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Social stability influences the association between adrenal responsiveness and hair cortisol concentrations in rhesus macaques

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Abstract

Hair cortisol concentrations are increasingly being used in both humans and nonhuman animals as a biomarker of chronic stress. However, many details regarding how hair cortisol concentrations relate to the dynamic activity and regulation of the HPA axis are still unknown. The current study explores 1) how the regulation of the HPA axis in infancy relates to hair cortisol concentrations (HCC) in infancy 2) whether this relationship persists into adulthood under conditions of social stability, and 3) how social instability impacts these relationships. All subjects were rhesus monkeys housed in large social groups at the California National Primate Research Center, and all had participated in a 25-hr. long BioBehavioral Assessment (BBA) at 3–4 months of age when four plasma samples were taken to assess HPA regulation, in particular cortisol responses to 1) 2-hour social separation and relocation, 2) sustained challenge, 3) dexamethasone and 4) ACTH administration. In Study 1, hair samples were collected at the end of the BBA testing from 25 infant rhesus monkeys from 2 different stable social groups. In Study 2, hair samples were obtained at three timepoints from 108 adults from 3 different stable social groups (1 in the Spring/Summer and 2 in the Fall/Winter) to examine the temporal stability of the relationship between HCC and HPA axis regulation. In Study 3, subjects included 31 infants and 33 adults from a single social group experiencing social instability following the same procedures as in Studies 1 and 2.Generalized linear models were used to determine if infants' HPA axis activity and regulation predictedHCC in infancy (Study 1), in adulthood with animals living in stable social conditions

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Conflict of Interest

All authors declare that they have no conflicts of interest.

(Study 2) or in animals living in an unstable social group (Study 3). Results indicated that for both infants and adults living in stable social groups, HCC are associated with the adrenal response to ACTH in infancy. Samples collected in the winter also had higher HCC than those collected in summer. In the unstable social group, adult hair cortisol levels were higher than in the stable social groups. Additionally, there were no consistent relationships between HCC and infant HPA axis regulation among adults or infants living in a group experiencing social instability. These results suggest that the aspects of the HPA axis that drive HCC may differ depending on context. Under stable, non-stressed conditions there seems to be a trait- like association between adrenal responsivity and HCC in infancy and adulthood. However, this association may be reduced or eliminated under conditions of social stress.

Keywords

Hair cortisol; adrenal responsivity; plasma cortisol; social stability; social stress

The concentration of cortisol in hair is increasingly being used in a wide variety of species as a biomarker of chronic stress. Taxa examined so far include domesticated pets and livestock (Accorsi et al., 2008; Comin et al., 2011; Keckeis et al., 2012), mammalian wildlife (Ashley et al., 2011; Bechshøft et al., 2011; Koren et al., 2008; Martin and Réale, 2008), non- human primates (Davenport et al., 2006; Dettmer et al., 2014; Hamel et al., 2011; Laudenslager et al., 2011), and humans (e.g. D'Anna-Hernandez et al., 2011; Dettenborn et al., 2010; Sauvé et al., 2007; Van Uum et al., 2008). The ability to quantify cortisol concentrations in hair provides multiple advantages over previously used methods involving blood or saliva; hair is easy and non-invasive to collect, can be stored at room temperature, and requires only one sample to provide a measure of long term activation of the hypothalamic-pituitary-adrenal (HPA) axis.

In humans, studies have demonstrated that hair cortisol concentrations (HCC) are altered in a number of physiological or disease states including Cushing's syndrome, metabolic syndrome, pregnancy, PTSD, and depression (D'Anna-Hernandez et al., 2011; Manenschijn et al., 2011; Thomson et al., 2010; Wester and van Rossum, 2015). In addition, hair cortisol concentrations are elevated under conditions of psychosocial stress in human and nonhuman animals (Davenport et al., 2006; Dettmer et al., 2014; Staufenbiel et al., 2013). For example, non-human primates undergoing relocation to a new building or facility show elevated HCC (Davenport et al., 2006; Fairbanks et al., 2011). In humans, HCC have been shown to be elevated in shift workers, the unemployed, and students experiencing major life events (see (Staufenbiel et al., 2013) for review).

While assessment of cortisol concentrations in hair has become increasingly common, many questions regarding how hair cortisol concentrations relate to the regulation of the HPA axis remain unanswered. Multiple pathways have been suggested for how cortisol is incorporated into hair (Kapoor et al., 2018; Stalder and Kirschbaum, 2012). The most commonly cited mechanism is that free cortisol can passively diffuse from the blood stream to be incorporated into actively growing hair (Meyer and Novak, 2012). Recent research by Kapoor and Ziegler (2018) using tritium labeled cortisol supports this mechanism in rhesus

monkeys (although this may be species dependent, see Keckeis et al., 2012). Alternatively, it is also possible that cortisol is deposited into the hair through sweat or sebaceous gland secretions, or through exogenous exposure to glucocorticoid substances. Finally, there is evidence that cortisol can be synthesized and secreted at the level of the skin and hair follicle (Ito et al., 2005; Sharpley et al., 2009).

While HCC are commonly used as a biomarker of chronic stress, a number of questions remain regarding how the activity and regulation of the HPA axis relates to HCC. Previous research has demonstrated that cortisol measurements derived from multiple salivary samples (e.g. area under the curve), 24-hour urinary cortisol, and fecal cortisol are correlated with HCC (Accorsi et al., 2008; D'Anna-Hernandez et al., 2011; Davenport et al., 2006; Sauvé et al., 2007) providing support for the idea that HCC reflect chronic HPA axis activity. However, decades of research suggests stress not only can result in chronic elevated cortisol levels but can alter the regulation of the HPA axis. Chronic stress can lead to *hyper*cortisolemia which can be seen, for example, in some types of depression (Yehuda et al., 2004). In other cases, however, chronic stress can lead to *hypo*cortisolemia as seen in PTSD (Yehuda, 2006) and in chronic social stress in rhesus monkeys (Capitanio et al., 1998b). These contrasting impacts of chronic stress on HPA axis activity and regulation make it challenging to use cortisol as a simple biomarker of chronic stress. Therefore, we need a better understanding of how dynamic HPA axis activity and regulation are related to HCC in both stressed and non-stressed individuals in order to provide a better foundation on which to interpret results from studies using HCC as a metric of stress.

The current study aims to improve our understanding of how activity and regulation of the HPA axis relates to HCC across time and social context. In Study 1 we first examine the relationship between HPA axis regulation and hair cortisol concentrations in infancy among infants reared in stable social groups. In Study 2 we examine whether this relationship is stable over time by examining the association between HPA axis regulation measured in infancy and HCC in adults living in stable social groups. Finally, in Study 3 we examine whether the presence of social instability alters the relationship between HCC and HPA axis regulation in both infants and adults.

1 Material and Methods

Data for the current paper derived from three independent lines of research. Hair samples for Study 1 were collected as part of a research project examining the effects of variation in maternal care due to birth timing on infant physiological and behavioral development (Vandeleest et al., 2013b). Hair samples for Studies 2 & 3 were collected in conjunction with a larger project examining how social network structure and social perturbations (i.e. removal of individuals from a social group) impact health. Specifically, this study involved examining whether removal of natal males (Groups E & F; Beisner et al., 2011a) or defragmentation of matrilines (Groups C & D; Beisner et al., 2011b), would improve group stability and impact the health of individuals from those social groups. Finally, the data described above were combined with a long-term project assessing the consequences of variation in biobehavioral organization in infancy that has assessed more than 5000 animals since 2001. The results from the BioBehavioral Assessment (BBA) have been linked to a

wide variety of factors including risk for asthma, abnormal behavior, and prenatal stress exposure (Capitanio et al., 2005; Herrington et al., 2016; Vandeleest et al., 2011).

1.1 Subjects and Housing

All subjects were housed outdoors in large (0.2 ha) enclosures at the California National Primate Research Center (CNPRC). Each enclosure contained a single naturalistic social group of 125–200 animals of age and sex distributions similar to the wild. Enclosures contained multiple climbing structures, perches, and shelters from the rain and wind. Ample food was provided twice per day and water was available ad libitum. All subjects were participants of a BioBehavioral Assessment program (Section 1.2, below) when they were 3–4 months of age. All procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the UC Davis Institutional Animal Care and Use Committee.

1. 1.1 Study 1: HCC and HPA regulation in infancy in stable social groups—

Subjects for Study 1 included 28 infant rhesus monkeys (*Macaca mulatta*) born in 2009 and raised in one of two different social groups (Groups A and B; see Table 1). All animals were 3–4 months of age. Both social groups in Study 1 had been stable (i.e. no major outbreaks of aggression or changes in leadership) for at least 2 years prior to sample collection.

1. 1. 2 Study 2: HCC and HPA regulation in adults in stable social groups—

Subjects for Study 2 included 108 adults (3+ years old) from 3 different social groups studied in 2013–2014 that had also been assessed through the BBA program (See section 1.2) in infancy (Groups C - E; see Table 1 for details). This subset of animals comprised 43% of all adults housed in these 3 groups. All the social groups in Study 2 had been stable (i.e. no major outbreaks of aggression or changes in leadership) for 1–5 years prior to samples being collected. One group was composed of specific pathogen free animals (N = 34) but analysis revealed no differences in HCC between animals in the two conventional and the single SPF groups, so we combined data from all three groups. None of the females from any of the groups were pregnant at the time samples were collected. Approximately 65.5% of females from Groups D & E had given birth in the 3–6 months prior to study.

1. 1. 3 Study 3: HCC and HPA regulation in an unstable social group—

Samples were available for infants (N = 31, 79% of all infants) and adults (N = 33, 33% of all adults) from one group that experienced social instability in 2014 (Group F; see Table 1). Instability was evidenced by a dissociation of the status and aggression networks, a trait that seems to be indicative of future outbreaks of widespread aggression (Beisner et al., 2015; Chan et al., 2013) and altered social and physiological measures compared to the other groups studied in 2013–2014 (Balasubramaniam et al., 2018; Balasubramaniam et al., 2016). Notably this group suffered a social collapse 1 month before to 2 months after hair sample collection for infants and 2 weeks after the Follow-up hair sample was collected (see section 1.3 below) for adults. Fourteen of the twenty adult females were pregnant or lactating during the study.

1.2. Biobehavioral Assessment and Plasma Cortisol

HPA axis activity and regulation was assessed via blood samples during a standardized 25-hour biobehavioral assessment when infants were 3–4 months of age. The detailed methods are described elsewhere (Capitanio et al., 2006; Golub et al., 2009; Vandeleest et al., 2013a). Briefly, infants were separated from their mothers and relocated to a novel indoor testing environment where a battery of tests was administered to assess the infant's behavioral and physiological reactivity. At the end of the 25-hr testing period infants were reunited with their mothers and returned to their natal social groups. During the testing period four blood samples were drawn from each monkey via femoral venipuncture following brief manual restraint (see Figure 1) to assess HPA axis responses to 1) social separation and relocation, 2) sustained challenge, 3) dexamethasone, and 4) ACTH administration. The first blood sample was drawn at 11:00 am (1 ml), approximately 2 hours after maternal separation and relocation to the testing environment, and presumably reflects the immediate cortisol response to these events. A second blood sample was drawn at approximately 4:00 pm on the first day of testing (0.5 ml) and reflected the HPA axis response to the sustained challenge of social separation and the testing environment. After the second sample was drawn infants were injected with 500 μg/kg dexamethasone, a synthetic glucocorticoid, intramuscularly (American Regent Laboratories, Inc., Shirley, NY). A third blood sample (0.5 ml) was drawn at 8:30 am the next morning to measure the effectiveness of HPA axis negative feedback (i.e. the suppression by dexamethasone of endogenous cortisol release). After the third sample was drawn infants were injected with 2.5 IU ACTH (Organon, Inc. West Orange, NJ) intramuscularly and a final blood sample (0.5ml) was obtained 30 minutes later. Sample 4 reflected the capacity of the adrenal cortex to respond to ACTH stimulation. Blood was collected in unheparinized syringes, was immediately transferred to ETDA tubes and centrifuged at 4°C at 1277 RCF for 10 min, and plasma was then extracted and stored at −80°C until assay.

1. 3 Hair sample collection

For infants (Study 1), at the end of BBA testing and after the collection of Sample 4 was completed, hair was collected from the posterior vertex region of the neck (as described in Davenport et al., 2006) of each infant while awake and manually restrained and stored in tinfoil at room temperature until cortisol extraction.

For adults (Studies 2 & 3), hair samples were collected in 2013–2014. Sampling occurred on a single morning during which all animals in a social group were anesthetized and biological samples were collected (including hair samples) and physicals were conducted at three time points:1) Baseline (1 week before permanent removal of 1–20 group members; N=135), 2) Post- perturbation (i.e. 5 weeks after permanent removals; N=110), 3) Follow-up (13 weeks after permanent removals; N=123). All hair samples were collected from the posterior vertex of the neck, and repeated sampling only included hair regrowth from the previous sampling. Hair samples were collected 3–12 years after the BBA plasma cortisol measurements. Samples were collected between September and January for two of the social groups in Study 2 (Groups D and E) and March and July for one Study 2 group (Group C) and the Study 3 group (Group F).

1. 4 Cortisol Assay

1. 4. 1 Plasma Cortisol Assay—Due to changes to assay kit manufacturing, infant plasma samples from infant subjects in Study 1 and all adult subjects in Studies 2 & 3 were assayed using commercial radioimmunoassay kits (Siemens Medical Solutions Diagnostics, Los Angeles, CA) while samples from infants born in 2014 (Study 3) were assayed using a quantitative competitive immunoassay. For samples collected before 2014, plasma samples were diluted 1:6 in PBS gel buffer prior to assay. Plasma concentrations of cortisol were estimated in duplicate. Assay procedures were modified with the addition of 0.5 and 2.5 μg/dL concentrations of standards along with the provided range of 1.0 to 50 μg/dL. Assay sensitivity has been determined to be 0.26069 μg/dL. Intra- and inter-assay CVs were 5.18% and 8.30%, respectively. For samples collected after 2014, cortisol was measured utilizing a quantitative competitive immunoassay which employs direct chemiluminescent technology on the ADVIA Centaur CP platform (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The assay consists of dimethyl acridinium ester labeled cortisol which competes for binding to a polyclonal rabbit anti-cortsiol antibody bound to a monoclonal mouse anti-rabbit antibody covalently coupled to paramagnetic particles. The immune complex is captured and separated by application of a magnetic force. Addition of acid (hydrogen peroxide and nitric acid) and base (sodium hydroxide) reagents produce a chemically induced light emission measured by luminometer in relative light units (RLUs). The RLUs are inversely proportional to the amount of cortisol in the unknown sample. Samples were diluted 1:10 with ADVIA Centaur Multi-Diluent 3 (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) prior to analysis to obtain accurate results. Analytical sensitivity is 0.2 ug/dL. Inter-assay CV was 5.0% and intra-assay CV was 2.4%. Multiple regression was used to compare results from RIA and chemoluminescent assays and demonstrated a high correspondence between results from the two assays ($N = 32$, $R^2 = 0.880$).

1. 4. 2 Hair Cortisol Assay—Hair cortisol samples collected in 2009 were extracted using methods described in Davenport (2006), and assayed using a salivary cortisol kit (EIA, Salimetrics, Carlsbad, CA, USA). The inter-assay CV was 5.41%. Briefly, hair was washed twice using Isopropanol and then dried for 5–7 days. Hair was then ground into a fine powder using a Retsch ball mill grinder. Cortisol was then extracted for 18–24 hr (mean = 20.67 hr) using 1 mL methanol. After extraction, samples were centrifuged at 21,100 RCF and 600 μL of supernatant was transferred into a new tube, dried down and samples were reconstituted with 450μL of assay buffer. For samples collected in 2009, hair was ground in 10ml stainless steel jars with one 12mm steel ball for 10 min at 30 Hz. Fifty mg of powdered hair was then transferred to a microcentrifuge tube for the cortisol extraction step. For samples collected in 2013–2014, 35mg of hair was ground in a 2 mL microcentrifuge tube using 2– 7mm steel balls at 30 Hz for 10 min. Samples were re- weighed and the extraction step was then conducted using these same tubes. Validation of the processing alterations was conducted and found that samples ground using the methods from 2009 and 2013 had an inter-method CV of 8.7% (2 of 16 samples had CV > 15%) and the cortisol yield for samples processed in the microcentrifuge tubes was on average 108% of the yield from samples ground in steel jars.

1.5 Data Analysis

Data were analyzed using generalized linear mixed models in SAS. The outcome for each analysis was HCC which was modeled using a negative binomial distribution. Separate analyses were run for each timepoint that HCC were measured. Control variables included sex, age at BBA assessment (Capitanio et al., 2005), and age at hair collection for adults to control for the elapsed time between infant HPA axis assessment and adult hair sampling. Additionally, the impact of pregnancy status during the period of hair growth and lactation status were examined where relevant (i.e. for the Study 2 Baseline and all Study 3 analyses involving adults) but did not predict HCC or impact the effects in the models. Predictors included cortisol concentrations from the four blood samples collected during infant BBA testing. Only animals with valid data from all four BBA samples were included in the analysis. In addition, one adult from Group F had HCC > 500 pg/mg at Baseline and was excluded from the analysis. Including group as a random effect was examined for analyses associated with Studies 1 and 2, however inclusion did not result in a Δ AIC ≥ 2 and therefore was not included in the final models.

Model building proceeded in multiple steps. The first step involved the evaluation of random effects and control variables. Each control variable was used to predict HCC and only variables that reduced AIC from a null model by at least 2 were included in subsequent models. Next, BBA blood cortisol samples were used to predict HCC. Due to the dependence of cortisol levels from Samples 2–4 on the cortisol levels from the previous sample, evaluation of the predictive value of Samples 2–4 used a nested procedure. This is necessary because how much cortisol can increase after ACTH stimulation (Sample 4) depends on how much it was suppressed by dexamethasone administration (Sample 3). For example, to examine whether BBA Sample 2 predicted HCC, a nested model (A) was first built that contained all control variables, random effects, and the effect of Sample 1. Then a second model (B) was built that added Sample 2 and the change in AIC from model A to model B was examined with a change in AIC of at least 2 indicating better model fit. Final models were chosen based on AIC scores.

Finally, we ran two sets of analyses for Study 3. First, ANOVAs were run to examine whether hair cortisol levels for adults were different for the unstable group and the stable groups at each of the three adult timepoints (i.e. Baseline, Post-perturbation, Follow-up). If season was not a significant predictor in the GLMMs described above, all 4 groups of adults were included in the ANOVA with season and stability as predictors. If season was a significant predictor for a timepoint in Study 2 models, then only the stable group observed in the same season as the unstable group (i.e. spring) were compared in the ANOVA. Second, we use GLMMs as in Studies 1 & 2 to examine whether any aspects of HPA axis activity or regulation as measure by the BBA in infancy predict HCCs.

2. Results

2. 1 Study 1: HCC and HPA regulation in infancy in stable social groups

Results indicated that measures reflecting HPA axis regulation predict HCC for infants born into stable social groups in 2009. The best fit model (ΔAIC = 3.57) included plasma cortisol

samples 3 and 4 (see Table 2). Specifically, HCC were predicted by the increase in cortisol levels from sample 3 to sample 4 in BBA (see Table 3), a reflection of the capacity of the adrenal to respond to ACTH stimulation (Figure 2). None of the control variables predicted HCC and therefore they were not included in final models.

2. 2 Study 2: HCC and HPA regulation in adulthood in stable social groups

For adults in stable groups, the increase in plasma cortisol from sample 3 to sample 4 in BBA during infancy predicted HCC in adulthood at all three timepoints (see Table 3): Baseline (ΔAIC = 5.10), Post-perturbation (ΔAIC = 2.49), and Follow-up (ΔAIC = 13.50). Regression coefficients for sample 4 indicated that an increase in 1 μg/dL cortisol for sample 4 led to a 0.31, 0.21, and 0.29 pg/mg increase, for Baseline, Post-perturbation, and Follow-up, respectively (see Figure 3). The models for Post-perturbation and Follow-up timepoints also indicated that hair samples collected during Fall-Winter had higher HCC than those collected in Spring-Summer.

2. 3 Study 3: HCC and HPA regulation in an unstable social group

Adult HCC were higher in the unstable group compared to the stable groups. At Baseline ANOVA indicated that HCC were significantly higher in the unstable group compared to the three stable groups ($F(1,133) = 7.40$, $p = 0.007$, $R^2 = 5.3\%$). For Post-perturbation and Follow- up, comparisons were only done within the same season, as Study 2 indicated a significant effect of season. At the Post-perturbation timepoint, results indicated a trend for HCC to be higher in the unstable vs the stable group observed in spring ($F(1,56) = 2.79$, $p = 0.10$, $R^2 = 4.8\%$). Finally, HCC were significantly higher in the unstable group than the stable group at the Follow-up timepoint ($F(1,65) = 8.32$, $p = 0.005$, $R^2 = 11.3$).

For infants born into the social group that experienced social collapse, none of the measures of infant HPA axis activity and regulation predicted HCC (see Table 2). For adults in this group, model results were inconsistent across the three sampling periods (Table 2). For the Baseline timepoint, the best fit model (ΔAIC = 4.14) indicated that sample 1 predicted HCC, showing that greater plasma cortisol levels in response to maternal separation and relocation to a testing environment in infancy predicted HCC in adulthood (see Table 3). Males also had lower HCC than females at Baseline. At the Post-perturbation timepoint the best fit model was the null model indicating that HCC were not predicted by any of the variables tested. Finally, the best fit model predicting HCC at the Follow-up timepoint (ΔAIC = 3.73) indicated that a greater increase in plasma cortisol from morning to afternoon (Sample 1 to Sample 2) during BBA in infancy predicted higher HCC in adulthood.

3 Discussion

Concentrations of hair cortisol are related to the capacity of the adrenal gland to respond to ACTH stimulation, but this relationship depends on the stability of the social group within which an animal resides. For infants in stable social groups, higher cortisol levels after an ACTH injection were associated with higher concentrations of cortisol in hair that was collected at the same time as the plasma samples (Study 1). This relationship was found to be stable over time (up to 13 years later) in adults living in stable social groups (Study 2). In

contrast, HCC were higher in adults living in a group experiencing instability and HPA axis activity in infancy was not consistently associated with HCC in infants or adults (Study 3).

These results suggest that hair cortisol is, at least in part, a reflection of adrenal responsiveness to ACTH stimulation under non-stressed conditions. Multiple factors impact the functional tone of the HPA axis including central mechanisms (e.g. hippocampal glucocorticoid receptor density or CRH expression in the paraventricular nucleus of the hypothalamus) and peripheral mechanisms (e.g. adrenal hyperplasia) (Anacker et al., 2014; Herman and Tasker, 2016; Ulrich-Lai et al., 2006). The fact that the adrenal response to ACTH was associated with HCC under stable conditions suggests that HCC may be largely a reflection of peripheral regulatory mechanisms. While chronic stress is known to alter these peripheral regulatory mechanisms (e.g. through adrenal hyperplasia and hypertrophy) (Ulrich-Lai et al., 2006), the animals in Study 1 were unlikely to be experiencing chronic stress due to their young age (3–4 months old) and the stable characteristics of the social group in which they lived. Instead the results from Studies 1 and 2 suggest that greater adrenal responsiveness to ACTH in infancy may reflect individual differences in peripheral regulatory mechanisms that persist to adulthood and influence daily cortisol release. This possibility, however, requires further study to more directly examine the mechanisms underlying the observed relationship.

The similarity in results from infants in Study 1 and adults from Study 2 suggest that the mechanisms governing cortisol release in response to ACTH stimulation at the adrenal gland are trait-like and can persist across the lifespan given stable conditions. Although few studies have examined HPA axis activity longitudinally, there is evidence of a trait-like component of the HPA-axis. Shirtcliff et al. (2012) found that 13% of the variation in cortisol levels was attributable to trait-like stability across childhood to adolescence in humans. Plasma cortisol and ACTH levels were also found to be stable over time under consistent environmental conditions, although they showed greater individual variation across context (Capitanio et al., 1998a).Finally, HCC have been shown to exhibit trait-like qualities in both humans and non-human primates (Fairbanks et al., 2011; Grant et al., 2017; Novak et al., 2017; Stalder et al., 2012).

Consistent with the literature, social stress was associated with higher HCC levels. A wealth of previous research has demonstrated that chronic stress is associated with elevated HCC (Meyer and Novak, 2012; Stalder et al., 2017). Although chronic stress has been associated with HCC, the impact of different subtypes of stressors (e.g. social, physical, or environmental) still requires more research (Stalder et al., 2017). Current evidence for the impact of socially derived stressors on HCC indicates that social status, population density, receipt of social aggression, and rearing environment impact HCC (Carlitz et al., 2014; Dettmer et al., 2014; Dettmer et al., 2012; Koren et al., 2008; Yamanashi et al., 2013). The current research extends these findings to suggest that overall group stability also affects HCC. These results also support other findings from our research group that indicate that this group was likely experiencing a period of instability, considered a population-level social stressor, in the months leading up to an outbreak of severe, widespread aggression (Beisner et al., 2015; Chan et al., 2013).

Not only were HCC higher on average among adults living in an unstable social group, infants and adults from this group did not show the same relationship between adrenal responses to ACTH and HCC as did those in the stable group. Instead, results indicate no association or possibly an association between HPA axis reactivity (BBA Samples 1 & 2) and HCC. Under conditions of stress it is possible that more frequent or prolonged central activation of the HPA axis may drive HCC rather than peripheral anatomy (e.g. adrenal size or ACTH receptor density). Additionally, chronic stress exposure can alter both central HPA axis regulation as well as adrenal physiology (Herman and Tasker, 2016; Ulrich-Lai et al., 2006). In addition to differences between stable and unstable groups, effects for adults from stable groups varied in their strength of association across the sampling timepoints. At the Post- perturbation timepoint, 5 weeks after permanent removal of 1–20 animals from the group, adrenal responses to ACTH and HCC showed a weaker association than those before the removals (Baseline) or the ones 3 months later (Follow-up; Figure 3). This further supports the idea that the mechanisms underlying HCC may be different between non-stressed and stressed conditions.

Finally, season was found to be an important predictor of adult HCC, particularly for samples collected in the Spring (May-July) versus Fall (November-January). Seasonal variation in cortisol release has been demonstrated across a wide variety of species, although the climactic triggers are still unknown (Ingram et al., 1999; Schiml et al., 1999; Walker et al., 1997). One contributor is likely variation in temperature. Cold temperatures have been associated with cortisol release, likely due to cortisol's role in thermogenesis (Maickel et al., 1967; Werner and Vens-Cappell, 1985). Our results provide additional evidence for seasonal variation in cortisol levels, possibly due to temperature variations. While Baseline HCC were not influenced by season, this is possibly due to early spring and early fall temperatures in Northern California being roughly comparable. Daily temperatures (low-high) average 45–69.2 °F in March-April vs. 51.8–84 °F September-October (Arguez et al., 2010). The effects of season emerge during timepoints where daily temperatures are maximally different in summer and winter. Average temperatures for months corresponding to the Post-perturbation and Follow-up timepoints were 54.9–87.7 °F (May-June) for groups observed in Spring and 39.5–57.6 °F (November-January) for Fall groups. This is consistent with previous findings that higher plasma cortisol levels during BBA were related to exposure to lower median temperatures in infants (Vandeleest et al., 2013a). Alternatively, hair collected at Baseline included hair in different growth states and therefore could reflect a wider range of time frames (including pregnancy for some females), potentially obscuring seasonal effects (Harkey, 1993).

Altogether these results suggest that the aspects of the HPA axis that drive HCC may differ depending on context. Under stable, non-stressed conditions there seems to be a trait-like association between adrenal responsivity and HCC in infancy and adulthood. However, this association may be reduced or eliminated under conditions of social stress where other aspects of the HPA axis may be more important in driving HCC. Additionally, seasonal climate variations are important to consider when examining long term cortisol secretion through HCC.

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Highlights

- Adrenal sensitivity to ACTH predicts Hair cortisol concentrations in infancy
- Adrenal sensitivity in infancy predicts adult hair cortisol concentrations
- Social stress eliminates the adrenal sensitivity - hair cortisol association
- Hair cortisol was higher in winter and fall than summer and spring.

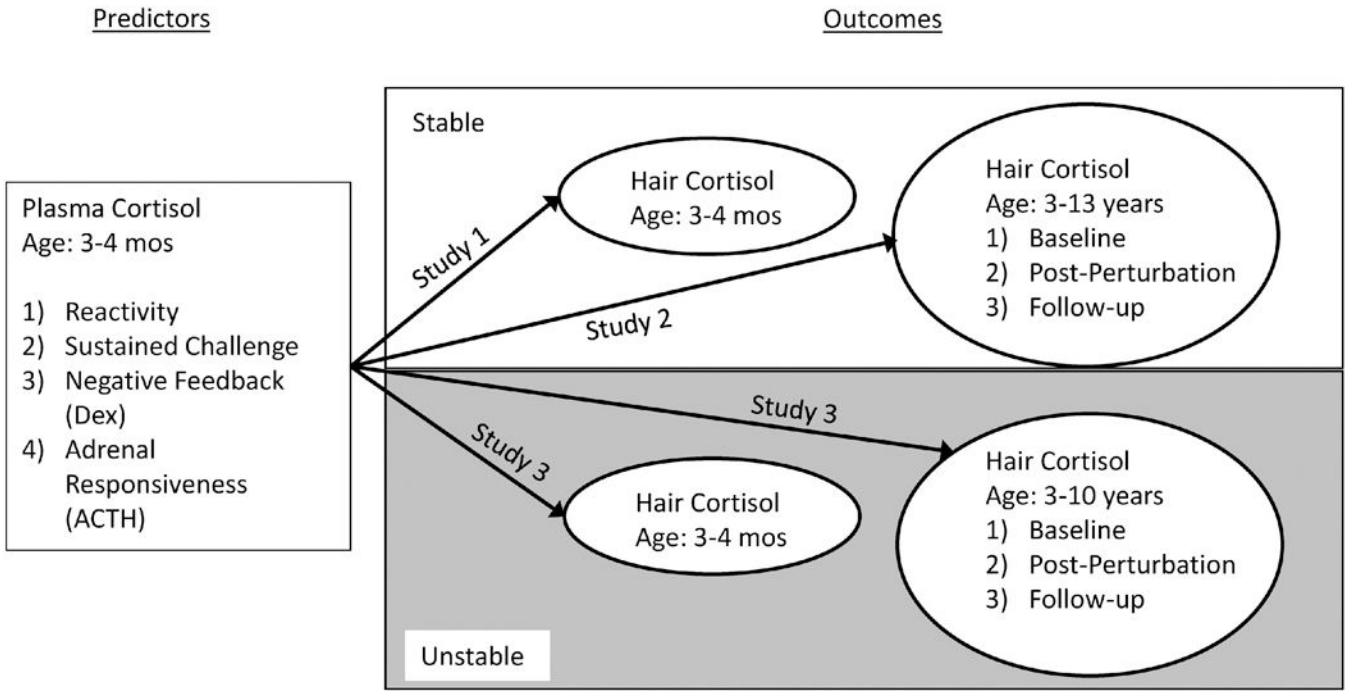

Figure 1. Study Design

Three studies examined the relationship between HPA axis activity and regulation measured in plasma in infancy and hair cortisol concentrations. In Study 1, plasma cortisol was used to predict hair cortisol concentrations in infants reared in stable social groups. In Study 2, the temporal stability of these associations was examined using hair samples collected at three time-points from adults living in stable social groups. In Study 3 the impact of social context on the observed relationships from Studies 1 & 2 were examined in infants and adults living in a group experiencing social instability.

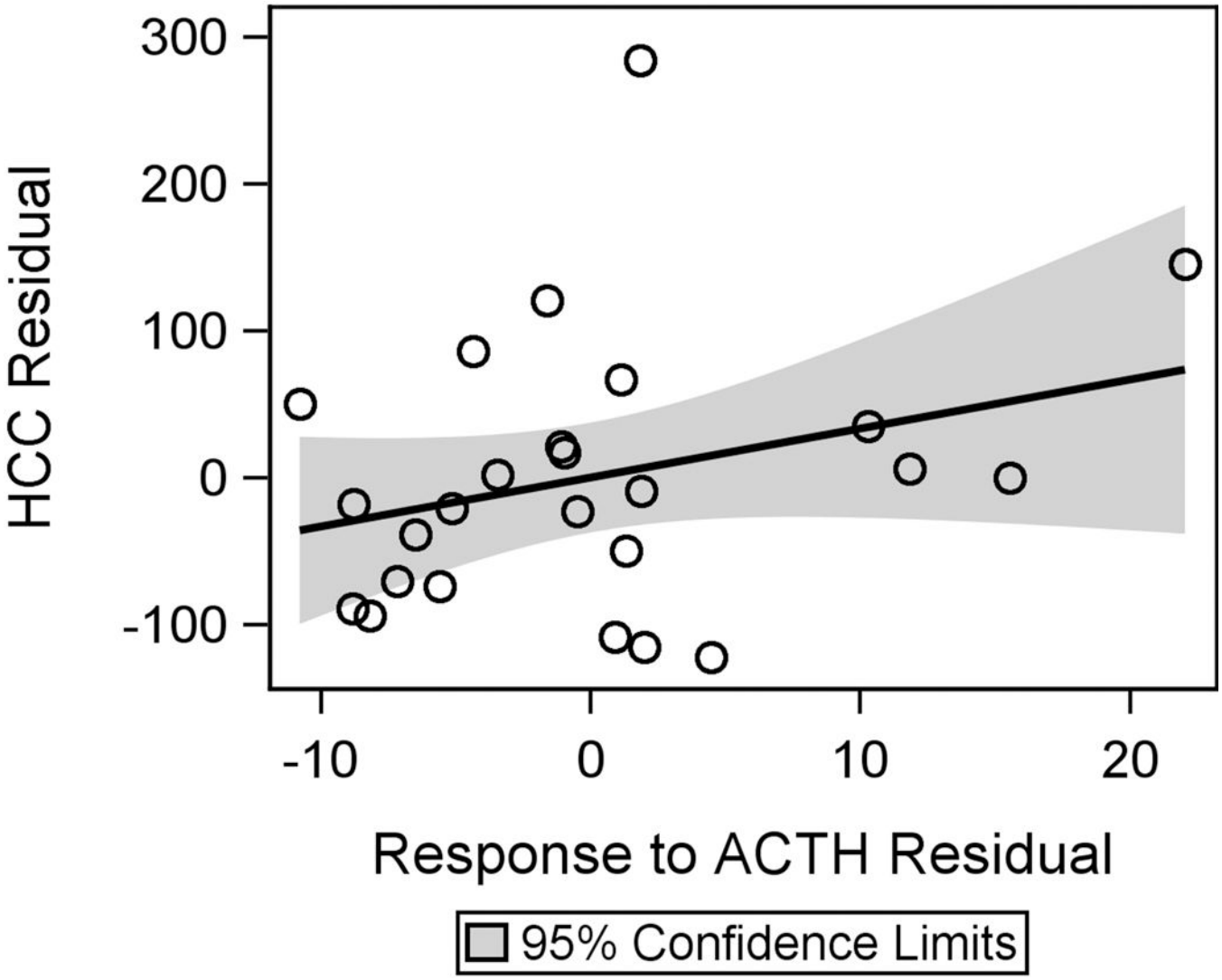

Figure 2. Study 1. Adrenal responsivity to ACTH and hair cortisol concentrations in infancy.
The association between plasma cortisol responses to ACTH stimulation (sample 4) and hair cortisol concentrations in infancy. Data are presented as residuals controlling for plasma responses to dexamethasone (sample 3). Shaded areas indicate 95% confidence limits.

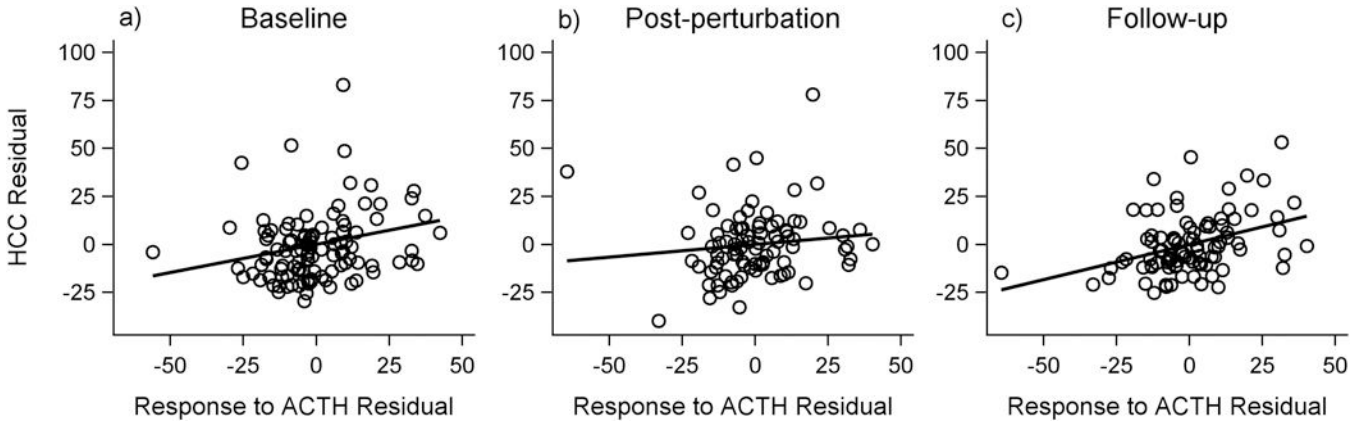

Figure 3. Study 2. Adrenal responsivity in infancy and adult hair cortisol concentrations.

The association between plasma responses to ACTH administration in infancy (sample 4) and adult HCC at a) baseline, b) after a social perturbation, c) during the follow-up period after social perturbation. Data are represented as residuals controlling for plasma responses to dexamethasone in infancy (plots a-c) and season (plots b-c).

Table 1:

Subjects and Descriptive Statistics

	Study 1			Study 2				Study 3	
	Infants		Totals	Adults			Totals	Infants	Adults
Year (Season)	2009	2009		2013 (Spring)	2014 (Fall)	2013 (Fall)		2014 (Spring)	
Group	A	B		C	D	E		F	F
N (male)	15 (10)	10 (4)	25 (14)	39 (10)	47 (15)	22 (3)	108 (28)	31 (17)	33 (13)
BBA sample 1 (SD)[1]	82.52 (26.31)	76.91 (19.47)	80.28 (23.53)	81.54 (25.41)	75.38 (26.33)	83.35 (16.39)	79.64 (24.41)	92.36 (21.0)[4]	82.36 (15.18)
BBA sample 2 (SD)[1]	82.01 (22.81)	77.93 (23.37)	80.38 (22.64)	92.50 (31.64)	87.01 (32.53)	96.85 (32.25)	90.99 (32.08)	101.03 (26.17)[4]	83.00 (21.50)
BBA sample 3 (SD)[1]	70.57 (27.03)	59.10 (20.73)	65.98 (24.90)	76.38 (32.65)	64.82 (21.30)	73.37 (29.37)	70.74 (27.78)	71.91 (21.96)[4]	62.42 (18.25)
BBA sample 4 (SD)[1]	93.77 (32.04)	86.96 (23.63)	91.04 (28.64)	106.38 (42.14)	100.31 (29.44)	104.77 (27.01)	103.41 (33.97)	100.39 (24.99)[4]	92.20 (22.47)
HCC at BBA (SD)[2]	170.84 (80.86)	240.15 (109.15)	198.57 (97.38)	-	-	-	-	175.55 (55.22)	-
HCC at Baseline (SD)[2]	-	-	-	58.89 (19.16)	67.06 (30.43)	60.50 (9.81)	62.81 (23.74)	-	77.24 (29.44)
HCC at Post-perturbation (SD)[2]	-	-	-	48.81 (12.90)	65.74 (19.32)	65.60 (22.87)	58.90 (19.63)	-	55.93 (19.61)
HCC at Follow-up (SD)[2]	-	-	-	45.03 (12.14)	69.08 (18.42)	69.84 (15.84)	60.00 (19.53)	-	55.30 (16.81)
Age at Hair Collection (range)[3]	0.29 (0.26–0.32)	0.29 (0.26–0.31)	0.29 (0.26–0.32)	6.6 (3–12)	7.4 (3–13)	6.3 (3–11)	6.9 (3–13)	0.29 (0.25–0.33)	5.4 (3–10)

[1] µg/dL

[2] pg/mg

[3] Age is in years

[4] Run using chemoluminescent assay

Table 2:

Models tested

		Study 1		Study 2				Study 3			
		Infants		Adults				Infants	Adults		
Model	Testing	2009		Baseline	Post-perturbation[1]	Follow-up[1]		2014	Baseline[2]	Post-perturbation	Follow-up
HCC = Sample 1	Sample 1: Response to separation and relocation	−0.57		1.55	2	0.37		1.99	**− 4.14**	1.98	−0.88
HCC = Sample 1 + Sample 2	Sample 2: Response to sustained challenge	1.99		0.59	0.2	1.39		1.97	− 0.85	0.87	**−3.73**
HCC = Sample 2 + Sample 3	Sample 3: Dexamethasone suppression test	1.55		1.15	1.1	1.9		1.87	1.4	1.71	2.64
HCC = Sample 3 + Sample 4	Sample 4: ACTH Challenge	**−3.57**		**−5.1**	**−2.49**	**−13.5**		0.33	0.66	1.83	1.5

Indicates a model with a drop in AIC > 2 when compared to the nested model.

[1]Controlled for significant effect season.

[2]Controlled for significant effect of sex.

Table 3:

Best Model Coefficients

	Study 1		**Study 2**				**Study 3**			
	Infancy		**Adults**				**Infancy**	**Adults**		
	2009		Baseline	Post-perturbation	Follow-up		2014	Baseline	Post-perturbation	Follow-up
N	25		107	87	91		31	28	23	32
Intercept	**4.6332**		**4.0624**	**3.7218**	**3.6103**		**5.1679**	**3.5693**	**4.0241**	**3.4483**
Sex (male)	-		-	-	-		-	**−0.22**	-	-
Season (Fall)	-		-	**0.27**	**0.3885**		-	-	-	-
Sample 1	-		-	-	-		-	**0.01017**	-	0.000763
Sample 2	-		-	-	-		-	**-**	-	**0.006037**
Sample 3	*−0.01892*		**−0.00617**	−0.00366	**−0.006**		-	-	-	-
Sample 4	**0.02065**		**0.004935**	**0.004258**	**0.006248**		-	-	-	-
Scale	0.1546		0.07569	0.05853	0.03367		0.08672	0.05989	0.07947	0.03938

p < 0.05, *p < 0.1*