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Research Article

Effects of Construction Noise on Behavior and Cortisol Levels in a Pair of Captive Giant Pandas (*Ailuropoda melanoleuca*)

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Studies of the effects of ambient noise on animals have found variable results. A study was conducted at the Smithsonian's National Zoological Park to determine what effect short-term demolition work would have on the behavior and cortisol excretion of giant pandas. Behavioral and endocrine differences were examined during the presence and absence of demolition work being conducted on an adjacent exhibit complex. High frequency noise was significantly louder on work days compared to non-work days. Panda activity budgets differed significantly between work and post-work periods, although in different ways. The male's use of substrates and locations that might be associated with refuge or shelter changed during the study; the female did not show similar changes. He spent more time in the enclosure adjacent to the work site rather than a more distant enclosure during the demolition period whether work was occurring or not. The behavior of both animals was more often characterized as "restless" during, as opposed to before or after the work period. In general, cortisol excretion increased during the study in both animals but this was likely a seasonal effect in the male. In many cases, significant short-term increases in cortisol were temporally associated with certain kinds of construction noises or specific physiological events. Variability in cortisol secretion fluctuated during the study for both animals but in differing patterns. These results demonstrate that

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demolition noise was associated with behavioral and some physiological changes in giant pandas, and these changes were individual-specific. Zoo Biol 0:1–18, 2006.

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INTRODUCTION

Studies of the effects of ambient noise on the well-being of animals have had variable results. Studies of captive and free-living non-human mammals have uncovered only mild or transitory effects [Krausman et al., 1998; Owen et al., 2004]; however, in rats exposure to noise throughout copulation and pregnancy caused a decrease in birth rates [Sato et al., 1982]. Children exposed to chronic, loud (>60 dB) noise have demonstrated motivational deficits in problem solving puzzles, higher systolic blood pressure, greater heart rate reactivity to acute stressors, higher overnight cortisol levels, report more stress symptoms, and show significant decreases in quality of life [Evans et al., 2001]. In their review of studies of the effect of aircraft noise on wildlife, Kempf and Hueppop [1996] suggest that interpretation of results from field studies is difficult because of species-specific sensitivity to noise, problems in measuring sound levels, challenges in separating the effects of acoustic and visual stimuli, and difficulties in interpreting behavioral responses. They conclude that animals can apparently adapt to high noise exposure and that noise is a less important disturbance than visual stimuli [Reijnen et al., 1995]. Noise is associated with changes in song characteristics [Ill'chev et al., 1995], decreases in reproduction and density [Reijnen et al., 1995, 1996, 1997; Forman and Deblinger, 2000], and reduced feeding and weight gain [Esmail, 1997] in some bird species whereas other bird species seem to be unaffected or only temporarily disturbed by ambient noise [Delaney et al., 1999]. Whether or not ambient noise has a negative impact on welfare probably depends on a variety of factors including individual and species differences, as well as characteristics of the stimulus.

As part of a major revitalization effort, the Smithsonian's National Zoological Park demolished an existing exhibit complex adjacent to the giant panda (*Ailuropoda melanoleuca*) exhibit. We sought to assess the impact of this construction disturbance on the pandas' behavior and cortisol levels because later, significant construction work would take place around the pandas, including work on the panda holding facility itself. Behavioral and cortisol analyses have increasingly been used together to assess well-being in captive animals or to assess their responses to environmental change or events (clouded leopards, *Neofelis nebulosa* [Wielebnowski et al., 2002]; giant pandas, *Ailuropoda melanoleuca* [Owen et al., 2004, 2005b]; leopard cats, *Felis bengalensis* [Carlstead et al., 1993a,b]). A wide range of behaviors have been identified in various taxa as indicators of stress (e.g., stereotypies) [Mason, 1991]. Similarly, changes in frequency or duration of "normal" behaviors may represent responses to stress (e.g., resting and self-grooming) [Jesberger and Richardson, 1985; Maestripieri et al., 1992; Carlstead et al., 1993a,b]. In our study we focus on behaviors that have been related to stress in giant pandas and other species as well as changes in normal behavior during exposure to acoustic disturbance. Although neither of these measures may indicate poor well-being, we believe that they are useful tools in identifying stimuli or events that could eventually reduce well-being if left uncontrolled [sensu Owen et al., 2005]. The most commonly used, non-invasive physiologic index of stress is the measurement of glucocorticoid metabolites in urine or feces. Glucocorticoids are released when the body experiences increased energetic demands, for example to mount a behavioral response to a perceived stressor. Both behavior and glucocorticoid levels may temporarily change in normal response to an acute stressor; however, when animals are exposed to a chronic stressor, their ability to cope with it may be impaired, with adverse behavioral and physiological consequences [Moberg, 2000]. In addition, exposure to one chronic stressor may sensitize the individual to other, unrelated stressors [Koolhaas et al., 1997; Bhatnagar and Dallman, 1998; Harris et al., 2004].

We hypothesized that the pandas' activity budgets and cortisol levels would differ between days with and without ongoing site demolition work. We generally predicted that the pandas would spend more time moving around their enclosures and engaged in abnormal or anxiety-related behavior when demolition work was occurring. We hypothesized that the pandas' use of certain substrates in the enclosure used for shelter, refuge, or positive experiences would be higher when demolition was taking place. Finally, we also hypothesized that the pandas would preferentially spend more time at enclosure locations farthest away from the work site.

Because glucocorticoids have been found to be associated with stress or physiological arousal [Mason, 1968; Hennessy and Levine, 1979; Hennessy et al., 1979], we expected to find higher levels of urinary cortisol during the work period compared to before work started and after it ended. In addition, we suspected that significant increases in cortisol might also be associated with particularly disruptive events or noises during the demolition. If pandas fail to habituate to the demolition, we expected to quantify an increase in cortisol excretion over time during the demolition project. It has been demonstrated, however, that there is normal seasonal variation in glucocorticoid production [Romero, 2002] in vertebrates. In giant pandas, Owen et al. [2005a] found that corticoid excretion increased from fall to winter, the period during which our study took place, but the difference in fall (September 21–December 20) and winter (December 21–March 20) means was not significant. Some of our own data on the National Zoological Park (NZP) giant pandas from other years besides the current study indicates that there are seasonal influences on cortisol and that the fall–winter period is a time when cortisol is increasing (Monfort and Brown, unpublished data). Finally, we hypothesized that evaluating variability in cortisol excretion over time, rather than simply assessing mean cortisol levels, might be informative for assessing long-term stress or physiological arousal [Carlstead and Brown, 2005]. If an individual animal exhibits repeated, short-term elevations in cortisol as a result of exposure to intermittent noxious stimuli, this may reflect a lesser or impaired ability to adapt or cope to the stressful stimuli. We hypothesized that, under normal circumstances, this ability would be consistent, and thus variability in cortisol, as measured by standard deviations (SD) of cortisol values, would not increase or decrease significantly over time. Essentially, we predicted that changes in the variability of cortisol excretion within an individual during the demolition project would reflect changes in the ability to maintain relatively stable levels of cortisol.

MATERIALS AND METHODS

Subjects and Housing

Two giant pandas (*Ailuropoda melanoleuca*), a 6-year-old male born in 1997 (Studbook #458) and a 5-year-old female (Studbook #473) born in 1998, were the subjects of this study. The pandas had daily access to two similarly-sized, large outdoor enclosures (Yard 1 and Yard 2) connected by a door that remained open. Yard 2 was adjacent to the demolition site. Each yard contained shade trees, dead trees for climbing, a pool of water, and an artificially cooled cave (caves were only cooled during summer months). Bamboo was placed in both yards before putting the pandas outdoors for the day at 0800.

Demolition Project

An old exhibit complex for Australian animals was demolished to prepare for construction of a new exhibit complex for Asian species. The demolition period lasted 100 days (7 November 2002–14 February 2003), but the number of consecutive days on which demolition work was done changed significantly during the study period due to bad weather and holidays. Before January 6, the work week averaged 2.8 ± 1.14 days whereas after this date, work weeks averaged 5.3 ± 0.82 days ($U = 94.5$, $n = 6,13$, $P = 0.003$). We therefore divided behavior and cortisol data from the demolition period into study blocks (pre- and post-January 6). Period 1 encompasses data from the pre-work period (October 13–November 6), Period 2 is the first phase of demolition with short work weeks (November 7–January 5), and Period 3 is the second phase of demolition with longer work weeks (January 6–February 14). Period 4 is comprised of data collected after demolition ended (February 15–March 11).

Sound Measurement

We measured sound levels throughout each observation period using a CEL Instruments sound meter (model 573; Casella/CEL Inc., Amherst, NH). Decibel measurements in the range of 30–110 dB were taken across an L-weighted broadband frequency spectrum. Each data point was the mean noise amplitude recorded over a one minute interval. The sound meter gave us the dB level of noise over the entire broadband spectrum and the dB level of noises at discrete frequencies (range = 16–16,000 Hz). We averaged these points to produce a mean dB for each frequency of sound for each session. Acoustic characteristics of work and non-work days were compared using confidence interval (CI) analysis with non-work days as the standard.

Behavior

We conducted behavioral observations 6 days/week from 0800–1000 from November 7, 2002 when demolition started until February 28, 2003, 14 days after demolition ended. We have no behavioral data from before the start of demolition (Period 1). Each week consisted of at least 1 day on which no demolition work was done. Observations were collected using focal animal sampling [Altmann, 1974] by two observers, each collecting data on one animal. Inter-observer reliability averaged 90%, and a criterion of 85% agreement with the principal investigator was required for participation in the study. Observers used instantaneous scan sampling at 1-min intervals to record behavior, substrate, and location of the animal (Yard 1 or 2). Behaviors recorded included feed, rest, walk (including running and climbing), exploration (sniffing, licking, biting objects or substrates, manipulating objects with paws), alert, social behavior, door-directed behavior, stereotypy, and other.

Each minute we also noted the use of four substrates of particular interest (grottos, concrete, trees/deadfalls, and cage mesh) in addition to an "other" category. These four substrates were selected because we thought they might represent refuge, shelter, or safety for the pandas. The grottos in each yard are smaller, semi-enclosed spaces similar to caves that are used by pandas in the wild [Schaller et al., 1985] for sleep and cub rearing. There is a concrete apron along the back of each yard. This is an area that is not only far from the public but also close to the indoor areas and places where keepers interact with the pandas. We thought that the concrete area might be associated with positive experiences for the pandas. Trees and deadfalls were included because young pandas often use trees for refuge from predators [Schaller et al., 1985]. The cage mesh at the back of the exhibit was included because it shared characteristics with the concrete area and the trees. The cage mesh could be associated with positive experiences for the pandas and could be climbed in case of perceived threat.

We tallied scent marks, self-grooming bouts, urinations/defecations, and certain vocalizations (bleat and honk) that have been reported by other investigators to be associated with stress [Kleiman, 1983; Kleiman and Peters, 1990; Maestripieri et al., 1992; Castles et al., 1999; Barros et al., 2001; Momozawa et al., 2003; Kanari et al., 2005] using all occurrences sampling. The bleat vocalization is usually associated with anticipation of some resource and is associated with appeasement or reassurance. Giant pandas honk when they are distressed and this call is associated with alarm or indecision. These vocalizations and their contexts have been described previously by Kleiman [1983] and Kleiman and Peters [1990].

Because repeated observations of the same animals violate the assumption of independence for conventional statistical analyses, we opted to use CI analyses where possible to try and establish differences. Where CI could not be used (e.g., in assessing trends over time), we opted for conventional statistical tests. We urge caution in generalizing these results to other giant pandas. Two types of behavioral analyses were carried out. First, behavioral data were converted to percentages and comparisons were made for each animal on work versus non-work days during the 100-day demolition period using a 95% CI around the mean value for the non-work days as the standard. Work day means that fell outside of this CI were considered significantly different [Tarou et al., 2005]. Confidence interval analysis was also used to compare behavioral measures during Periods 2 (short weeks) and 3 (long weeks) to Period 4 (post-demolition). Data for the post-work period were used as standards for comparison.

Because exploration behavior could be influenced by how often the pandas received enrichment, we reviewed daily keeper reports and calculated the mean number of enrichments given per day on a weekly basis to the pandas for 2 weeks before, during, and 2 weeks after the demolition period. Each day was considered an independent data point for two reasons. First, multiple keepers worked in the panda facility during this period and their scheduling was more or less random. Second, the level and type of enrichment provisioning each day varied with the availability of materials, keeper assessments of the animals' behavior, and the amount of time that keepers had to do enrichment. We analyzed enrichment data by ANOVA and post-hoc Holm-Sidak tests. Our goal was to establish whether the giant panda keepers at NZP provided enrichment in such a way that it could have systematically affected the behavior of the two giant pandas in their care.

Cortisol

Urine samples were aspirated from the floor of the pandas' overnight indoor enclosures using a syringe, placed in a plastic storage tube, and stored frozen until analysis. To account for an approximately 12–24 hr excretion lag time, cortisol values were back-dated 1 day. To account for daily fluctuations in urine concentration, hormone concentrations (ng/ml) were divided by creatinine concentrations (mg/ml); hormone concentrations were expressed as mass of hormone excreted per mg of Cr excreted (ng hormone/mg Cr) [Taussky, 1954; Monfort et al., 1989]. For Cr determinations, 50 μl urine (diluted 1:20 in phosphate buffer) was added in duplicate to 96-well flat bottom microtiter plates, combined with 0.05 ml of 0.4 N picric acid and 50 μl of 0.75 N NaOH, and incubated at room temperature (25°C) for 30 min. Optical density was measured at 490 nm (reference 620 nm) using a microplate reader [Dynex MRX; Chantilly, VA] and urine samples were compared to a series of creatinine standards evaluated in duplicate (100–6.25 μg/ml Cr). Urine samples with Cr concentrations <0.1 mg Cr/ml were considered too dilute and were excluded from analysis.

Urinary cortisol levels were assessed using an enzyme immunoassay method described previously [Munro and Lasley, 1988]. The cross-reactivities for the employed polyclonal antiserum [R4866; C. Munro, University of California, Davis, CA] were: cortisol (100%), prednisolone (9.9%), prednisone (6.3%), cortisone (5%), and corticosterone, desoxycorticosterone, 21-desoxycortisone, testosterone, androstenedione, androsterone, and 11-desoxycortisol (all $<1\%$) [Young et al., 2004]. Antiserum was diluted 1:8,500 in coating buffer (0.05 M $NaHCO_3$, pH 9.6) and 50 μl was added to 88 wells of the 96-well microtiter plates [Nunc-Immuno, Maxisorp; Fisher Scientific, PA]. Unabsorbed antibodies were washed away from the plate after being stored at 4°C for 12–18 hr. Cortisol standards (50 μl; range = 0.039–20 ng/ml diluted in assay buffer, 0.1 M $NaPO_4$, 0.149 M NaCl, 0.1% albumin bovine serum, pH 7.0) and sample (50 μl, 1:20 dilution in diluent buffer, 0.1 M $NaPO_4$, 0.149 M NaCl, pH 7.0) were added in duplicate to the plates. Cortisol horseradish-peroxidase ligand (50 μl, 1:20,000 in assay buffer) was then added to all wells and the plate was allowed to incubate a room temperature for 1 hr, before being washed five times. Substrate solution (100 μl, 0.4 mM 2,2′-azino-di-[3-ethylbenzthiazoline sulfonic acid] di ammonium salt, 1.6 mM H_2O_2, 0.05 M citric acid, pH 4.0) was added to each well and the plates were allowed to incubate at room temperature for 15–30 min. The optical densities of the plates were then read on a microplate reader at 405 nm (reference, 540 nm). Intra-assay variations were $<10\%$, and inter-assay variations were 15% and 19% at 30% and 68% binding, respectively.

In addition to cortisol data from the demolition period, we had cortisol data from the 25 days before and after demolition. We also analyzed cortisol data using CI analysis; we compared cortisol data from Periods 2–4 to Period 1. In addition, we compared cortisol data from the pre-demolition, demolition, and post-demolition periods using CI. Linear regression analysis was used to determine if there was a relationship between cortisol and day of demolition (e.g., 1 to 100) for each animal.

We plotted the SD of cortisol values by period for each animal to determine whether variability in cortisol excretion during the study was stable.

An iterative process was used to identify elevated peaks in cortisol [Brown et al., 1994] to determine if these coincided with particular types of demolition noise or events. Briefly, the mean and SD of all cortisol values were determined, and values falling beyond two SD from the mean (i.e., peaks) were considered significantly different and excluded from further calculations. The process was repeated until no new significant peaks are detected. The contractor's daily log was obtained to determine what types of demolition work were occurring on a given day. Means are presented ± 1 SD unless otherwise noted.

RESULTS

Behavioral analyses for the female are based on 68 work days, 15 non-work days during demolition, and 10 days after demolition. For the male, analyses are based on 67 work days during demolition, 21 non-work days during demolition, and 10 days after demolition. Cortisol data are based on 11 pre-demolition days and 69 work days for both animals. Cortisol data were available for 25 non-work days during the demolition period for the female and 23 non-work days for the male. Post-demolition cortisol data were available for 17 days and 16 days for the female and male, respectively. Review of the contractor's log showed that there were six categories of demolition work that occurred during the study. These were: indoor demolition work (November 7–22, 2002), ditch digging (November 25–December 7, 2002), jack-hammering (December 9, 2002–January 4, 2003), wall and metal beam cutting (January 6–18, 2003), dumpster loading/site cleanup (January 20–31, 2003), and grading of the site (February 2–10, 2003). These differing types of work took place consecutively during the project. Period 2 of the study included the indoor demolition, ditch digging, and jack hammering noise (November 7, 2002–January 4, 2003). Period 3 included wall and beam cutting, demolition of the structure, dumpster loading, site clean up, and grading work (January 6–February 10, 2003).

Work Days Versus Non-Work Days During Demolition

For most sound frequency categories, decibel levels on work days were generally louder than non-work days (Table 1), but most frequency ranges did not differ using CI analysis. The exceptions were sounds in the 2,000, 8,000, and 16,000 Hz categories. Work days were characterized by louder sound in the 2,000 Hz category. The majority of measurements in the 8,000 and 16,000 Hz categories on non-work days were below the sensitivity level of the meter (i.e., virtually no high frequency sounds were measured), so we compared to loudest 1% of sound measurements in these ranges between work and non-work days. For both categories, work days were louder.

Many behaviors differed between work and non-work days in the male. He spent more time walking, grooming, scent marking, and engaged in stereotypy and door-directed behavior on work days (Table 2). The male spent less time resting on work days, and most all occurrence-sampled behaviors were more frequent in the male on work days compared to non-work days (Table 2). There were no differences in the male's use of different substrates in the enclosures between work and non-work days (Table 3). He spent more time in Yard 2 on work days (68.6%)

TABLE 1. Comparison of acoustic characteristics between work and non-work days

Type of noise (Hz)	Non-work days (dB)	Work days (dB)
Broadband	72.1 ± 1.73	72.2 ± 0.58
16	67.3 ± 2.57	64.8 ± 1.22
32	68.1 ± 2.15	67.9 ± 0.74
63	65.2 ± 1.68	67.5 ± 0.48
125	62.5 ± 1.70	64.4 ± 0.76
250	54.8 ± 1.79	59.3 ± 0.99
500	55.4 ± 2.22	59.5 ± 1.20
1,000	53.3 ± 1.75	57.1 ± 1.16
2,000	49.9 ± 1.18	53.5 ± 1.02[a]
4,000	47.5 ± 1.04	50.6 ± 1.10
8,000[b]	<30 (48.5 ± 1.19)	52.3 ± 1.21[a]
16,000[b]	<30 (46.3 ± 0.48)	50.8 ± 1.08[a]

Values are mean ± SE.
[a]Significantly different from non-work days.
[b]The loudest 1% of measurements are compared for these frequencies because most measurements taken in these frequency ranges on non-work days did not register any sound.

TABLE 2. Activity budgets and behavior of the male and female panda on work vs. non-work days during the demolition period

	Male		Female	
Behavior	Work mean	Non-work CI	Work mean	Non-work CI
Rest	14.15*	17.76–29.32	15.64*	18.44–34.06
Feed	38.07	31.52–46.22	31.73	21.31–35.35
Walk	13.66*	6.72–11.45	14.91*	8.34–14.52
Explore	7.41	4.44–8.98	8.51	4.14–11.22
Alert	9.59	5.30–9.78	13.67	7.45–15.65
Groom	1.21*	0.42–1.16	0.26	0–4.38
Scent mark	0.47*	0.04–0.38	0.05	0.00
Social	9.48	4.37–16.47	9.49	3.14–19.36
Stereotypy	1.51*	0–0.44	0.00	0.00
Door-directed	0.35*	0–0.13	0.62*	0–0.53
Events per observation				
Scent marks	2.06*	0.13–1.47	0.29	0–0.35
Grooming bouts	3.39*	0.36–1.64	0.88	0–2.80
Bleats	3.79*	0–1.44	0.00	0.00
Honks	0.60	0–0.62	0.27	0.00
Stereotypic pirouettes	8.45*	0–1.58	0.10	0–0.23
Urination/defecation	0.54*	0–0.32	1.25*	0.29–1.13

*Differences in behavior from non-work days.

compared to non-work days (CI = 40.4–58.6%), whereas the reverse was true for Yard 1 (work days: 31.5%, non-work days: CI = 41.4–59.6%). There was no difference in mean cortisol between work (77.2 ng/mg Cr) and non-work days (CI = 48.4–91.4 ng/mg Cr).

TABLE 3. Use of various substrates in the enclosure by the male and female panda on work vs. non-work days

	Male		Female	
Substrate	Work mean	Non-work CI	Work mean	Non-work CI
Tree/deadfall	18.15	12.15–30.35	4.96	2.91–21.37
Mesh	0.37	0	0.22	0–0.75
Grotto	0.54	0–11.52	0.55	0–8.65
Concrete	12.54	4.52–18.23	17.83	8.34–26.27

There are no differences between work and non-work days for either animal.

Only four behaviors differed between work and non-work days in the female. On work days, she spent more time walking and engaged in door-directed behavior and less time resting compared to non work days (Table 2). She also urinated and defecated more on work days compared to non-work days. There were no differences in her use of different substrates (Table 3) or the two enclosures to which she had access (Yard 1 work days: X = 32.1%, non-work days: CI = 12.3–38.5%; Yard 2 work days: X = 67.9%, non-work days: CI = 61.5–87.7%). Her mean cortisol did not differ between work (65.8 ng/mg Cr) and non-work (CI = 46.5–81.3 ng/mg Cr) days.

Behavioral and Hormonal Responses Over Time

There were a number of differences in behavior between Periods 2 (short work weeks) and 3 (long work weeks) and the post-demolition period (Period 4). The male spent more time feeding, exploring, and engaged in stereotypic behavior in Period 2 compared to period 4 (Fig. 1). He spent more time walking, exploring, and engaged in door-directed behavior in Period 3 compared to Period 4. There was a significant difference in enrichment provisioning during the study ($F_{2,15} = 5.40$, $P = 0.02$); enrichment was provided significantly more often during the demolition period (0.89 enrichments/day) compared to after demolition ended (0.14 enrichments/day, $P = 0.01$). There was no difference in enrichment provisioning between pre-demolition (0.57 enrichments/day) and post-demolition periods or between pre-demolition and demolition periods. Stereotypic pirouetting was more frequent in Period 3 (X = 6.5/hr) than Period 4 (X = 2.8/hr). He spent more time on the concrete apron at the back of the enclosure during Periods 2 and 3 compared to Period 4 (Fig. 2a). There were no differences in his use of Yards 1 and 2 across periods.

Mean cortisol values for the four periods of the study are presented in Figure 3. The male's cortisol levels in Periods 2–4 were all higher than the level during Period 1 before demolition. Day of work was significantly associated with increasing cortisol levels in the male, though the slope of the regression was low ($F_{1,43} = 5.27$, $P = 0.03$, cortisol $= 1.728 + 0.003 \times$ day).

The female spent more time walking and alert in Period 3 compared to Period 4 (Fig. 4). She fed more after demolition was over (Period 4) than during either demolition period, whereas she spent more time resting in Period 2 compared to Period 4. The female deposited more scent marks during Period 2 compared to Period 4. She spent more time in the trees and deadfalls during demolition compared to afterward (Fig. 2b). She also spent more time on the concrete apron during Period

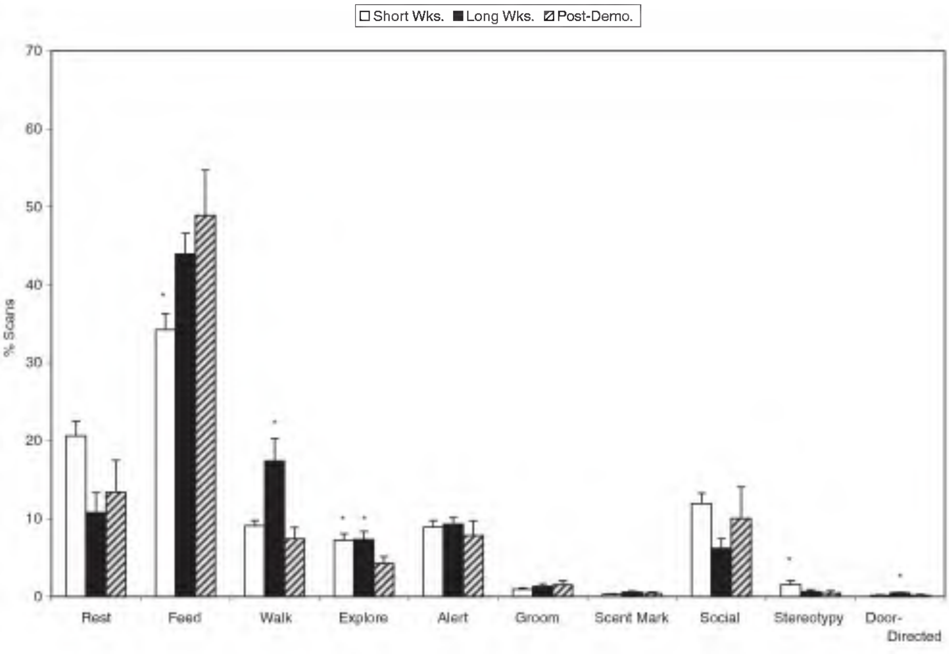

Fig. 1. Activity budget of the male giant panda during Periods 2 (short work weeks), 3 (long work weeks) and 4 (post-demolition) of the study. *Differences in behavior from the post-demolition period.

3 compared to Period 4. There were no differences in her use of Yards 1 and 2 across periods.

The female's mean cortisol values for each period are presented in Figure 3. Periods 2 and 4 were higher than Period 1. Her Period 3 cortisol was almost significantly higher than her Period 1 cortisol (Period 3: 63.22 ng/mg Cr, upper confidence limit for period 1: 64.17 ng/mg Cr). There was no significant association between day of work and cortisol in the female ($F_{1,57} = 2.16$, $P = 0.15$, cortisol = 1.85–0.002 × day).

In reviewing observer notes and daily keeper reports, we found only one note of the male panda displaying "restless" or "upset" behavior in the 25 days before demolition and none for the female. During the demolition period, there were 14 records of the male appearing restless. There were 11 records of the female appearing restless or spooked by specific noises (a leaf blower, drill, metal structure demolition). During the instances where a particular noise frightened the female, she would spend significant time (up to 1 hr) walking around the enclosure, interspersed with short bouts of running. She would also visit the concrete area at the back of the enclosure and look in at the keeper work area. In some cases she would move toward the male and remain in proximity to him for some time. After demolition was over, we found three records of the male being restless and two for the female. Abnormal mucous stool was observed once in the 25 days before demolition in the female. Each animal had one abnormal mucous stool during the demolition phase and one after the demolition ended (Fig. 5).

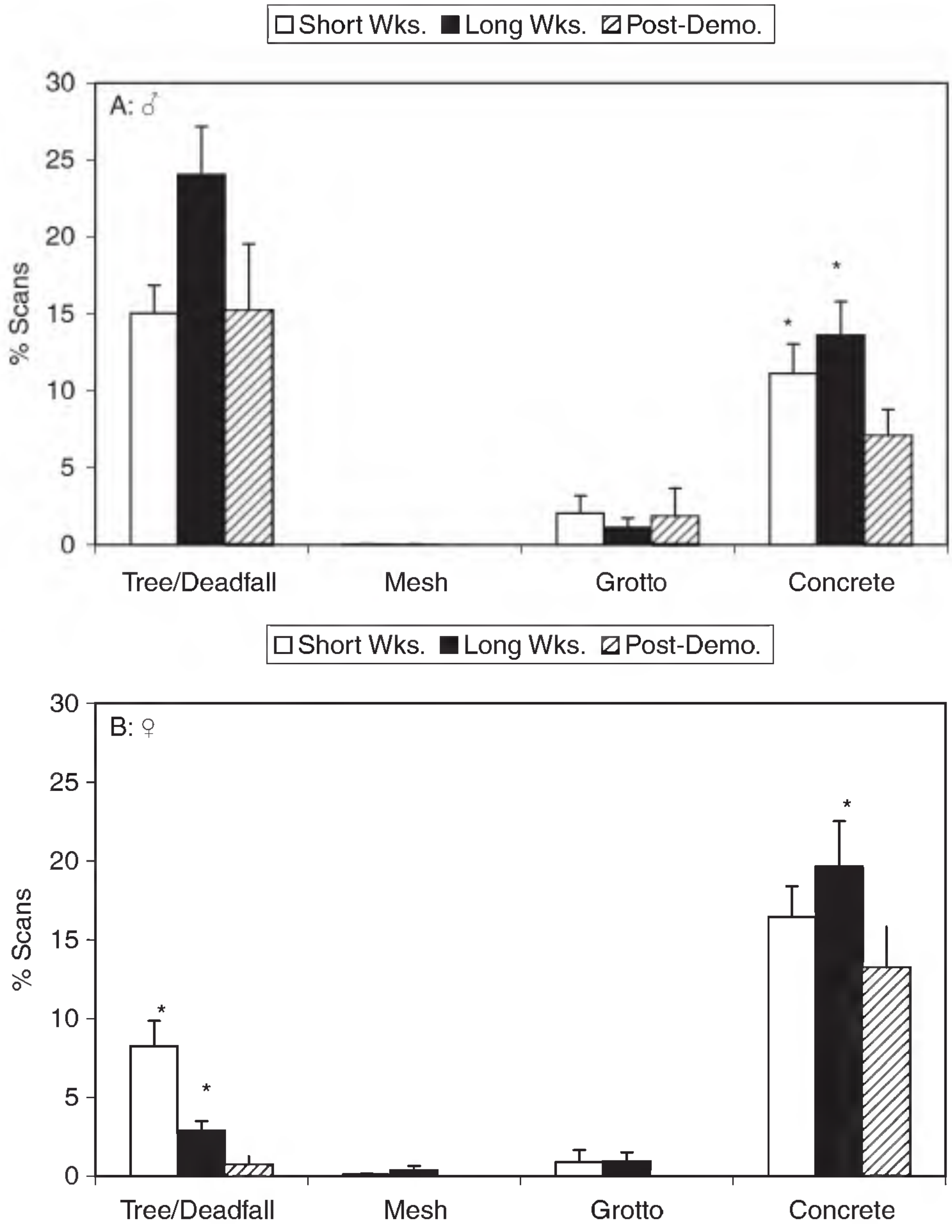

Fig. 2. Use of various substrates in the enclosure during Periods 2–4 of the study by the male (**A**) and female (**B**). *Differences in substrate use from the post-demolition period.

The complete cortisol profile for the male shows three cases of elevated cortisol followed by return to baseline levels and the beginning of a significant increase when the study was ending (Fig. 5). Two of his peaks occurred during the work period, whereas the third, and largest peak, occurred after the demolition had ended. His first peak occurred on November 28, after the first 3 days of ditch digging work at the site. No specific behavioral changes or events were noted on this day by

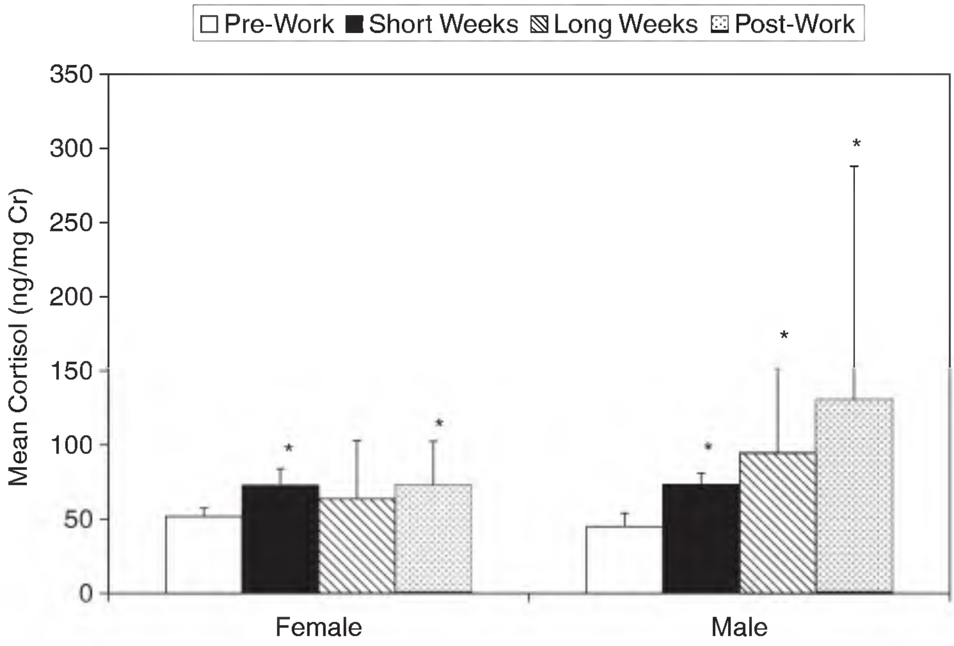

Fig. 3. Mean cortisol during the four periods of the study in the male and female giant panda. *Differences in cortisol from the pre-demolition period.

keepers or observers. His second peak on January 13 was observed on the first day of wall and metal beam cutting work. The male's third peak seems to have started 5 days after demolition ended and lasted for 3 days on February 19–21. He was observed to pass two abnormal mucoid stools on February 20, one day after the largest peak in cortisol. No environmental events were recorded that could account for this peak.

There are four occurrences of increased cortisol in the female's profile. Three of these occurred during the work, whereas a fourth, smaller increase occurred after the work had finished. Her first peak occurred in the first 3 days of demolition work, but she also passed two abnormal mucous stools 2 days after the peak in cortisol. Her second peak occurred over a 5-day period from December 6–11. We could not identify a unique sound on the days before December 6; however, jack hammering and drilling began on December 9 and 10. The third peak also took place over a period of days from January 22–28. Loading of dumpsters with demolition debris began on January 20 and site clean up began on January 27. We did not identify an auditory stimulus associated with her fourth peak in February after the work had ended; however, keepers noted that social play with the male on the two peak days was rougher than usual.

Variability in male cortisol as measured by SD followed an exponentially increasing pattern over the course of the study (Period 1 cortisol variability [CV] = 37.3, Period 2 CV = 40.3, Period 3 CV = 57.4, Period 4 CV = 157.4). The female's profile reflects a pattern of increased cortisol variability during the work period followed by a decrease in variability after work had ended although it did not return to pre-work levels (Period 1 CV = 18.5, Period 2 CV = 46.5, Period 3

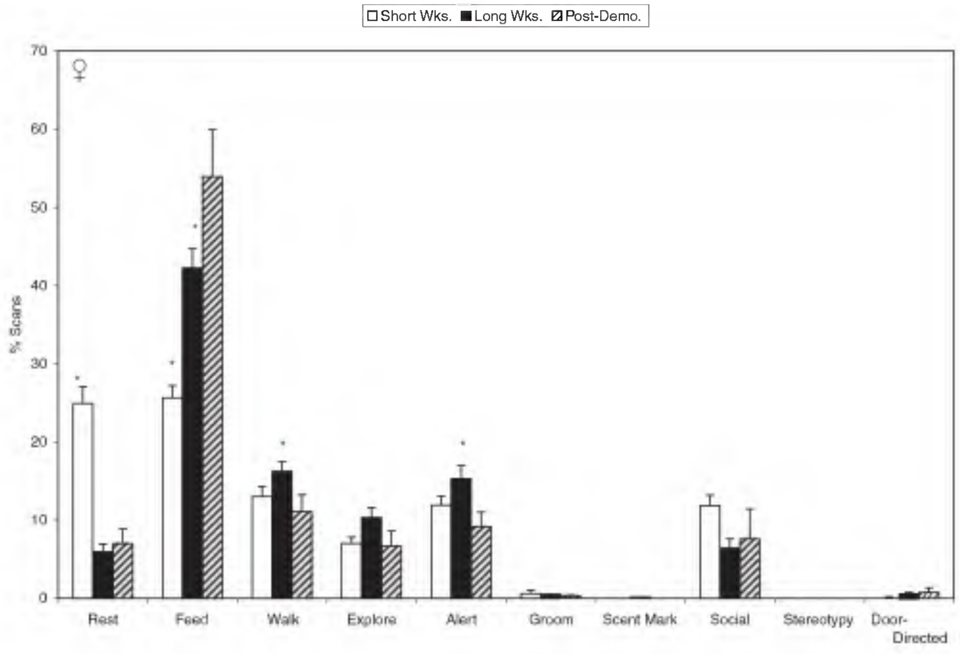

Fig. 4. Activity budget of the female giant panda during Periods 2 (short work weeks), 3 (long work weeks) and 4 (post-demolition) of the study. *Differences in behavior from the post-demolition period.

CV = 39.9, Period 4 CV = 29.6). Cortisol variability was similar in magnitude for the male and female, except during the post-demolition period.

DISCUSSION

In this study we sought to determine if demolition noise or activity was associated with changes in behavior or glucocorticoid excretion in a pair of giant pandas. We documented very few differences in acoustic characteristics of work versus non-work days, but all of the differences we did find were in the higher frequency categories, suggesting that any behavioral or hormonal responses we observed might be related to high frequency noises.

We found that the pandas' activity budgets differed between days with and without ongoing demolition. Additionally we observed changes in activity budgets between the demolition and post-demolition periods. Both pandas were generally more active and exhibited more door-directed behavior when demolition was occurring. The male exhibited more stereotypic behavior when demolition was ongoing; the female has never shown stereotypic behavior. Both animals exhibited more stress or anxiety-related behaviors (e.g., scent marking, grooming, bleating, urination/defecation) during demolition. The female rested more during the first phase of the demolition compared to the post-demolition period. Her initial response to the demolition may have been to become less active, a response seen in other species when exposed to chronic stress [Jesberger and Richardson, 1985; Carlstead et al., 1993b].

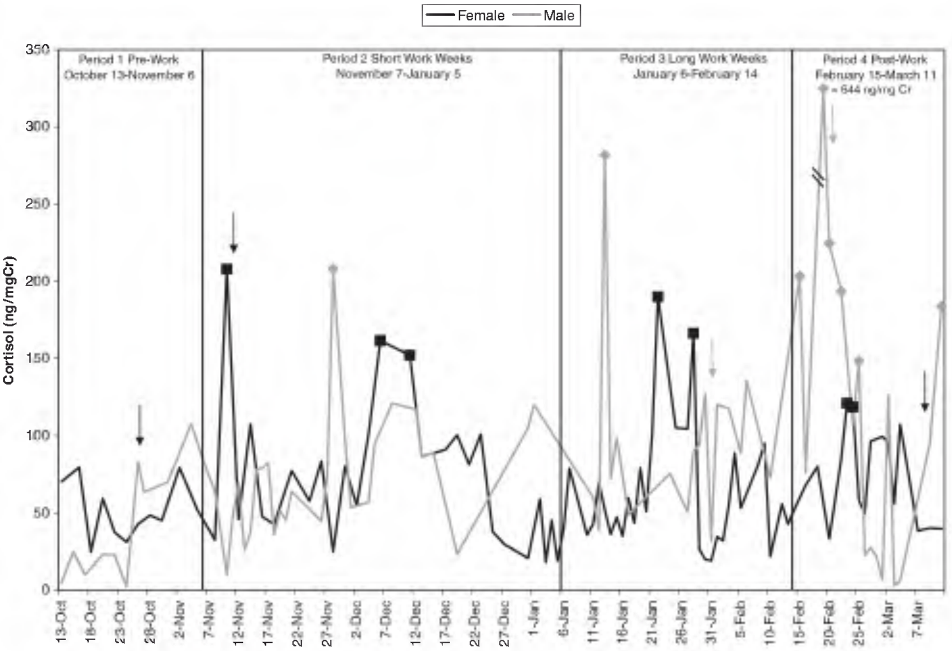

Fig. 5. Cortisol profiles of the male and female giant panda. Cortisol values that are elevated above baseline are represented by squares (female) or diamonds (male). Arrows represent the occurrence of mucous stools in the female (black arrows) and male (shaded arrows).

It is interesting that the male engaged in more exploration behavior during the demolition period than after it ended. Perhaps the presence of many unfamiliar odors (from people, over-turned earth) stimulated this behavior, but it is likely that increased exploration behavior was the result of differing amounts of enrichment given to the pandas during the study. According to the keepers, the increase in the amount of enrichment given to the pandas during the demolition period was a response to changes in the pandas' behavior, particularly the male's behavior, which suggested stress or agitation. The majority of enrichments given are devices that require the pandas to work to obtain food. The types of work include licking and gnawing on objects and manipulating them with paws, behaviors that were categorized in our ethogram as exploration.

There was also support for our prediction that the pandas would use substrates or areas in the enclosure that might be associated with safety or positive experiences more when work was taking place. We did not find any support for our prediction that the pandas would spend most of their time in the enclosure most distant from the demolition site; in fact, the reverse was true. Yard 2, closest to the demolition site, contains a highly preferred sleeping site for the female during the fall and winter. The pandas generally prefer to be in the same enclosure as one another regardless of the time of year (Powell, unpublished data). This could explain why the male tended to spend most of his time in the enclosure adjacent to the demolition site.

Mean cortisol in both animals increased throughout the phases of the study and reached their peak after demolition had ended; interestingly, their behavior was returning to "normal" in the post-demolition period. Although this increase may have been due to demolition noise, an alternative explanation is that we are documenting a seasonal effect rather than a response to demolition noise. Owen et al. [2005a] demonstrated an increase in corticoids throughout the fall and winter periods in giant pandas at the San Diego Zoo, but they were not able to detect significant differences in cortisol between these seasons using analysis of variance. When we analyzed male cortisol data for the same 100-day period from other years (Monfort and Brown, unpublished data) using regression analysis, we get a significant, positive slope in each year (results not shown), and the slopes are similar in magnitude. This increase in cortisol each year generally occurs in November and lasts through winter, the period corresponding to rut-like behavior in the male and the onset of the breeding season. Because the magnitude, direction, and timing of these changes in the male's cortisol excretion are similar across years, it seems more likely that the gradual increase in cortisol seen during this study is a normal physiological event and not a reaction to noise. Seasonal effects on cortisol do not seem to be a likely explanation for our results in the female. Regression analysis showed no significant change in cortisol over time during this study or during same period the following year (results not shown). We do see seasonal changes that generally correlate with the female's estrus cycle, but in all years for which we have data, this increase happens 1–2 months after the study period being analyzed.

The fact that the male demonstrated an unusually large, sustained elevation in urinary cortisol excretion after demolition suggests that exposure to the demolition noise over the previous 100 days may have sensitized him to other stressful stimuli [Koolhaas et al., 1997; Bhatnagar and Dallman, 1998; Harris et al., 2004]. The female also exhibited a significant peak in cortisol after demolition ended, but it was similar in magnitude to previous peaks, suggesting that she was not sensitized to other stimuli as the male may have been. We could not identify an acoustic stimulus associated with the male's large cortisol peak on February 19; however, there was a snow storm 4 days before the peak, and his cortisol profile indicates that the start of the cortisol surge occurred on February 15. The pandas' enjoy snowfall and spend significant time playing when snow is on the ground. Perhaps this elevation in cortisol reflects the increased metabolic demands of vigorous physical activity in colder weather.

It is interesting to note that some of the significant increases in cortisol were temporally associated with the pandas' passing abnormal mucous stools. The etiology of these events is poorly understood, but mucous stools have not been documented in wild giant pandas. When excreting mucous stools, the animals were markedly lethargic and showed very little interest in food or social interaction for several hours. Each panda passed two mucous stools either during or after demolition, a rate slightly higher than usual for both animals. These animals have the same diet and are exposed to the same environments. Although data were insufficient to conclude that demolition activities induced mucous stools, our data suggest that the relationship between mucous stools and cortisol excretion should be explored further.

The pandas seemed to have differed in their ability to regulate glucocorticoid responses over time. It has been suggested that mechanisms should have evolved to minimize fluctuation in cortisol, given the dramatic effects that glucocorticoids have on physiological processes [sensu Ladewig, 2000]. Variability in cortisol excretion followed an exponential function in the male, suggesting that his ability to regulate physiological responses to a particular stressor (demolition noise) was adequate, but long-term exposure to that stressor sensitized him to other stressful stimuli [Harris et al., 2004]. The pattern of cortisol variability over time in the female suggests that she had a more limited ability to regulate her physiological responses to stress. She was only slightly better able to regulate her responses during the third period of the study, and her cortisol variability after work had ceased was still higher than pre-demolition levels. We suggest that it is important to consider not only average responses to stressors but also variability in the response over time when evaluating welfare.

Although these results demonstrate that demolition noise was associated with some changes in behavior and endocrine physiology, we do not feel that the pandas experienced a significant decline in welfare during the demolition period. The magnitude of most of the behavioral changes was small, and both animals seemed to mount normal physiological responses to stress. Owen et al. [2004] found changes in behavior and stress hormones in giant pandas subjected to varying levels of noise from visitors at the San Diego Zoo; however, they too concluded that these changes were small and not likely to be of biological significance. During their first 1–2 years of life, the Washington pandas were exposed to a major construction project at their facility in China. This could explain why their responses to ambient noise are relatively mild. Alternatively, giant pandas may be relatively unaffected by ambient noises. Neither of the giant pandas at the National Zoo has developed any behavioral or physiological problems as a result of construction noise. In the absence of overt pathology, regular behavioral and physiological monitoring should be adequate for identifying and mitigating the risk of stress-related problems. Managers should be cognizant of not only the appearance of abnormal behaviors but also changes in the frequency of or time engaged in normal behaviors.

We are not suggesting that giant pandas are unresponsive to ambient noise. On several occasions, the pandas have been alarmed by noises in proximity to their enclosure. These noises are typically of short duration, loud, or high frequency. In general, the response to these noises has been heightened alertness, increased locomotion (sometimes lasting up to 1 hr post-stimulus), and in rare instances, production of vocalizations that are thought to be associated with distress. Although this behavior could be considered as negative by human observers, such a response could be adaptive. An exploratory behavioral response (vigilance and increased locomotion) to a stressor would assist the animal in obtaining information about its environment, including the source of the stressor. The stressor also elicits a physiological response (in this case increases in glucocorticoids) that may impact the animal's ability to learn. There is a vast literature on the effects of stress on learning, and the mechanisms of how glucocorticoids and other hypothalamo–pituitary–adrenal axis hormones promote adaptive responses to environmental changes through learning. The mechanisms are complex and not completely understood; however, it is clear that glucocorticoids play a role in the regulation of memory consolidating processes [Roozendaal, 2000; Wolf, 2003; Shors, 2004] and may enhance memory formation. Therefore, we suggest that caution is warranted when interpreting changes in behavior and glucocorticoids in response to putative stressors. Negative stress may only be identified when animals seem to suffer biological costs (e.g., impaired reproduction or immune function) from responding to stressors [Moberg, 2000].

CONCLUSIONS

Demolition work in proximity to the giant panda exhibit at the National Zoo affected the activity budgets and stress-related behaviors of the pandas, and behavioral and adrenal responses to demolition noise were individual-specific. Although both giant pandas exhibited acute responses to acoustic stimuli, the welfare of neither seemed to be significantly decreased by demolition noise.

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