the zoo literature, is transportation—though it includes many of the potential stressors that were reviewed. In the animal welfare literature, it is well recognized that transportation, which involves an abrupt and dramatic change in environment, is a common cause of animal distress (Fraser, 1979). However, the effect of transportation on animals in the zoo has not been well studied (Dembiec, Snider, & Zanella, 2004). For zoo-housed animals, interinstitution transportation, and even relocation within an institution, is a common occurrence. It may be particularly important to assess this practice in zoo animals because we might expect wild animals in captivity to have a stronger stress response to confinement and movement associated with transportation than domestic species. Also, endangered animals are often transported between zoos for breeding purposes to maintain the genetic variability of the captive population. It is especially important to monitor the stress response of these animals because stress can affect reproduction (von Borell, Dobson, & Prunier, 2007). For many animals, transportation may result in an acute stress response that allows an animal to adapt to the abruptly changing environment, but any long-term consequences of transportation need to be carefully analyzed as a possible threat to welfare.

There is an extensive and detailed literature concerning the animal welfare implications of transportation and varied parameters of pre- and posttransport management of farm animals, including pigs (Stephens & Perry, 1990; Warriss, 1998), sheep (Baldock & Sibly, 1990; Parrott, Hall, & Lloyd, 1998; Parrott, Lloyd, & Brown, 1999), cattle (Tarrant, 1990), horses (Stewart, Foster, & Waas, 2003), and deer (Grigor, Goddard, Littlewood, & Macdonald, 1998; Weeks, 2000). Studies of transportation have also been conducted with animals in the laboratory, most notably mice and rats (Hayssen, 1997; Tuli, Smith, & Morton, 1995; van Ruiven et al., 1998). Wolfensohn (1997) has reviewed some of the sparse literature on transportation effects on primates. A small number of studies have empirically examined the effect of transportation on captive wildlife to evaluate this necessary component of zoo management, including tigers (Dembiec et al., 2004), elephants (Laws et al., 2007), and spotted hyenas (Goymann, Mostl, Van't Hof, East, & Hofer, 1999). With the exception of some of the horse, rodent, and primate literature, all of these studies pertain to road transportation.

All transportation stress authors are in agreement that myriad variables, from genetics to time since last feeding, influence the degree and nature of an animal's response to transportation, and all emphasize the importance of species differences in behavioral and physiological reactions, even within a taxon (Wolfensohn, 1997). Somewhat surprising is the general consensus that the acute phase of loading and unloading animals during transportation is less distressing than the period of confinement in a moving vehicle, when elevations in heart rate and cortisol were typically highest (Parrott et al., 1998; Tarrant, 1990). Although some animals appear to habituate during the course of lengthy transport to certain stimuli associated with the process, they may sensitize, or become more vulnerable to the effects of other stressful stimuli. Pigs, for example, did not habituate to high-intensity noise and vibration. When given control over noise and vibration via operant training, they more frequently turned off such stimulation near the end of a trial (Stephens & Perry, 1990). Most studies showed that animals recovered relatively quickly from the acute effects of transportation stress as long as they were not subjected to overcrowding or high temperatures, which were associated with injuries and fatalities. Increased resting, feeding, and drinking were common posttransport reactions. Emphasis was placed on the fact that management techniques—including handling, transport design, space allowances, driver skill, and rest periods—have considerable influence on the degree of stress that animals experience.

In contrast to the case of farm animals, captive wildlife transportation is sometimes over long distances by air. A far more diverse set of wildlife than domestic species is subjected to transportation, and individual zoo animals may be transported multiple times during their lifetime. More studies of transportation in varied species of wildlife are needed, as these may contribute to our ability to minimize the distress associated with this common management procedure and improve the welfare of captive wildlife. Zoological researchers should apply the welfare lessons already learned in other arenas (Swaisgood, 2007) while continuing to investigate issues pertinent to some zoo animals, such as air transportation.

We studied the effects of air transportation on four giant pandas (*Ailuropoda melanoleuca*). Giant pandas are an endangered species (International Union for Conservation of Nature, 2011), and captive breeding is a major focus of conservation efforts (Ellis, Pan, Xie, & Wildt, 2006). Additionally, China loans giant pandas to institutions in other countries to generate resources for conservation (Ellis et al., 2006). The loan program and captive breeding program necessitate transportation of giant pandas by air, but no previous studies have assessed how giant pandas respond to this form of transportation. When two pairs of giant pandas were transported from China to the United States for loans, we measured behavior during a 30-day period (before and after transportation) and urinary cortisol concentrations during transportation and for 30 days posttransport to assess how these animals responded to transport and to their new environment.

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METHODS

Subjects

The subjects were 2 male and 2 female subadult giant pandas. All subjects were 2 to 3 years of age. Female Lun Lun (studbook #452, birth date August 25, 1997), and male Yang Yang (studbook #461, birth date September 9, 1997), were born and housed at the Chengdu Research of Giant Panda Breeding (Chengdu Research Base) in Sichuan, People's Republic of China (PRC) until shipment to Zoo Atlanta, Atlanta, Georgia, on November 5, 1999. Female, Mei Xiang (studbook #473, birth date July 22, 1998), and male, Tian Tian (studbook #458, birth date August 27, 1997), were born and housed at the China Conservation and Research Center for the Giant Panda in Wolong, Sichuan, PRC, until shipment to the National Zoological Park, Washington, DC, on December 6, 2000.

Diets for all subjects remained the same throughout data collection for this study except that different species of bamboo were provided prior to and after transport. The bamboo species fed in China were not available in the United States, and so the U.S. institutions provided other bamboo species that giant pandas are known to eat. Thus, although the amount of fresh bamboo offered remained the same in the pre- and posttransport conditions, the subjects did have to adjust to eating different species of bamboo.

At the Chengdu Research Base, Lun Lun and Yang Yang were housed together for 1 year prior to transport. For the 30-day period before transport, they were housed together in an outdoor enclosure measuring 3,460 m^2 from 0800 to 1700. From 1700 to 0800, they were housed together in an indoor enclosure measuring 70 m^2. They were on exhibit to visitors while they were in the outdoor enclosure. At Zoo Atlanta, they were housed together in four adjacent indoor dens measuring a total of 40 m^2 for the 1st week after their arrival. For the remainder of the data collection period, they were housed in these dens from 1700 to 0800 and given access to two large indoor day-rooms (126 m^2 total) or two outdoor enclosures (557 m^2 total) from 0800 to 1700. The indoor temperature was maintained at approximately 15°C, and the subjects always had access to indoor space that could not be viewed by visitors.

At the China Conservation and Research Center for the Giant Panda, Tian Tian and Mei Xiang were housed together during the day for 4 months and separated at night. For the month prior to transport, they were housed separately for quarantine until 3 days before transport when they were reunited and housed together again during the day. While housed apart, they were in adjacent enclosures with auditory, visual, and olfactory access to each other. Tian Tian occupied an outdoor enclosure measuring approximately 250 m^2 and an indoor den approximately 10 m^2, and Mei Xiang occupied an outdoor cement enclosure approximately 20 m^2 and an indoor den, approximately 10 m^2, 24 hr per day. Three days prior to transport, Mei Xiang was introduced to Tian Tian in his enclosure. When reunited, the subjects reacted affiliatively to each other and engaged in a play bout shortly after the reunion. When they chose to be outdoors, they could be viewed by visitors. At the National Zoo, the pandas were housed together from 0700 to approximately 1500 in two indoor day rooms (96 m^2 total) with two adjoining dens (20 m^2 total). From 1500 to 0700 the pandas were separated and housed in one of the day rooms with an adjoining den. The indoor temperature was maintained at approximately 15°C and the subjects always had access to indoor space that could not be viewed by visitors.

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Transport Procedure

Subjects transported to Zoo Atlanta. Lun Lun and Yang Yang were transported individually in steel shipping crates. Each crate measured 152 cm × 83 cm × 92 cm. The crates were placed in their indoor enclosure 19 days prior to the shipment date. The pandas were sometimes fed in the crates and were allowed to explore them. They were never locked in the crates until the day of shipment, which was November 4, 1999. On this day, at 0700, they were baited into the crates with food and locked inside. The crates were driven by truck approximately 25 km to Chengdu International Airport. The duration of road transport was about 60 min. They were manually loaded onto a hydraulic lift and then onto a commercial airplane to Beijing. They were on the plane for 1.5 hr before takeoff. The duration of the flight from Chengdu to Beijing was approximately 2 hr. In Beijing, the subjects were deplaned using a hydraulic lift and loaded onto a baggage trolley. Then they were transferred to, and loaded via, hydraulic lift onto an empty cargo plane. Because of flight schedules, they waited on the cargo plane for 17 hr before takeoff and then flew 8 hr from Beijing to Anchorage, Alaska. There was a 2-hr layover in Anchorage for refueling and inspection by the United States Fish and Wildlife Service, the United States Department of Agriculture, immigration and customs. The subjects then flew 5.5 hr from Anchorage to Atlanta. In Atlanta, the pandas were unloaded from the plane and loaded into vans using a hydraulic lift. They were driven 16 km to Zoo Atlanta, a journey of approximately 20 min. Finally, the pandas were unloaded from the vans using a hydraulic lift and released into indoor enclosures in the giant panda building at approximately 1100 (Eastern time) on November 5. The duration of the entire journey was approximately 40 hr.

Throughout the entire transportation procedure, three veterinarians accompanied the subjects; two of these individuals were familiar to the subjects. They were given water, fresh bamboo, and a milk-based gruel (their regular diet at the Chengdu Research Base) routinely throughout the trip. They were the only cargo on the plane from Beijing to Atlanta. Temperature and humidity in the cargo bay were adjusted according to observations of the subjects' behavior made by the veterinarians. The temperature was maintained between 10 and 15.5°C.

Subjects transported to National Zoological Park. On December 6, 2000, Mei Xiang and Tian Tian were also transported individually in steel shipping crates measuring 189 cm × 139 cm × 127 cm. On the day of transport, the pandas were lured into smaller rolling crates using food rewards and then shifted into the larger, overseas transport crates. The crates were loaded by hand onto trucks and driven to the Chengdu International Airport; the duration of the road transport was about 3 hr. They were flown from Chengdu to Anchorage, Alaska, where there was a 2-hr layover for refueling and inspection by the United States Fish and Wildlife Service, United States Department of Agriculture, immigration and customs. The pandas were provided with fresh water, bamboo, apples, and leaf eater biscuits throughout transport and maintained at a temperature of approximately 10°C. The pandas were then flown to Dulles International Airport in Virginia, transferred by hydraulic lift to enclosed cargo trucks, and driven to the National Zoo. The total transport time was approximately 24 hr. The pandas were unloaded at the zoo and released into indoor enclosures at approximately 1700 on December 6.

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DATA COLLECTION

Behavioral Data

For subjects Lun Lun and Yang Yang, a total of 21 hr of behavioral data were collected during the 30-day period prior to transport, and 38 hr were collected in the 30-day period after transport. For subjects Mei Xiang and Tian Tian, a total of 42 hr of behavioral data were collected during the 30-day period prior to transport, and 42 hr were collected in the 30-day period after transport. Instantaneous sampling at 1-min intervals was used to record behavioral data (Altmann, 1974; Crockett, 1996). Behaviors recorded were as follows: inactive, active, stereotypic, feed, and social (see Table 1). Behavioral observations were conducted from 0900 to 1400 at Zoo Atlanta and from 0800 to 1800 at National Zoological Park and were balanced across those hours. Two observers collected behavioral data on Lun Lun and Yang Yang. One observer collected behavioral data on Mei Xiang and Tian Tian. Interobserver reliability was measured for the three observers using the index of concordance (Martin & Bateson, 1993). Observers were considered reliable with each other when the

TABLE 1
Behaviors Included in Each Category

Behavior Category	*Zoo Atlanta*	*National Zoo*
Inactive	Rest, stationary alert	Rest, stationary alert
Active	Climb, carry object, groom, locomote, locomotor play, object exam, olfactory investigate	Climb, groom, walk, locomotor play, object play, moving explore, stationary explore, run
Stereotypic	Pace, self suck	Pace, other stereotypic
Feed	Feeding on bamboo, biscuits, produce, vegetation, or other	Feeding on bamboo, biscuits, produce, vegetation, or other
Social	Play-fight, sexual	Friendly, aggressive, sexual

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index of concordance was greater than 0.85 for all behaviors. Paper and pencil were used to collect behavioral data. A stopwatch provided an auditory cue for instantaneous sampling at 1-min intervals.

Cortisol Data

Urine samples were aspirated from enclosure floors using a clean, dry syringe. Approximately 2 ml of urine were collected for each sample. An effort was made to collect samples that were not contaminated by water, feces, or debris. Each sample was placed in a polypropylene vial labeled with the animal's name, date, and time of collection. Samples were stored at −20°C until analyzed. Samples were collected approximately daily in the 30-day period prior to transport, during transport, and in the 30-day period after transport. Samples collected prior to transport were lost before being shipped to the United States for assay. Therefore, only samples collected during and after transport were assayed.

One sample was collected from Lun Lun and 1 sample was collected from Yang Yang during the flight from Beijing to Anchorage. Twenty-four samples were collected from Yang Yang and 26 samples were collected from Lun Lun posttransport. One sample from Tian Tian was collected during air transport; no samples were recovered from Mei Xiang during transport. Sixteen samples were collected for Mei Xiang and 11 samples were collected for Tian Tian posttransport.

Urine samples from all subjects were measured for urinary corticoids using radioimmunoassay at the Center for Reproduction of Endangered Species, Zoological Society of San Diego. Cortisol values are reported in nanograms per milligram of creatinine. Details about the assay procedure used are provided in Owen, Swaisgood, Czekala, Steinman, & Lindburg (2004).

STATISTICAL ANALYSIS

Behavioral Data

Because problems with pseudoreplication (using multiple data points from 1 subject as independent data points) and data pooling (combining nonindependent data; Kuhar, 2006) would have been prominent if inferential statistics were applied to the behavioral data, we instead chose to report descriptive statistics—means and standard errors—for pre- and posttransport behavioral data. Directional change was determined through visual inspection of the plotted data.

Cortisol Data

To examine the pattern of change in cortisol concentrations during the transport and posttransport period, data were analyzed using an autoregression procedure (Martin & Bateson, 1993). This method examines the change in a dependent measure over time above and beyond the correlation that generally occurs between temporally related data points. In other words, data points that are consecutive in time may be correlated simply because of their temporal relationship. The autoregressive method controls for a potential lack of independence between time points by including an autocorrelation predictor (Rho) in the analysis.

Cortisol values were expected to be higher during transportation (Days 1–2 for Zoo Atlanta and Day 1 for National Zoo) than during the posttransport period (Days 3–30 for Zoo Atlanta and Days 2–30 for National Zoo).

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RESULTS

Table 2 presents the mean percentage of time subjects engaged in behaviors before and after transport. The female subjects (Lun Lun and Mei Xiang) spent more time active after transport, whereas the male subjects (Yang Yang and Tian Tian) spent less time active after transport (Figure 1). Subsequently, the male subjects displayed increases in inactive behavior after transport (Figure 2). Only 2 subjects, Lun Lun and Tian Tian, engaged in stereotypic behavior before or after transport. They both exhibited a decrease in the mean percentage of time engaged in stereotypic behavior after transport (Figure 3; Lun Lun: pre = 1.9% to post = 0.6%; Tian Tian: pre = 20.5% to post = 1.3%). Two subjects, Lun Lun and Tian Tian, increased their time spent feeding following transportation, whereas Yang Yang and Mei Xiang exhibited a decrease in the percentage of time spent feeding (Figure 4). There was an increase in social behavior for Mei Xiang and Tian Tian after transport to the National Zoological Park (Figure 5),

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TABLE 2
Mean Percentage of Time Subjects Performed Each Behavior Category Pre- and Posttransport

	Mei Xiang				*Tian Tian*				*Lun Lun*				*Yang Yang*			
	Pre		*Post*		*Pre*		*Post*		*Pre*		*Post*		*Pre*		*Post*	
Behavior	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Inactive	44.9	7.4	35.1	6.1	29.8	4.8	45.9	10.5	64.2	7.0	65.1	5.2	54.7	7.3	76.6	4.6
Active	18.4	4.2	23.0	3.1	23.1	4.3	10.9	1.9	16.6	4.2	18.4	3.0	11.3	3.7	9.3	1.9
Stereotypic	0.0	0.0	0.0	0.0	20.5	4.2	1.3	0.9	1.9	1.3	0.6	0.4	0.0	0.0	0.0	0.0
Feed	34.7	7.8	31.0	3.0	18.4	3.3	32.5	7.0	0.9	0.4	9.1	3.4	12.0	5.0	5.8	2.8
Social	0.0	0.0	5.9	2.8	2.6	2.6	6.2	3.5	8.8	3.7	4.2	1.5	20.3	5.9	5.3	2.2

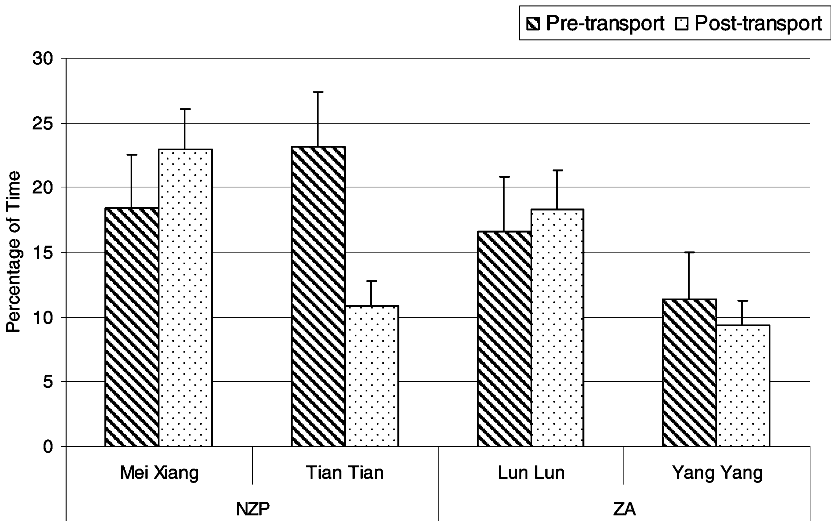

FIGURE 1 Mean percentage of time subjects were active pre- and posttransport. NZP = National Zoological Park, ZA = Zoo Atlanta.

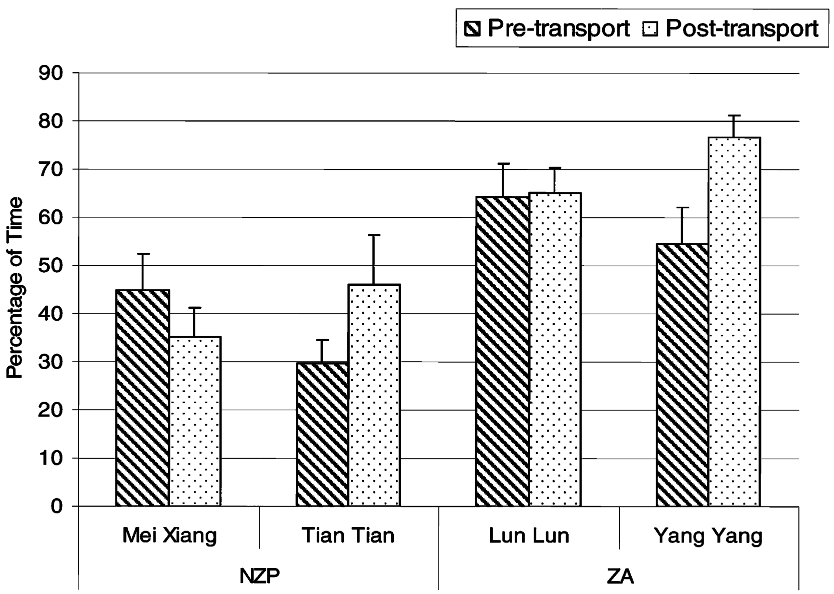

FIGURE 2 Mean percentage of time subjects were inactive pre- and posttransport. NZP = National Zoological Park, ZA = Zoo Atlanta.

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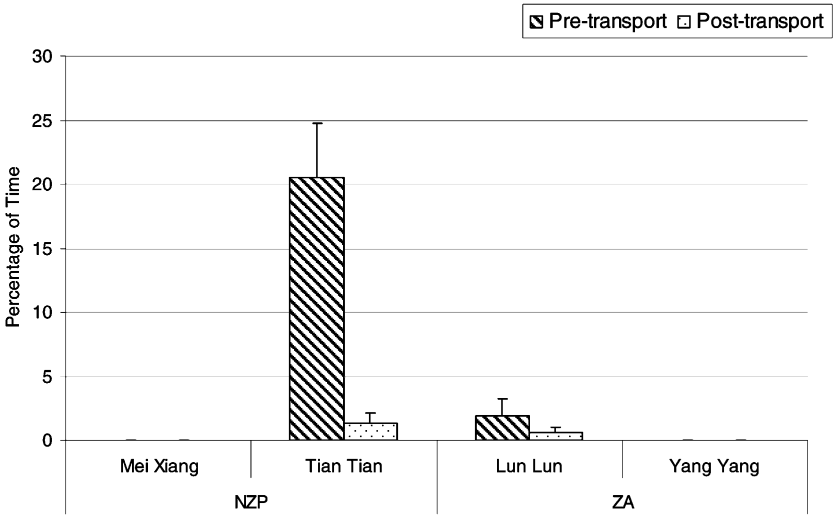

FIGURE 3 Mean percentage of time subjects engaged in stereotypic behavior pre- and posttransport. NZP = National Zoological Park, ZA = Zoo Atlanta.

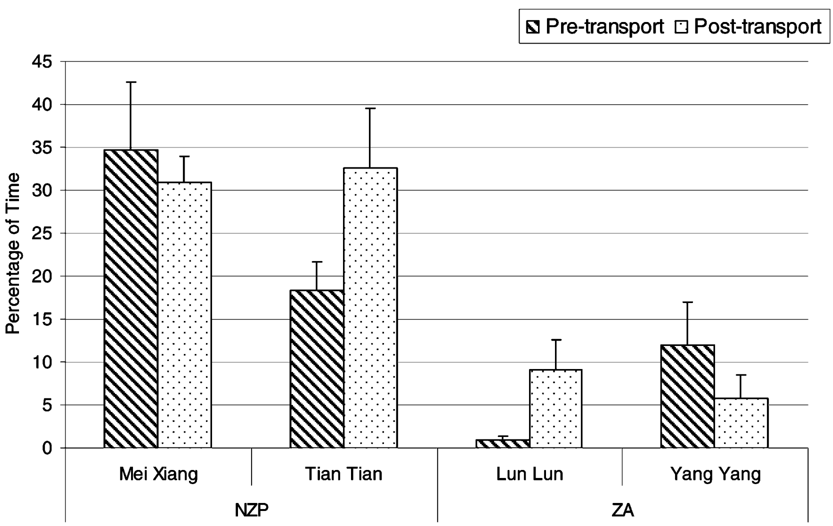

FIGURE 4 Mean percentage of time subjects fed pre- and posttransport. NZP = National Zoological Park, ZA = Zoo Atlanta.

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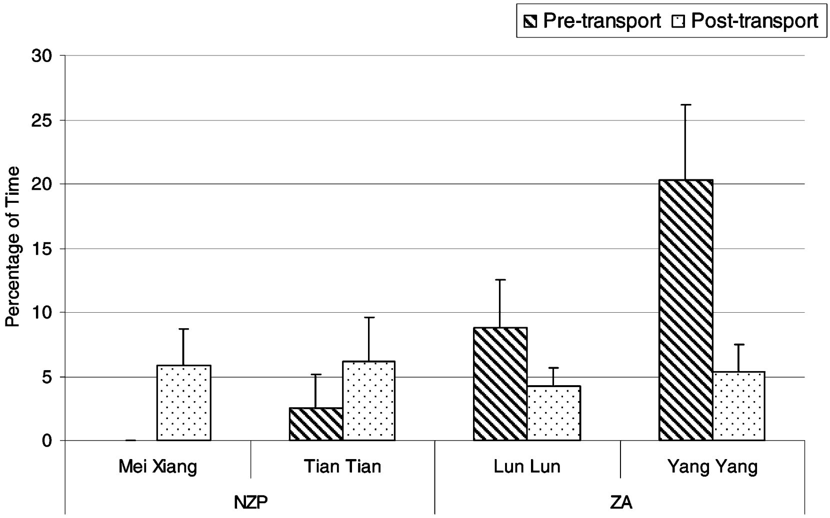

FIGURE 5 Mean percentage of time subjects engaged in social behavior pre- and posttransport. NZP = National Zoological Park, ZA = Zoo Atlanta.

although only one observation session was conducted on Mei Xiang and Tian Tian after they were introduced in China, and Tian Tian was the focal animal for that session; there are no data on social interaction for Mei Xiang prior to transport. Thus, these results should be interpreted with caution. Conversely, there was a decrease in social behavior for the subjects transported to Zoo Atlanta.

The independent measure "transport" was a significant predictor of "cortisol" at the $\alpha = .05$ level for subjects Lun Lun ($p \approx .000$, Figure 6) and Tian Tian ($p \approx .003$, Figure 7). Thus, their cortisol concentrations were significantly higher during the transportation period than during the period after transportation. Yang Yang displayed a more variable pattern of cortisol (Figure 6), and "transport" was not a significant predictor of "cortisol" at the $\alpha = .05$ level. A urine sample was not collected for subject Mei Xiang during transportation, so her data were not analyzed statistically. Following transport, she displayed a relatively flat cortisol pattern (Figure 7).

Over the 30 days following transportation, average cortisol levels were lower for Zoo Atlanta subjects (Lun Lun: $M = 100.7$ ng F/mg Cr; Yang Yang: $M = 98.5$ ng F/mg Cr) than National Zoo subjects (Mei Xiang: $M = 217.2$ ng F/mg Cr; Tian Tian: $M = 295.2$ ng F/mg Cr) (Table 3). Variability was similar for Lun Lun ($SE = 15.0$), Yang Yang ($SE = 11.3$), and Mei Xiang ($SE = 17.4$), whereas cortisol levels varied more for Tian Tian ($SE = 62.8$; Table 3).

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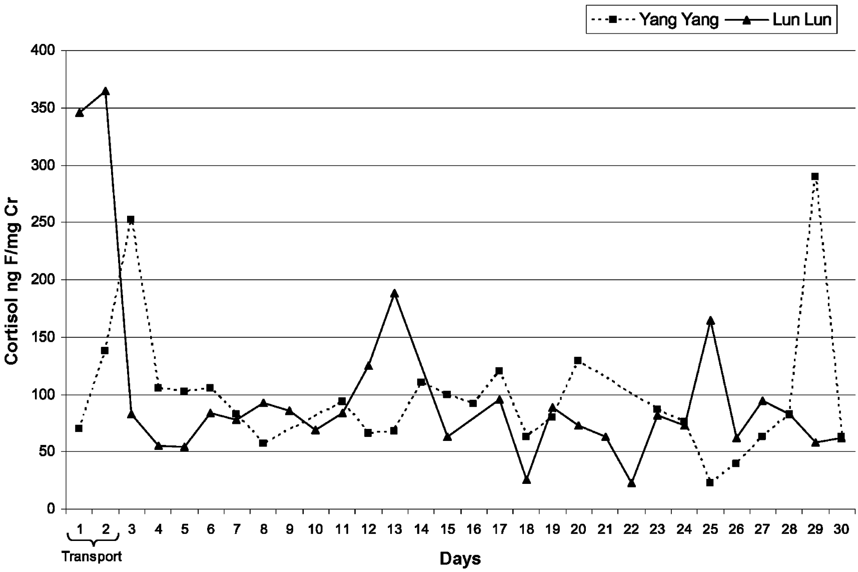

FIGURE 6 Urinary cortisol concentration for Lun Lun and Yang Yang during (i.e., first two data points) and following transport to Atlanta.

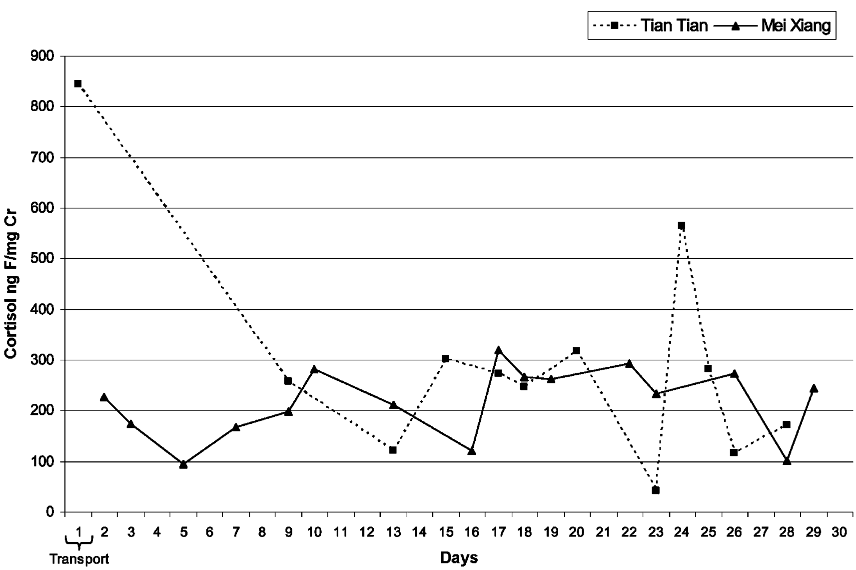

FIGURE 7 Urinary cortisol concentration for Mei Xiang and Tian Tian during (i.e., first data point) and following transport to Washington, DC.

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TABLE 3
Mean, Standard Error, and Range of Cortisol (ng F/mg Cr) for All Subjects

Subject	*M*	*SE*	*Range*
Mei Xiang	217.2	17.4	94.7–320.2
Tian Tian	295.2	62.8	42.9–845.3
Lun Lun	100.7	15.0	23.0–364.3
Yang Yang	98.5	11.3	22.7–298.9

DISCUSSION

This study is the first to investigate the influence of transportation stress in giant pandas and reports the patterns of behavioral and physiological change exhibited by 4 individuals following transfer from China to the United States. Our findings are generally consistent with the findings from other studies of the effects of transportation. For example, we observed that patterns of behavioral change varied across individuals, which is similar to previous research assessing transportation in captive wildlife (Dembiec et al., 2004). However, we observed some relevant and interesting patterns of behavior change.

The 2 subjects who engaged in stereotypic behaviors exhibited a decrease in the time spent engaged in stereotypic behavior following transport. This finding is in contrast to a previous study in which giant pandas exhibited increased stereotypic behavior following increased cortisol levels (Liu et al., 2006). However, stress is just one possible cause of stereotyped behavior (Mason, Clubb, Latham, & Vickery, 2007) and may not be the underlying motivation in these individuals. For example, a lack of environmental stimulation may also lead to abnormal behaviors such as stereotypy (Broom, 1991), and novel scents and objects have been shown to significantly reduce stereotypic behavior (Meehan, Garner, & Mench, 2004). In the current study, the subjects were exposed to a wide variety of new sights, sounds, and scents during and after transport. It is possible that the novel environment may have alleviated an underlying cause of stereotypic behavior, lack of stimulation, and resulted in the observed decrease following transportation. However, more data are needed to fully investigate the observation that stereotypic behavior decreased following transportation.

The amount of time spent engaged in social behavior following transport was similar for all 4 subjects (range 4.2–6.2%). Mei Xiang and Tian Tian exhibited an increase in social behavior following transportation. This finding is not surprising given that this pair was separated for most of the 30 days before transport. Thus, we would expect an increase in social behavior when they were housed together daily. Subjects Lun Lun and Yang Yang displayed a decrease in social behavior after transport. Yang Yang experienced illness following transportation, and his decreased social behavior may have been attributable to illness, acute distress from the move, or a combination of the two. His decreased social behavior likely resulted in the observed decrease in Lun Lun's social behavior. Although social behavior decreased after transport for Lun Lun and Yang Yang, the time they spent in social behavior following transport remained high (see Figure 5).

The amount of time spent feeding was substantially lower for Lun Lun and Yang Yang before and after transport than it was for Mei Xiang and Tian Tian. Lun Lun displayed an increase in feeding after transport, but Yang Yang's feeding decreased. This may have resulted from bouts of gastrointestinal illness Yang Yang displayed periodically prior to and after the move. Mei Xiang also displayed a decrease in time spent feeding after the move; however, it was a small change compared with Yang Yang. Tian Tian displayed a significant increase in feeding behavior after transport, which may be an indication that he adjusted well to his new environment because decreased food consumption is a reliable indicator of stress intensity in species such as rats (Adam & Epel, 2007; Armario, 2006). Although the same amount of bamboo was provided each day, the subjects were given different species in the United States than in China. Previous research has found that pandas exhibit preferences for different species of bamboo and that these preferences vary among individuals (Tarou, Williams, Powell, Tabet, & Allen, 2005). In the current study, some subjects may have adjusted more quickly to the change in bamboo species than others, which is another factor that might have influenced changes in feeding behavior.

Although the data were not analyzed in a manner that quantified sex differences, an interesting pattern emerged through visual inspection of the data. Both males exhibited a decrease in active behavior, and concurrently an increase in inactive behavior, following transportation. Both females exhibited an increase in active behavior. It is not unexpected that the pattern of response differed for these subjects given that sex has been associated with differential behavioral and physiological responses to stress in other species, such as rats, humans, and goats (Aoyama, Negishi, Abe, Maejima, & Sugita, 2003). However, further studies are necessary to explore whether there really may be sex differences in the response to stress in the giant panda.

Cortisol results were much as predicted. Urine samples were collected from 3 of the subjects during transport. For 2 of the subjects, cortisol values were significantly higher during transportation than during the remainder of the 30-day period. For the 3rd subject, the second highest cortisol value was recorded on the day following transportation. Cortisol levels generally declined following the elevated values during or immediately following transport, suggesting a decreased stress response across the days following transportation and relocation. This finding is consistent with previous studies conducted with other captive wildlife (Dembiec et al., 2004; Goymann et al., 1999; Millspaugh et al., 2007). In a previous study investigating construction noise and stress in Mei Xiang and Tian

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Tian, cortisol concentrations were also found to decrease after an initial spike (Powell, Carlstead, Tarou, Brown, & Monfort, 2006). However, the potential stressor continued over a period of several weeks, and thus cortisol remained higher than baseline levels throughout the construction period. Unfortunately, in the current study it is difficult to know what baseline cortisol levels were because the samples collected prior to transport in China were lost before they could be assayed.

Both males exhibited a second peak in urinary cortisol late in the 30-day period following transportation. Yang Yang and Lun Lun spent the greatest percentage of time play-fighting the day before this spike occurred in Yang Yang's cortisol. The increased arousal associated with play may account for this increase in cortisol. However, Lun Lun did not show a similar increase in cortisol at this time. The day before Tian Tian's spike in cortisol he was observed pacing and bleating prior to being reunited with Mei Xiang. However, this behavior was not unusual for him, and so it may not explain why he experienced a large increase in cortisol. Mei Xiang's urinary cortisol was variable across the 30-day period following transport, which is not entirely surprising—especially because cortisol could not be measured for her during transport. For animals being transported to a new location, stress responses may continue beyond the response to transportation to include responses to the new environment, new animal care staff, and other novel stimuli (Dembiec et al., 2004); further, stressful events may sensitize an individual to other stressful stimuli that occur later (Koolhaas, Meerlo, De boer, Strubbe, & Bohus, 1997; Powell et al., 2006). These factors may also partially explain this subject's different pattern of cortisol levels.

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CONCLUSIONS

Overall, physiological and behavioral changes suggest that giant pandas did not experience significant distress following transportation from China to the United States. For 2 subjects, cortisol values were highest during the flight but decreased significantly following the actual transportation. The 4 giant pandas exhibited different behavioral patterns during the study, but no changes indicative of long-term stress were observed in the period following transportation. On the contrary, for some subjects, the transportation resulted in behavioral improvement based on a decrease in stereotypic behavior. All animals are in good health and both pairs of giant pandas have succeeded in producing offspring since the transportation. Previous studies of captive giant pandas in the United States, including the 2 National Zoo subjects in this study, have shown that giant pandas are able to recover quite quickly from stressful events or stimuli (Owen et al., 2004; Powell et al., 2006).

There are limited generalizations that may be made from a study of 4 animals. Nonetheless, this study presents the behavioral and physiological responses of 4 giant pandas to transportation and a change in environment, and we hope that this research will serve as a starting point for future investigations. We believe that the extensive planning involved in developing the most efficient travel routes, and the care taken in the process of conveying these animals, contributed to the apparently brief disruptions in their behavior and endocrine mobilization. We hope that similar measures will be performed on other giant pandas who are transported long distances in the future so that we can continually refine our methods of management and handling during transportation by reference to objective scientific data. Furthermore, the accumulation of descriptive data would eventually allow for an accurate statistical meta-analysis of the effect of transportation stress on behavior in the captive giant panda population (Kuhar, 2006).

Zoological institutions need to be aware of the potential stress caused by transportation and its implications for animal welfare, especially for individuals moved for breeding purposes. More research including a wider variety of species is needed to develop a complete understanding of transportation stress in captive wildlife to ensure the welfare of these species.

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