Population density-dependent hair cortisol concentrations in rhesus monkeys (*Macaca mulatta*)

A.M. Dettmer[1],[*], **M.A. Novak**[2], **J.S. Meyer**[2], and **S.J. Suomi**[1]

[1]Eunice Kennedy Shriver National Institute for Child Health and Human Development, National Institutes of Health, Poolesville, MD, 20837 USA

[2]Department of Psychology, University of Massachusetts Amherst, Amherst, MA, 01003 USA

Summary

Population density is known to influence acute measures of hypothalamic-pituitary-adrenal (HPA) axis activity in a variety of species, including fish, deer, birds, and humans. However, the effects of population density on levels of chronic stress are unknown. Given the fact that exposure to chronically elevated levels of circulating glucocorticoids results in a host of health disparities in animals and humans alike, it is important to understand how population density may impact chronic stress. We assessed hair cortisol concentrations (HCCs), which are reliable indicators of chronic HPA axis activity, in rhesus monkeys (*Macaca mulatta*) to determine the influence of population density on these values. In Experiment 1, we compared HCCs of monkeys living in high-density (HD; 1 monkey/$0.87m^2$) and low-density (LD; 1 monkey/$63.37m^2$) environments (N=236 hair samples) and found that HD monkeys exhibited higher hair cortisol across all age categories (infant, juvenile, young adult, adult, and aged) except infancy and aged ($F_{(5)}$=4.240, p=0.001), for which differences were nearly significant. HD monkeys also received more severe fight wounds than LD monkeys (χ^2=26.053, p<0.001), though no effects of dominance status emerged. In Experiment 2, we examined how HCCs change with fluctuating population levels across five years in the adult LD monkeys (N=155 hair samples) and found that increased population density was significantly positively correlated with HCCs in this semi-naturalistic population ($r_{(s)}$=0.975, p=0.005). These are the first findings to demonstrate that increased population density is associated with increased chronic, endogenous glucocorticoid exposure in a nonhuman primate species. We discuss the implications of these findings with respect to laboratory research, population ecology, and human epidemiology.

Keywords

cortisol; stress; chronic; population density; rhesus monkey

---

[*]Correspondence: Amanda M. Dettmer, 16701 Elmer School Rd., Poolesville, MD, 20837; dettmera@mail.nih.gov; Tel: 301-443-9944; Fax: 301-496-0630.

Conflict of interest

We declare that none of the authors has any financial or other relationships that might lead to a conflict of interest in relation to this study or to the content of this manuscript.

Introduction

Population density is known to affect various indices of a population's health. Animal studies have demonstrated that increased population density results in reduced viability in fruit flies (Lewontin, 1955); in reduced growth rates in land snails (Cameron and Carter, 1979), trout (Jenkins et al., 1999), and deer (Pettorelli et al., 2002); and in reduced reproductive success in numerous species including song sparrows (Arcese and Smith, 1988), guppies (Dahlgren, 1979), and red deer (Bonenfant et al., 2002). In humans, increased population density predicts emerging infectious disease events (Jones et al., 2008).

One potential underlying mechanism for these deleterious effects of population density may be exposure to chronically elevated circulating glucocorticoids. Increased population density may act as a stressor due to increased competition for resources including food, mates, and shelter, among others. Evidence for this notion comes from a vast body of literature describing the detrimental effects of long-term exposure to elevated glucocorticoids (i.e., corticosterone and cortisol). Chronic exposure to elevated glucocorticoid levels, whether via persistent environmental stress or repeated administration of glucocorticoids, results in reduced fetal growth (Jobe et al., 1998), growth suppression (Allen, 1996; Emack et al., 2008), suppressed immune function (Dhabhar, 2009), reduced reproductive function (Carragher et al., 1989; Chrousos et al., 1998; Pervanidou and Chrousos, 2012), and is also neurotoxic, particularly to the hippocampus (Bodnoff et al., 1995; Conrad, 2008; Sapolsky et al., 1990). Several animals have demonstrated increased glucocorticoid levels in response to increased population density, including fish (Li et al., 2012; Vijayan and Leatherland, 1990), voles (Novikov and Moshkin, 1998), chipmunks (Clulow et al., 1969), deer (Li et al., 2007), and primates (Pearson et al., 2007), including humans (Evans and Wener, 2007). One limitation of these studies, however, is that they have relied either on "point" measures of cortisol (i.e., serum/plasma/salivary cortisol) or on indices that reflect less than 1 day of adrenocortical activity (i.e., urinary and fecal cortisol). Thus, while it is likely that population density influences chronic HPA axis activity, this prediction has not been examined outright.

In 2006, our laboratory developed and validated an assay for quantifying cortisol concentrations in the hair of rhesus monkeys (Davenport et al., 2006). This technique has since been applied in studies of chronic HPA axis activity (i.e., accumulation of cortisol into the hair shaft over the past several months, as opposed to minutes or hours as in the point samples mentioned above) in both animals (Bechchøft et al., 2012; Davenport et al., 2008; Malcom et al., 2012; Meyer and Novak, 2012; Novak et al., 2013) and humans (O'Brien et al., 2012; Vanaelst et al., 2013). Studies utilizing hair cortisol have shown that HCCs decline during the first several years of life in several nonhuman primate species including rhesus monkeys (Dettmer et al., 2012; Feng et al., 2011), vervets (Laudenslager et al., 2012), and baboons (Fourie & Bernstein, 2011), and one study also demonstrated sex differences in HCCs in nonhuman primates during puberty and adulthood (Laudenslager et al., 2012). Moreover, several studies in both human and nonhuman primates have demonstrated increases in HCCs in response to stressful events including relocation (in monkeys; Davenport et al., 2008; Dettmer et al., 2012; Fairbanks et al, 2011), unemployment (in humans; Dettenborn et al., 2010), and chronic health conditions (in humans; Thomson et al., 2010; Van Uum et al., 2008). Thus, we sought to utilize the hair cortisol assay to test the hypothesis that population density affects chronic physiological stress in nonhuman primates. Specifically, we predicted that rhesus monkeys (*Macaca mulatta*) exposed to high-density (HD) housing arrangements would exhibit higher hair cortisol concentrations (HCCs) than those living in low-density (LD) conditions (Experiment 1), and that for monkeys living in the LD (i.e., more naturalistic) environment, changes in population density would predict HCCs (Experiment 2). We further sought to determine whether

behavioral indices of stress resulted from increased population density by examining the proportion of individuals in each housing condition that received severe fight wounds, as we predicted that increased population density would result in increased rates of aggression and thus increased fight wounds.

Materials and Methods

Experiment 1

Hair samples (N=236) were assayed from 152 rhesus macaques (*Macaca mulatta*; 30.5% male) born and reared at the Laboratory of Comparative Ethology in Poolesville, MD. Monkeys ranged in age from 0.5 to 18 years (mean±SEM=3.39±0.27 years) during hair sampling, which occurred between 2007 and 2013. Monkeys were housed in one of two conditions, described in detail below. Table 1 depicts subject characteristics for this experiment.

High-density (HD) environment—Monkeys (n=58) were housed in one of two HD enclosures depending on their age. The first type of enclosure, which housed infants (<1yr) and adults (5+ yrs) used for this study, was an indoor-outdoor enclosure constructed of galvanized steel mesh connected by guillotine doors and wood chips covering the floors. Eight to 10 adult females and one adult male, along with 5-6 infants up to 8 months of age, were housed together in this type of enclosure. Multiple perches, swings, and enrichment devices were provided. The indoor pen measured $2.44 \times 3.05 \times 2.21$m, and the outdoor pen measured $2.44 \times 3.0 \times 2.44$m. Animals were given free access between indoor and outdoor pens except when confined to one half for cleaning (twice per week), laboratory protocol procedures, or inclement weather (e.g., 4°C or below, a very rare occurrence). Inside lighting was maintained on a 12:12 cycle (0700-1900), and the outdoor portion of the enclosure was exposed to ambient lighting. Monkeys were fed Purina High Protein Monkey Chow (#5038) twice daily and received water *ad libitum.* Supplemental fruit and other foraging materials such as peanuts or sunflower seeds were provided daily. Monkeys from four of these types of enclosure were studied, with population density in each enclosure being approximately one monkey per 0.87m^2.

The second HD enclosure was used to house young monkeys from the time of weaning through adolescence; thus monkeys lived in this enclosure from 8 months (when they were moved from the first HD enclosure) through 24 months of age. These monkeys represented all infants born in a cohort (i.e., infants born each year; approximately 40-50 per year) from all of our laboratory's rearing conditions: mother-peer-reared (MPR; these infants were reared in the first type of HD enclosure), peer-reared (PR), and surrogate-peer-reared (SPR). PR and SPR monkeys were reared in a neonatal nursery from birth through 8 months (Shannon et al., 1998; Dettmer et al., 2012); these infants were not included in the present study. This second HD enclosure was also indoor/outdoor; the indoor enclosure measured $7.3 \times 3.4 \times 3.7$m and was equipped with perches, barrel, swings, and wood shavings on the floor. The outdoor enclosure was a circular corn-crib measuring 5.03m in diameter $\times$ 5.48m high, and was also equipped with perches, barrels, and swings but had a bare cement floor. Cleaning and lighting schedules, as well as food, water, and indoor-outdoor access were the same as for the first HD enclosure described above. Population density in this enclosure was approximately one monkey per 1.05m^2.

Low-density (LD) environment—Monkeys in this environment (n=94) lived in a 5-acre outdoor field enclosure with access to three indoor enclosures measuring $2.74 \times 5.79 \times 4.27$m, where food, foraging enrichment, and indoor shelter were provided. Indoor enclosures were equipped with swings and perches. The field station consisted of a grass-

covered substrate and natural foliage (e.g., cedar trees, honeysuckle bushes, tall grasses) as well as climbing apparatus (e.g., jungle gym, swings/perches) and small shelters (e.g., corn-cribs and tree huts) for outdoor access to shelter from inclement weather. Monkeys in the LD condition were born and reared in this enclosure throughout the duration of the study. Cleaning and lighting schedules, as well as food, water, and indoor-outdoor access were the same as for the first HD enclosure described above, with the exception that animals were not locked inside for inclement weather or for cleaning of shelters located in the field station. That is, monkeys in the LD enclosure had plentiful space to relocate during these cleaning times and as such were not greatly disturbed by these procedures. The population in this enclosure averaged 74.4 monkeys over the five years of the study; thus population density was approximately one monkey per 63.37m^2.

Subjects in each housing condition were divided into the following age groups: infant (<1yr, n=37), one-year-olds (1yr, n=80), two-year-olds (2yr, n=61), young adults (5-9yr, n=30), adults (10-14yr, n=24), and aged (15+yr, n=6). Table 1 depicts the subjects by age and housing condition. Because very few samples were available for three- and four-year-old monkeys in the HD condition, these ages were not included in analyses.

Fight wounds—We examined veterinary records of each subject in Experiment 1 to determine the incidence of fight wounds in the HD and LD conditions. From these records, we calculated the proportion of animals in each age category that received severe fight wounds, i.e., those requiring veterinary treatment, as these types of wounds indicated extreme levels of aggression and thus served as an index of behavioral stress.

Experiment 2

Hair samples (N=155) were collected from adult rhesus monkeys (4 years or older at the time of sampling; mean±SEM=12.10±3.4% male), born and reared in the LCE's 5-acre field enclosure (LD condition), in February of 2007, 2008, 2009, 2010, and 2013. Because we have observed an age-related decline in HCCs in both our HD and LD populations (see Figure 1), and because others have also observed age-related declines in HCCs across development (Feng et al., 2011; Fouric & Bernstein, 2011; Laudenslager et al., 2012), we included only adult animals (4 years or older) to determine whether population density affected HCCs in adult monkeys. Thus, the sample sizes (i.e., number of adult hair samples) for each year were as follows: 2007, n=37; 2008, n=34; 2009, n=32; 2010, n=22; 2013, n=30 (total N=155 hair samples). Owing to changes in research personnel in 2011 and the first part of 2012, hair samples for February in these years were not available. Of importance to this experiment, a matrilineal overthrow occurred in this population in March 2009, resulting in the removal of most of one of the three major families living here over the next 6-8 months (i.e., the family that was overthrown). Subjects for this experiment were housed in the same conditions as in the LD condition for Experiment 1 (and many animals contributed the same samples to both experiments).

The total population in the field station was recorded at the time of each hair sample to correlate population density with HCCs.

Dominance status

Because dominance status has been shown to influence chronic stress in adult monkeys (Michopoulos et al., 2012; Sapolsky, 2005; Shively et al., 1997), we assessed the influence of dominance status on HCCs for both Experiments 1 and 2. Adult male and female monkeys were classified as high- (1), middle- (2), or low-ranking (3) by animal care staff, who observed each animal on a daily basis for health status and who made routine notes of agonistic interactions (e.g., threat, displace, chase, fight/tackle, bite) between subjects. High-

ranking monkeys were those that never or very rarely received agonistic behaviors from other monkeys; middle-ranking monkeys were those that received agonistic behaviors from high-ranking monkeys but not from low-ranking monkeys and directed agonistic behaviors to low-ranking monkeys; and low-ranking monkeys never or rarely directed agonistic behaviors toward other monkeys but most often received agonistic behaviors from high- and middle-ranking monkeys. These rankings were in place prior to the onset of the study; inter-observer reliability was achieved by having trained researchers record all agonistic interactions between monkeys in daily, 60-min observation sessions. After these observations, interactions were entered into a database and Elo ranks were calculated for each social group (Elo, 1978). Elo-rating is a method for ranking individuals with numerous advantages over conventional matrix-based methods that has been established for use in behavioral research (Neumann et al., 2011). Inter-observer reliability between research staff conducting the formal observations was achieved at or above 85%; the dominance rankings produced by Elo rankings were in agreement with those conducted by animal care staff at 90%. In the HD condition, of the 27 adult animals for which dominance status were available, 9 were high-ranking, 9 were middle- and 9 were low-ranking. In the LD condition, of the 25 adult animals for which dominance data were available, 10 were high-ranking, 12 were middle- and 3 were low-ranking.

Hair sampling and cortisol assay

Hair samples for both experiments were collected between February 2007 and February 2013. Hair was always shaved from the same location on the back of the neck during routine health exams. Samples were placed in an aluminum foil pouch and stored in the dark at room temperature until assay, which occurred within one month of hair collection (Davenport et al., 2006; Dettmer et al., 2012). Our laboratory validated, and has since published numerous studies on, the hair cortisol assay in rhesus monkeys (Davenport et al., 2006; 2008; Dettmer et al., 2009; 2012) and other animals (Bechshøft et al., 2011). Briefly, hair samples were weighed, washed twice with isopropanol, allowed to air dry for 5-7 days, then ground to a fine powder with a ball mill grinder (MM200; Retsch, Newtown, PA) before being incubated in methanol for 24hr to extract cortisol from the samples. Aliquots of the methanol extract were then dried down and reconstituted with assay buffer prior to being analyzed via enzyme immunoassay (EIA) using a salivary cortisol kit (#1-3002; Salimetrics, State College, PA). Resulting values (μg/dL) were converted to pg/mg for analysis. Inter- and intra-assay coefficients of variation were <8% based on aliquots of the same extracted pooled hair sample analyzed repeatedly across assays. For Experiment 2, annual HCC values for monkeys in the field station (LD condition) were correlated with maximum population density in a given year.

Statistical analysis

Experiment 1—All HCC values were log transformed to meet the assumptions of normality prior to analysis. Univariate analysis of variance (ANOVA) was employed to test for the effects of housing condition (HD vs. LD), age, sex, and dominance status on HCCs. Post-hoc Tukey's HSD tests were used to determine statistically significant differences in HCCs for age and dominance status. Chi-square tests examined differences in wounding rates in HD and LD monkeys across each age category.

Experiment 2—The sample sizes (i.e., number of available hair samples from adult monkeys) for each year were as follows: 2007, n=37; 2008, n=34; 2009, n=32; 2010, n=22; 2013, n=30 (total N=155 hair samples). Two outliers, an individual from 2008 and one from 2013, each of which had HCCs at least three times as high as the highest value in that year, were identified and removed from the dataset prior to analysis. All HCC values were log transformed to meet the assumptions of normality prior to analysis. Univariate ANOVA was

employed to test for the effects of year, sex, and dominance status on HCCs. Post-hoc Tukey's HSD tests were used to determine statistically significant differences in HCCs based on year and dominance status. Spearman correlations assessed the relationship between total population density and average values of adult HCCs across the five years (2007, 2008, 2009, 2010, 2013); thus five pairs of values were used in the correlation analysis.

Results

Experiment 1

Significant main effects of housing condition (LD vs. HD; $F_{(1)}$=98.79, p<0.001) and age category (<1, 1yr, 2yr, young adult, adult, aged; $F_{(5)}$=29.48, p<0.001) on HCC were revealed. HD monkeys exhibited significantly higher HCCs than LD monkeys (125.89±6.50 vs. 70.71±3.28 pg/mg; Table 2). Infants, 1-year-olds, and 2-year-olds exhibited higher HCCs than any adults (Infants: 158.23±9.38 pg/mg; 1yr: 94.91±5.96 pg/mg; 2yr: 77.08±4.59 pg/mg; young adults, adults, and aged monkeys' average HCC ranged from 56.13±4.70 to 63.02±8.00 pg/mg). No effect of sex on HCCs was present (p>0.50). A significant housing × age category interaction was revealed ($F_{(5)}$=4.240, p=0.001) such that HD monkeys exhibited significantly higher hair cortisol than LD monkeys at 1yr, 2yr, 5-9yr, 10-14yr (all p's<0.001), and 15+ yr ($t_{(5)}$=2.942, p=0.021), and the difference was nearly significant for infants ($t_{(35)}$=1.907, p=0.067; Figure 1; Table 2). Interactions for housing × sex, age category × sex and housing × age category × sex were not significant (p's>0.50). No significant main effects for dominance status emerged (p>0.50), but a significant housing × dominance interaction was revealed ($F_{(2)}$=3.28, p=0.047) such that HD monkeys exhibited higher hair cortisol concentrations than LD monkeys for all dominance ranks (all p's≤0.01; Table 2). Figure 1 depicts HCC values for HD and LD monkeys across age categories. Table 2 shows higher HCCs for HD as compared to LD monkeys by age category, sex, and dominance.

Overall, a significantly greater proportion of HD than LD monkeys received severe fight wounds (χ^2=26.053, p<0.001). Further analyses revealed that these differences were significant at 1yr (χ^2=4.881, p=0.041) and for young adults (5-9yr; ^{2}=9.159, p=0.004; Figure 2).

Experiment 2

Figure 3 shows HCC average values for adult monkeys housed in the LD condition across the five years of the study. Significant main effects of year ($F_{(4)}$=10.65, p<0.001) and sex ($F_{(1)}$=5.12, p=0.026) were revealed. No effect of dominance status was revealed, nor were there any interactions. Post-hoc tests revealed that HCCs in 2007 were greater than HCCs in 2010 and 2013; HCCs in 2008 were greater than HCCs in 2009, 2010, and 2013; and HCCs in 2009 were less than HCCs in 2008 and greater than HCCs in 2010 (all p's<0.05). For clarity, asterisks in Figure 3 indicate significant differences from the previous year. Overall, HCCs were higher for adult females (55.81±2.29 pg/mg) than for males (45.39±4.06 pg/mg).

HCCs were significantly positively correlated with total population density values across the five years of the study ($r_{(s)}$=0.975, p=0.005; Figure 4).

Discussion

We discovered that higher population density is associated with increased hair cortisol concentrations (HCCs), a reliable indicator of integrated HPA axis activity, in captive rhesus monkeys. This was true for HD monkeys as compared to LD monkeys, as well as for LD

monkeys living in a semi-naturalistic environment and experiencing fluctuations in population density. Although the present findings are correlational in nature, they strongly suggest that population density influences chronic levels of circulating glucocorticoids, which has important implications for nonhuman primate well-being, population ecology, and human health.

Monkeys living in a high-density (HD) environment exhibited higher HCCs than those living in a low-density (LD) environment at all ages we examined. In addition, we observed an age-related decline in HCCs that has been previously observed in primates, including humans, with the highest levels recorded during infancy (Davenport et al., 2003; Feng et al., 2011; Grunau et al., 2007; Laudenslager et al., 2012). Regarding the near-significant differences in HCCs at different population densities in infancy (Figure 1), it is possible that the physiological effects of dense living conditions are not realized until later in development, though further experiments assessing HCCs more frequently throughout the first year of life would be necessary to determine this relationship. Moreover, in human infants, corticosteroid binding globulin (CBG) levels are low, resulting in higher levels of "free" unbound cortisol in plasma (Hadjian et al., 1975; Gunnar and Donzella, 1992); if the same is true in nonhuman primates, it could explain the high HCC levels we observed in infancy, as HCC levels are also thought to reflect the "free" fraction of cortisol in the bloodstream (Davenport et al., 2006). Despite the high levels of HCCs in infancy in both housing conditions, the fact that HCCs were higher amongst HD monkeys in all age groups suggests that a more dense living arrangement influences long-term HPA axis activity regardless of age. We also determined that even semi-free ranging monkeys living in a very LD environment showed increased HCCs as population density increased; as population levels declined, HCCs declined as well. Thus it appears that monkeys living in a variety of housing conditions are susceptible to the chronic stress that may result from long-term crowding, as well as from fluctuations in population density. Our data showing a higher incidence of fight wounds in HD vs. LD monkeys support this notion. Our laboratory is currently conducting studies to determine the relationship between population density, HCCs, and other potential deleterious outcomes of high density including hair loss, behavioral withdrawal, and reproductive consequences (e.g., infant mortality). Such findings will have important implications for behaviorists, enrichment specialists, directors, and other staff and research personnel working with captive nonhuman primates, whether they are housed in zoos or in laboratory settings.

In Experiment 1, we observed no difference in HCCs between males and females. While at first glance these results conflict with other reports demonstrating lower HCCs in males than in females (e.g., Laudenslager et al., 2012), a closer examination reveals that Laudenslager et al. (2012) reported lower HCCs in males at puberty and beyond, whereas our results are likely driven by the fact that, in Experiment 1, we had very few samples from males 3 years and older (as in Laudenslager et al., 2012). We had more hair samples from male subjects aged 0–2yrs (n=75) than from young adult, adult, or aged males (n=4). Thus, the observed age-related decline in HCCs may be particularly noticeable for males in our study; it is likely that if we had been able to sample more pubertal and adult males we may have observed a similar sex difference to Laudenslager et al (2012). This notion is supported by our results from Experiment 2, which focused only on adults aged 4 years and older and showed lower overall HCCs in males than in females, which is in line with findings by Laudenslager et al. (2012).

The behavioral changes we observed (i.e., increased aggression as evidenced by increased fight wounds) brought on by increases in population density may themselves stimulate the HPA axis. Past studies in rhesus monkeys, including one by Boyce et al. (1998) at our LD facility examining the effects of short-term confinement, as well as work by Judge and De

Waal (1997) studying short-term increases in population density, report density-related increases in the incidence of fight wounds (Boyce et al., 1998) and certain patterns of aggression (Judge and De Waal, 1997). Our results are also in line with very recent findings in chimpanzees, in which higher HCCs were correlated with higher received rates of aggression (Yamanashi et al., 2013). While it is likely that the psychological stress resulting from increased aggression in our HD population may have resulted in increased long-term cortisol secretion, an alternative explanation is that HCCs were increased simply because in this setting, there was no place to hide and/or escape from aggressors as there was in the LD study. Our data are only correlational and thus cannot prove causality of this relationship, and further studies examining fluctuations in density in the HD condition are warranted to tease apart this relationship. However, it is probable that the "no room to hide" variable is partially a function of population density itself; had fewer animals been present in the HD condition, there would be less of a need to flee, and more space to do so if necessary. In support of this notion, Judge and de Waal (1997) found that as population density increased, female rhesus monkeys increased all types of behavior, including contact and non-contact aggression. Our HD environments consisted of 6-8 adult females and only 1 adult male; thus it is reasonable to assume that, as in Judge and de Waal's study (1997), increased population density results in increased aggression, which goes hand-in-hand with having no place to hide. Thus it is likely that many variables related to population density work in concert to raise HCCs in the HD condition.

The fact that we observed no relationship between dominance status and HCCs in either the HD or LD setting confirms previous reports showing no effect of dominance status on acute measures of cortisol in stable social groups of some other primates (Bercovitch & Clarke, 1995; Hoffman et al., 2010; Stavisky et al., 2001); however, it should be noted that the relationship between dominance and cortisol has been found to be species- and sex-dependent (Abbott et al., 2003). Rather, acute levels of cortisol (i.e., those measured in plasma, saliva, or urine) are higher in most individuals regardless of rank during periods of rank instability (Chamove & Bowman, 1978; Sapolsky, 1992).

Our findings extend beyond captive environments to naturalistic environments as well. The fact that we found that HCCs in semi-free ranging monkeys fluctuated closely with population density suggests that wild populations of primates, and indeed other animals, might experience similar physiological stress in crowded living conditions. Habitat destruction is one type of insult that may lead to overcrowding and, consequently, elevated adrenocortical activity. Indeed, wild populations of fish, deer, chipmunks, and birds all present with higher acute glucocorticoid levels in response to increased population density (Li et al., 2007; Nephew and Romero, 2003; Ramsay et al., 2006). In light of the findings we report here, it is possible that wild primates may experience chronic as well as acute physiological stress as population density increases. Given that exposure to chronically elevated circulating glucocorticoids results in reduced immune function and fecundity, amongst a host of other health issues (Chrousos and Gold, 1992), our results suggest that efforts aimed at reducing population density are especially critical for at-risk animal populations. Unfortunately, we were unable to assess the short-term effects of the matrilineal overthrow of March 2009 in this population, as hair samples were only available in the month prior to the overthrow and again one year later. However, it appears that the resulting removal of the overthrown family, which drastically reduced the population density in the LD condition, may have contributed to the significant decrease in adult HCCs from 2009 to 2010.

Humans, too, are susceptible to the stresses of crowded living conditions. Higher acute measures of cortisol have been reported for youth and young adults living in crowded residential dwellings (Aiello and Baum, 1979; Evans and Marcynyszyn, 2004). Elevated

baseline and stress-response plasma cortisol, as well as a greater prevalence of hypertension, have been reported for African adults undergoing urbanization (Malan et al., 1992; Opie et al., 2005), and greater perceived stress in the perinatal period has been reported for U.S. women living in urban versus rural areas (Zust et al., 2010). Thus, there is little reason to doubt that more crowded, urban living conditions would also result in higher chronic stress as compared to less crowded, rural living conditions. The chronic stress brought on by crowded living conditions may account for density-dependent transmission of many diseases (Bharti et al., 2011; Yang et al., 2012), as elevated cortisol levels are known to suppress immune function (Marik and Flemmer, 2012); this immunosuppression could exacerbate heightened disease vulnerability in overcrowded conditions. Accordingly, our findings have important implications for human epidemiology, particularly as the world's population continues to grow and cities are becoming more crowded (World Bank, 2012). Hair cortisol may indeed prove useful as a biomarker for a number of stress-related health disparities in humans and animals and will be an invaluable tool in studies of social pathology and medical epidemiology.

Acknowledgments

References

Abbott DH, Keverne EB, Bercovitch FB, Shively CA, Mendoza SP, Saltzman W, Sapolsky RM. Are subordinates always stressed? A comparative analysis of differences in cortisol levels among primates. Horm Behav. 2003; 43:67–82. [PubMed: 12614636]

Arcese P, Smith JNM. Effects of population density and supplemental food on reproduction in song sparrows. J. Animal Ecol. 1988; 57:119–36.

Aiello, JR.; Baum, A. The study of residential crowding. In: Aiello, JR.; Baum, A., editors. Residential Crowding and Design. Plenum Press; New York: 1979. p. 1-7.

Allen DB. Growth suppression by glucocorticoid therapy. Endocrin. Metab. Clinc. North Amer. 1996; 25:699–717.

Bechshøft TØ, Sonne C, Dietz R, Born EW, Novak MA, Henchey E, Meyer JS. Cortisol levels in hair of East Greenland polar bears. Sci. Total Environ. 2011; 409:831–4. [PubMed: 21144554]

Bechshøft TØ, Sonne C, Dietz R, Born EW, Muir DC, Letcher RJ, Novak MA, Henchey E, Meyer JS, Jenssen BM, Villanger GD. Associations between complex OHC mixtures and thyroid and cortisol hormone levels in East Greenland polar bears. Environ. Res. 2012; 116:26–35. [PubMed: 22575327]

Bercovitch FB, Clarke AS. Dominance rank, cortisol concentrations, and reproductive maturation in male rhesus macaques. Phys Behav. 1995; 58:215–221.

Bharti N, Tatem AJ, Ferrari MJ, Grais RF, Djibo A, Grenfell BT. Explaining seasonal fluctuations of measles in Niger using nighttime lights imagery. Science. 2011; 334:1424–27. [PubMed: 22158822]

Bodnoff SR, Humphreys AG, Lehman JC, Diamond DM, Rose GM, Meaney MJ. Enduring effects of chronic corticosterone treatment on spatial learning, synaptic plasticity, and hippocampal neuropathology in young and mid-aged rats. J. Neurosci. 1995; 15:61–9. [PubMed: 7823152]

Bonenfant C, Gaillard JM, Klein F, Loison A. Sex- and age-dependent effects of population density on life history traits of red deer *Cervus elaphus* in a temperate forest. Ecogrpahy. 2002; 25:446–58.

Boyce WT, O'Neill-Wagner P, Price CS, Haines M, Suomi SJ. Crowding stress and violent injuries among behaviorally inhibited rhesus macaques. Health Psychology. 1988; 17:285–9. [PubMed: 9619479]

Cameron RAD, Carter MA. Intra- and interspecific effects of population density on growth and activity in some helicid land snails (*Gastropoda: Pulmonata*). J. Animal Ecology. 1979; 48:237–46.

Carragher JF, Sumpter JP, Pottinger TG, Pickering AD. The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta L.* and *Salmo gairdneri Richardson*. Gen. Comp. Endocrin. 1989; 1989:310–21.

Chamove AS, Bowman RE. Rhesus plasma cortisol response at four dominance positions. Aggressive Behav. 1978; 4:43–55.

Chrousos GP, Gold PW. The concepts of stress and stress system disorders. JAMA. 1992; 267:1244–52. [PubMed: 1538563]

Chrousos GP, Torpy DJ, Gold PW. Interactions between the Hypothalamic-Pituitary-Adrenal Axis and the Female Reproductive System: Clinical Implications. Ann. Int. Med. 1998; 129:229–40. [PubMed: 9696732]

Clulow FV, DesMarais A, Vaillancourt J. Physiological correlations of population density in the eastern chipmunk (*Tamias striatus*) and the white-footed mouse (*Peromyscus leucopus*). Canad. J. Zool. 1969; 47:427–33.

Conrad CD. Chronic stress-induced hippocampal vulnerability: the glucocorticoid vulnerability hypothesis. Rev. Neurosci. 2008; 19:395–412. [PubMed: 19317179]

Dahlgren BT. The effects of population density on fecundity and fertility in the guppy, *Poecilia reticulata (Peters)*. J. Fish Biology. 1979; 15:71–91.

Davenport MD, Novak MA, Meyer JS, Tiefenbacher S, Higley JD, Lindell SG, Champoux M, Shannon S, Suomi SJ. Continuity and change in emotional reactivity in rhesus monkeys throughout the prepubertal period. Motiv. Emot. 2003; 27:57–76.

Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. Gen. Comp. Endocrinol. 2006; 147:255–61. [PubMed: 16483573]

Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. A rhesus monkey model of self-injury: effects of relocation stress on behavior and neuroendocrine function. Biol. Psychiatry. 2008; 15:990–6. [PubMed: 18164279]

Dettenborn L, Tietze A, Bruckner F, Kirschbaum C. Higher cortisol content in hair maong long-term unemployed individuals compared to controls. Psychoneuroendocrinology. 2010; 35:1404–9. [PubMed: 20471757]

Dettmer AM, Novak MA, Suomi SJ, Meyer JS. Physiological and behavioral adaptation to relocation stress in differentially reared rhesus monkeys: hair cortisol as a biomarker for anxiety-related responses. Psychoneuroendocrinology. 2012; 37:191–9. [PubMed: 21715101]

Dettmer AM, Novak MFSX, Novak MA, Meyer JS, Suomi SJ. Hair cortisol predicts object permanence performance in infant rhesus macaques (*Macaca mulatta*). Devel. Psychobiol. 2009; 51:706–13. [PubMed: 19771550]

Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. Neuroimmunomodulation. 2009; 16:300–17. [PubMed: 19571591]

Elo, AE. The rating of chess players, past and present. Arco; New York: 1978.

Emack J, Kostaki A, Walker CD, Matthews SG. Chronic maternal stress affects growth, behaviour and hypothalmo-pituitary-adrenal function in juvenile offspring. Horm. Behav. 2008; 54:514–20. [PubMed: 18674758]

Evans GW, Marcynyszyn LA. Environmental justice, cumulative environmental risk, and health among low- and middle-income children in upstate New York. Am. J. Public Health. 2004; 94:1942–44. [PubMed: 15514234]

Evans GW, Wener RE. Crowding and personal space invasion on the train: please don't make me sit in the middle. J. Envir. Psych. 2007; 27:90–4.

Fairbanks LA, Jorgensen MJ, Bailey JN, Breidenthal SE, Grzywa R, Laudenslager ML. Heritability and genetic correlation of hair cortisol in vervet monkeys in low and higher stress environments. Psychoneuroendocrinology. 2011; 36:1201–08. [PubMed: 21411232]

Feng X, Wang L, Yang S, Qin D, Wang J, Li C, Lv L, Ma Y, Hu X. Maternal separation produces lasting changes in cortisol and behavior in rhesus monkeys. Proc. Natl. Acad. Sci. 2011; 108:14312–17. [PubMed: 21844333]

Fourie NH, Bernstein RM. Hair cortisol levels track phylogenetic and age related differences in hypothalamic-pituitary-adrenal (HPA) axis activity in non-human primates. Gen. Comp. Endocrinol. 2011; 174:150–5. [PubMed: 21893059]

Grunau RE, Haley DW, Whitfield MF, Weinberg J, Yu W, Thiessen P. Altered basal cortisol at 3, 6, 8, and 18 months in infants born at extremely low gestational age. J. Pediatr. 2007; 150:151–56. [PubMed: 17236892]

Gunnar MR, Donzella B. Social regulation of the cortisol levels in early human development. Psychoneuroendocrinology. 1992; 27:199–220. [PubMed: 11750779]

Hadjian AJ, Chedin M, Cochet C, Chambaz EM. Cortisol binding to proteins in plasma in the human neonate and infant. Pedatr. Res. 1975; 9:40–5.

Hoffman CL, Ayala JE, Mas-Rivera A, Maestripieri D. Effects of reproductive condition and dominance rank on cortisol responsiveness to stress in free-ranging female rhesus macaques. Am. J. Primatol. 2010; 72:559–65. [PubMed: 20039328]

Jenkins TM Jr. Diehl S, Kratz KW, Cooper SD. Effects of population density on individual growth of brown trout in streams. Ecology. 1999; 80:941–56.

Jobe AH, Wada N, Berry LM, Ikegami M, Ervin MG. Single and repetitive maternal glucocorticoid exposures reduce fetal growth in sheep. Am. J. Obst. Gynecol. 1998; 178:880–5. [PubMed: 9609554]

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P. Global trends in emerging infectious diseases. Nature. 2008; 451:990–4. [PubMed: 18288193]

Judge PG, De Waal F. Rhesus monkey behavior under diverse population densities: coping with long-term crowding. Animal Behavior. 1997; 54:643–62.

Laudenslager MJ, Jorgensen MJ, Fairbanks LA. Developmental patterns of hair cortisol in male and female nonhuman primates: Lower hair cortisol levels in vervet males emerge at puberty. Psychoneuroendocrinology. 2012; 37:1736–39. [PubMed: 22497987]

Lewontin RC. Effects of population density and composition on viability in *Drosophila melanogaster*. Evolution. 1955; 9:27–41.

Li C, Jiang Z, Tang S, Zeng Y. Influence of enclosure size and animal density on fecal cortisol concentration and aggression in Père David's deer stags. Gen. Comp. Endocrin. 2007; 151:202–9.

Li D, Liu Z, Xie C. Effect of stocking density on growth and serum concentrations of thyroid hormones and cortisol in Amur sturgeon, Acipenser schrenckii. Fish Physiol. Biochem. 2012; 38:511–20. [PubMed: 21717129]

Malan NT, Eloff FC, Pretorius PJ, Van der Merwe JS, Huisman HW, Laubscher PJ. A comparison of endocrine reactions to different types of acute stressors in rural and urban black populations. Stress Medicine. 1992; 8:213–18.

Malcolm KD, McShea WJ, Van Deelen TR, Bacon HJ, Liu F, Putman S, Zhu X, Brown JL. Analyses of fecal and hair glucocorticoids to evaluate short- and long-term stress and recovery of Asiatic black bears (Ursus thibetanus) removed from bile farms in China. Gen. Comp. Endocrin. 2012; 185:97–106.

Marik PE, Flemmer M. The immune responses to surgery and trauma: Implications for treatment. J Trauma Acute Care Surg. 2012; 23:801–8. [PubMed: 22976420]

Meyer JS, Novak MA. Minireview: Hair cortisol: a novel biomarker of hypothalamic-pituitary-adrenocortical activity. Endocrinology. 2012; 153:4120–7. [PubMed: 22778226]

Michopoulos V, Higgins M, Toufexis D, Wilson ME. Social subordination produces distinct stress-related phenotypes in female rhesus monkeys. Psychoneuroendocrinology. 2012; 37:1071–85. [PubMed: 22244748]

Nephew BC, Romero LM. Behavioral, physiological, and endocrine responses of starlings to acute increases in density. Horm Behav. 2003; 44:222–32. [PubMed: 14609544]

Neumann C, Duboscq J, Dubuc C, Ginting A, Irwan AM, Agil M, Widdig A, Engelhardt A. Assessing dominance hierarchies: validation and advantages of progressive evaluation with Elo-rating. Animal Behav. 2011; 82:911–21.

Novak MA, Hamel AF, Kelly BJ, Dettmer AM, Meyer JS. Stress, the HPA axis, and nonhuman primate well-being: a review. Appl. Animal Behav. Sci. 2013; 143:135–49.

Novikov E, Moshkin M. Sexual maturation, adrenocortical function and population density of red-backed vole, *Clethrionomys rutilus* (Pall.). Mammalia. 1998; 62:529–40.

O'Brien KM, Tronick EZ, Moore CL. Relationship between hair cortisol and perceived chronic stress in a diverse sample. Stress Health. 2012 Epub ahead of print. doi: 10.1002/smi.2475.

Opie LH, Seedat YK. Hypertension in sub-Saharan African populations. Circulation. 2005; 112:3562–68. [PubMed: 16330697]

Pearson BL, Judge PG, Reeder DM, Smart L. Salivary cortisol and self-directed behavioral responses to short-term crowding in hamadryas baboons (*Papio hamadryas hamadryas*). Am. J. Primatol. 2007; 69(S1):92.

Pervanidou P, Chrousos GP. Metabolic consequences of stress during childhood and adolescence. Metabolism. 2012; 61:611–19. [PubMed: 22146091]

Pettorelli N, Gaillard JM, Van Laere G, Duncan P, Kjellander P, Liberg O, Delorme D, Maillard D. Variations in adult body mass in roe deer: the effects of population density at birth and of habitat quality. Proc. Royal Soc. London. B: Biol. Sci. 2002; 269:747–53.

Ramsay JM, Feist GW, Varga ZM, Westerfield M, Kent ML, Schreck CB. Whole-body cortisol is an indicator of crowding stress in adult zebrafish, *Danio rerio*. Aquaculture. 2006; 258:565–74.

Sapolsky RM. Cortisol concentrations and the social significance of rank instability. Psychoneuroendocrinology. 1992; 17:701–9. [PubMed: 1287688]

Sapolsky RM. The influence of social hierarchy on primate health. Science. 2005; 308:648–52. [PubMed: 15860617]

Sapolsky RM, Uno H, Rebert CS, Finch CE. Hippocampal damage associated with prolonged glucocorticoid exposure in primates. J. Neurosci. 1990; 10:2897–902. [PubMed: 2398367]

Shannon C, Champoux M, Suomi SJ. Rearing condition and plasma cortisol in rhesus monkey infants. Am. J. Primatol. 1998; 46:311–21. [PubMed: 9839904]

Shively CA, Laber-Laird K, Anton RF. Behavior and physiology of social stress and depression in female cynomologous monkeys. Biol. Psych. 1997; 41:871–82.

Stavisky RC, Adams MR, Watson SL, Kaplan JR. Dominance, cortisol, and behavior in small groups of female cynomologous macaques (*Macaca fascicularis*). Horm Behav. 2001; 39:232–238. [PubMed: 11300714]

Thomson S, Koren G, Fraser L-A, Rieder M, Friedman TC, Van Uum SHM. Hair analysis provides a historical record of cortisol levels in Cushing's syndrome. Exp. Clin. Endocrinol. Diabetes. 2010; 118:133–8. [PubMed: 19609841]

Vanaelst B, Michels N, De Vriendt T, Huybrechts I, Vyncke K, Sioen I, Bammann K, Rivet N, Raul JS, Molnar D, De Henauw S. Cortisone in hair of elementary school girls and its relationship with childhood stress. Eur. J. Pediatr. 2013 Epub ahead of print. PMID: 23371393.

Van Uum SHM, Sauve B, Fraser LA, Morley-Forster P, Paul TL, Koren G. Elevated content of cortisol in hair of patients with severe chronic pain: A novel biomarker for stress: Short communication. Stress: Int. J. Biol. Stress. 2008; 11:483–8.

Vijayan NM, Leatherlan JF. High stocking density affects cortisol secretion and tissue distribution in brook charr, Salvelinus frontinalis. J. Endocrinol. 1990; 124:311–8. [PubMed: 2155988]

Wold Bank. Urban population (% of total). 2012. Data retrieved May 22, 2013, from World DataBank Database, http://data.worldbank.org/indicator/SP.URB.TOTL.IN.ZS

Yamanashi Y, Morimura N, Mori Y, Hayashi M, Suzuki J. Cortisol analysis of hair of captive chimpanzees (*Pan troglodytes*). Gen. Comp. Endocrinol. 2013; 194:55–63. [PubMed: 24013009]

Yang K, LeJeune J, Alsdorf D, Lu B, Shum CK, Liang S. Global distriution of outbreaks of water-associated infectious diseases. PLoS Negl. Trop. Dis. 2012; 6:e1483. [PubMed: 22348158]

Zust B. Stress perception among rural and urban perinatal patients. Online J. Rural Nurs. Health. 2010; 10:70–9.
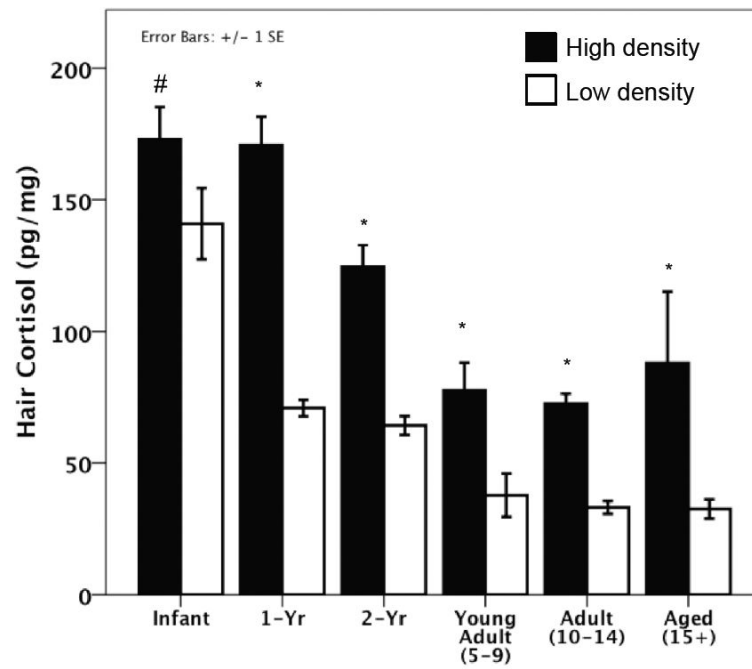
Figure 1.
Results for Experiment 1. Monkeys in the high-density (HD; solid bars) housing condition exhibited higher hair cortisol concentrations (HCCs) than monkeys in the low-density (LD; open bars) condition across all ages. *p<0.05, #0.05 p 0.10.

Figure 2.
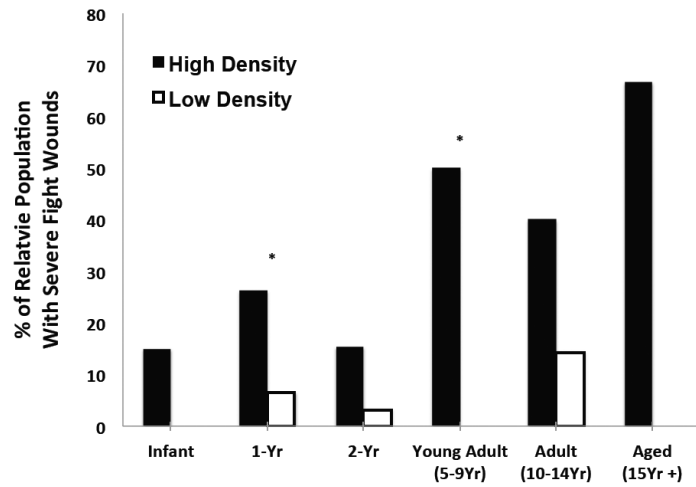Incidence of severe fight wounds across all ages for HD monkeys (solid bars) and LD monkeys (open bars). *p<0.05.
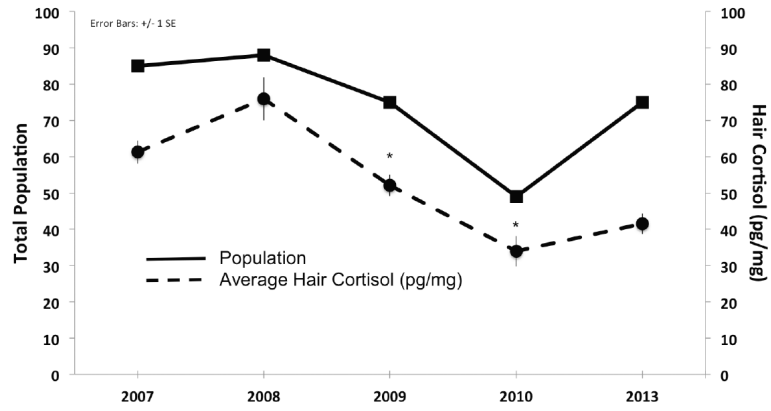
Figure 3.
Results for Experiment 2. For monkeys reared in a semi-naturalistic environment (LD condition), hair cortisol concentrations (HCCs) fluctuated with population levels. *indicates significant change in HCCs from previous year.
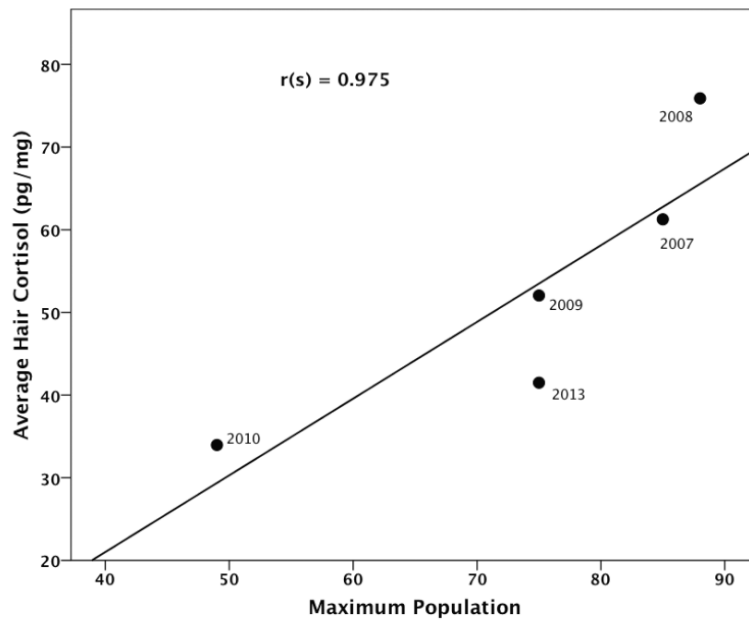
Figure 4.
For monkeys housed in the LD condition, hair cortisol concentrations (HCCs) were significantly positively correlated with maximum population ($r_{(s)}$=0.866, p=0.005). Numbers next to black circles indicate the year of sampling.

Table 1

Subject characteristics for Experiment 1. HD=high density; LD=low density.

Age Category	HD	LD	Total
Infant (<1yr)	20	17	37
1yr	19	60	80
2yr	13	46	61
Young Adult (5-9yr)	19	11	30
Adult (10-14yr)	14	10	24
Aged(15+yr)	3	4	6
Total	88	148	236

Table 2

Hair Cortisol concentrations (HCCs) for Experiment 1. All HCCs are reported as mean±SEM pg/mg.

		HD Hair Cortisol (n[a])	LD Hair Cortisol (n[a])	P[b]
	Infant	172.94±12.36 (20)	140.92±13.53 (17)	0.067
	1-yr	170.81±10.76(19)	70.88±3.14(60)	<0.001
Age	2-yr	124.55±8.33 (13)	63.73±3.73 (46)	<0.001
	Young Adult (5-9yr)	77.66±10.42 (19)	37.74±8.21 (11)	<0.001
	Adult (10-14yr)	72.56±3.75 (14)	33.12±2.46(10)	<0.001
	Aged (15+ yr)	87.93±27.19(3)	32.53±3.67(4)	0.075
Sex	Male	151.48±11.16(25)	66.31±4.28(54)	<0.001
	Female	115.73±7.61 (63)	73.14±4.61 (94)	<0.001
	High-Ranking	60.53±3.54(9)	40.09±8.90 (10)	<0.01
Dominance Status	Middle-Ranking	81.43±10.62(9)	30.55±2.60 (10)	<0.001
	Low-Ranking	77.50±5.14(9)	34.00±1.92(5)	<0.001
	TOTAL	125.89±6.50 (88)	70.65±3.32(148)	<0.001

[a](n)represents the number of hair samples used for analysis.

[b]P values indicate differences between HD and LD HCCs for each variable.