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A novelty seeking phenotype is related to chronic hypothalamic-pituitary-adrenal activity reflected by hair cortisol

Mark L. Laudenslager^a, Matthew J. Jorgensen^b, Rachel Grzywa^a, and Lynn A. Fairbanks^c

^a Department of Psychiatry, University of Colorado at Denver School of Medicine, Denver CO 80220

^b Department of Pathology, Section on Comparative Medicine, Wake Forest University School of Medicine, Winston-Salem NC

^c Department of Psychiatry & Biobehavioral Sciences, Semel Institute, University of California at Los Angeles, Los Angeles CA 90095

Abstract

Reduced hypothalamic pituitary adrenal (HPA) activity is associated with greater novelty seeking in humans. Hair cortisol represents an integrated proxy measure of total cortisol production/release over an extended period of time and may be a valuable tool for tracking the HPA system. Sampling approaches (collection of blood, saliva, urine, or feces) for socially housed nonhuman primates present a number of technical challenges for collection particularly when repeated sampling is necessary. Herein we describe a relationship between cortisol levels measured in hair collected from 230 socially housed female vervet (*Chlorocebus aethiops sabaeus*) monkeys and a free-choice novelty seeking phenotype. A predator-like object was placed at the periphery of the outdoor enclosures for 30 min and speed of approach (latency to approach within 1 m) and persistence of interest (number of 1 min intervals within 1 m) were scored. A composite Novelty Seeking score, combining these two measures, was calculated. The intra-class correlation coefficient (ICC=.68) for two different objects across years indicated that this score reflects a stable aspect of temperament. Hair samples were collected from each subject approximately 3–6 months following the second assessment; cortisol levels were determined from the hair. A significant inverse relationship of Novelty Seeking score with hair cortisol level ($p < .01$) was noted. The high hair cortisol groups had significantly lower Novelty Seeking scores than the low cortisol groups both years (p 's $< .05$). These results suggest that low average cortisol levels promote novelty seeking, while high average levels inhibit novelty seeking behavior.

Keywords

novelty seeking; risk taking; temperament; hair cortisol; nonhuman primate; vervet monkey; *Chlorocebus aethiops sabaeus*

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Corresponding Author: Mark L. Laudenslager, PhD, Department of Psychiatry, University of Colorado at Denver, School of Medicine, Denver CO 80220, Office Number 303-315-9276, FAX Number (303) -315-9125, mark.laudenslager@ucdenver.edu.

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1. Introduction

Individual differences in novelty seeking and response to novelty are predictive of risk for multiple psychiatric disorders, including anxiety disorders or alcohol and substance abuse (1–4). Animal models have used a variety of novelty paradigms to understand the basic mechanisms involved, often with differing results depending on the type of novelty test. For example, rodent models of substance use disorders have found that inescapable or free-choice novelty paradigms evaluate different components of the addiction process (5) and reflect different neurochemical mechanisms (6). High rates of locomotion in an inescapable novelty test predict initial tendency to use or self-administer cocaine, while preference for novel places in a free-choice novelty test predicts the transition from use to addiction (7).

In nonhuman primates, inescapable novelty tests have been used to study emotionality and anxious temperament, modeled after studies of behavioral inhibition in children (3). Inescapable novelty paradigms typically involve removing infant or adolescent monkeys from their home environment, placing them in an unfamiliar cage or small room, confronting them with novel objects or an unfamiliar human, and measuring behavioral responses. These tests have been used to identify effects of early experience, genetic, hormonal, and neurobiological systems on defensive and fearful behavioral responses (8–12). Several studies have shown that levels of serum cortisol following the test sessions are positively associated with levels of *anxiety-related behaviors* such as freezing observed during the test (8, 9, 13).

Free-choice novelty tests, in contrast, have been used to measure *novelty-seeking phenotype* in nonhuman primates and differ significantly from inescapable challenges. In free-choice tests, the monkeys are presented with access to a novel area or novel object in the familiar home environment, and subjects are free to approach and explore, or to remain at a distance. Latency to enter a novel area or to approach a novel object by juvenile and adolescent primates in free-choice tests has been related to mildly stressful early experiences in macaques, vervets and squirrel monkeys (14–17). Free-choice novelty tests in a pedigreed vervet monkey colony demonstrated that novelty-seeking is a heritable trait, with a portion of the genetic contribution attributable to the same polymorphism in the dopamine D4 receptor gene that has been related to novelty seeking in human primates (18). In contrast to results from inescapable novelty tests, there is little information on the relationship between free-choice novelty seeking and acute or chronic measures of hypothalamic-pituitary-adrenal axis (HPA) activity (16).

A normal response of the HPA axis to an acute stressor is marked by a rapid increase in plasma cortisol levels followed by a relatively rapid return to baseline (19). Both enhanced and/or blunted responses of the onset and/or offset of the HPA system suggest disrupted regulation which may lead to pathophysiology and behavioral disturbance in the organism (20). Identification of valid markers of long term HPA regulation/activation will add to understanding the relation of the HPA system to trait-like individual differences in response to novelty. Is there a valid measure that can be easily obtained from nonhuman primates which reflects long term activation of the HPA?

Cortisol measured in hair has been recently introduced to ethological research as a marker of long term activation of the HPA axis. Measurement of steroids in hair has been available for over three decades but generally required the use of mass spectrometry approaches (21, 22). Longitudinal evaluation of cortisol extracted from hair samples has the potential to serve as an alternative marker of chronic HPA activation (23) much like hemoglobin A1c reflects blood glucose control for an extended period of time (24). Development of commercially available, high sensitivity enzyme immunoassays has been used for measurement of cortisol

in low concentration in saliva (25). These same assays permit rapid and reliable assessment of cortisol in hair collected from nonhuman primates (26). Hair cortisol level in the nonhuman primate was found to be higher in association with a phenotype of self injury and was correlated with salivary and plasma cortisol (27). Hair cortisol represents a reliable marker of longer term cortisol release in mammals. Importantly, hair cortisol levels are not impacted by the acute sampling distress as is the case for plasma steroids and other hormones, particularly for nonhuman primates (28).

Here we determine if there is a relationship between cortisol measured in hair collected from socially housed female vervet monkeys and their response to a free-choice novelty test using potentially threatening objects placed at the periphery of their home cage enclosures.

2. Materials and Methods

2.1. Subjects

Subjects were 230 female vervet monkeys (*Chlorocebus aethiops sabaues*) (3–18 yr of age) living in 16 stable multigenerational, matrilineal social groups at the Vervet Research Colony (VRC). The VRC was originally established in Sepulveda, CA in 1975 with vervet monkeys captured from St. Kitts, West Indies. All subjects in the current study were born at the VRC and lived in social groups that were managed to reflect the natural social composition of vervet monkey groups in the wild. Infants and juveniles were raised by their mothers in one of 16 matrilineal social groups. Females remained in the social group with their mothers and female kin, while males were removed from the natal group at adolescence and transferred into new groups as adults for breeding. These procedures have produced a large, extended multi-generational pedigree (29, 30).

The monkeys were housed in outdoor enclosures varying in size from 30–117 square meters of ground area (mean = 61 m²), with adjacent indoor shelters. Each outdoor corral had one or two large platforms and multiple perches, climbing structures and enrichment devices. The social groups were undisturbed except for daily maintenance, behavioral tests, the annual veterinary exam, and clinical interventions as needed. The number of adult female subjects per social group varied from 6 to 26 (mean = 14.4, SD = 5.6). Of the 230 female subjects, two were pregnant and delivered 38 and 71 days after sample collection. Five others had given birth between 40–75 days prior to hair sample collection. None of these females were sampled within one month of delivery; a time frame associated with an increase in hair cortisol in this population (55) and humans (56). None had experienced experimental procedures or significant clinical interventions in the three months prior to sample collection. All procedures were approved by the UCLA and Department of Veterans Affairs Institutional Animal Care and Use committees.

2.2. Home Group Novelty Test

The Home Group Novelty test is a procedure to measure free-choice novelty seeking in the home enclosure (18). A novel and potentially threatening object was placed at the edge outside of the outdoor enclosure within reach of the animals but away from any of the preferred sitting or resting places. Novel objects were selected that were salient enough to arouse interest and curiosity, with some potential for fear. All subjects in the current analysis were tested twice, a year apart, using predator-like objects as the novel stimuli. During the test, the door to the indoor shelter was closed so all group members were present in the outdoor area. The novel object (a cloth snake in 2006 and a plastic tarantula in 2007) was placed in a wicker basket positioned outside the chain link fence of the home enclosure, at ground level. The latency to approach within one meter of the object, and the number of 1-minute intervals that each animal was observed within 1 meter of the object was scored for a

30-minute test session. A team of observers familiar with identifying individual monkeys made a consensus determination of who was within 1 meter for each interval. The area within 1 meter of the object only occupies a small portion of the home enclosure, and it requires voluntary action on the part of the monkeys to approach the object.

2.3. Hair cortisol

Hair samples were collected in December 2007 - January 2008, during the annual veterinary examination, 3–6 months after the second novelty test was completed. All members of a social group were transferred into a capture tunnel and anesthetized with 8–10mg/kg Ketamine hydrochloride. Using electric hair clippers, a 4 x 4 cm patch of hair was shaved from the center of the back between the shoulder blades, taking care not to damage the skin. The hair for each individual was wrapped in aluminum foil, stored in individual plastic bags, and stored in a dark, temperature controlled environment until overnight shipment to Colorado for analysis. This approach follows recommendations of the Society for Hair Testing (31).

Hair cortisol analysis followed the method of Davenport (26). All of the hair obtained from each subject was washed two times in 5 ml 99% isopropyl alcohol and allowed to dry for 4 or more days in glass tubes. From the washed hair a clump representing a mixture of long, short, and fine hairs was removed for grinding. The entire length of hair from proximal to distal end (5.6 ± 0.7 cm, range = 4.5–6.7 cm) was ground in a ball mill (Retsch MM200) at 25 hz for 15 min, 50 mg of the powdered hair was extracted overnight in 1ml 100% HPLC grade methanol, and 0.6 ml of the extraction medium was dried under a nitrogen stream at 38°C for 45 min. The dried samples were reconstituted in 0.4 ml assay buffer used in the enzyme immunoassay (EIA) (Expanded range, high sensitivity salivary cortisol EIA, #3001, Salimetrics LLC). Twenty five microliters of the reconstituted samples in assay buffer were pipetted in duplicate to the wells of the microtiter plate and assayed according to manufacturer's instructions (Salimetrics, LLC). Plates were read on a Biotek microtiter plate reader at 450 nm. Standard curves were determined using Gen Five software from which the unknowns were estimated. In order to establish assay reliability, a pool of ground human hair was extracted at the time of each assay and included on each plate with unknown samples. Within and between assay coefficients of variability for the pooled hair control were 4.0 and 9.1% respectively.

2.4. Data Analysis

The latency to approach and the number of 1-min intervals within 1 meter of the novel object were inversely correlated (snake, $r = -0.42$; tarantula, $r = -0.48$). A composite score combining these measures was computed for each year (range = 0 –60), with high scores indicating a greater tendency to explore the object: Novelty Seeking Score = $(30 - \text{latency}) + (\# \text{ intervals within 1 meter})$. The interclass correlation coefficient was used to assess the consistency of individual differences in Novelty Seeking across tests.

The association between Novelty Seeking and hair cortisol level was assessed by Pearson correlation. In order to identify a possible nonlinear relationship between novelty seeking and cortisol level, one way analysis of variance with hair cortisol group (low, middle, high) as the independent variable and Novelty Seeking score as the dependent variable was also run, including a test for linear contrasts between groups. A significant main effect of group was followed by pair-wise comparisons among groups, using Tukey tests. Both hair cortisol and novelty seeking data were screened for covariates, including age, dominance rank, group size, and reproductive status.

3. Results

3.1. Novelty Seeking

Figure 1 compares Novelty Seeking scores for subjects for the 2006 (snake) and 2007 (tarantula) home group novelty tests. Of the 230 subjects, 197 (85.6%) approached the stimulus object during both tests, 9 (3.9%) did not approach the object in both tests, and 24 (10.4%) approached the stimulus object in one year but not the other. The intra-class correlation of Novelty Seeking scores was significant across years ($r = 0.68$, $df = 229$, $p < 0.001$) indicating the presence of a stable aspect of behavioral temperament that reflects reduced latency to approach and interest in novel and potentially risky stimuli.

Novelty seeking scores were inversely related to subject age for the range considered here (snake, $r = -0.29$, $df = 228$, $p < 0.001$; tarantula, $r = -0.27$, $df = 228$, $p < 0.001$). The high year to year consistency in scores remained after controlling age ($ICC = 0.65$, $df = 229$, $p < 0.001$). There were no significant effects of dominance rank on Novelty Seeking scores for either year (rank coded as high, middle, low: snake, $r = 0.11$, ns; tarantula, $r = .004$, ns), and Novelty Seeking was unrelated to the number of adult females in the group (snake, $r = -.05$, ns; tarantula, $r = 0.01$, ns).

3.2. Hair cortisol

Hair cortisol levels from samples collected approximately 3–6 months following the second novelty seeking test were normally distributed as shown in Figure 2. Hair cortisol levels ranged from 22.4 to 101.2 pg/mg (mean = 52.8, $SD = 12.3$), and was unrelated to female age (3–18 years of age) at the time of hair collection [Pearson's $r = 0.08$, ns]. There were no significant effects of female dominance rank on hair cortisol levels (rank coded as high, middle, low: $r = -0.01$, $df = 228$, ns), and the seven females who delivered an infant two to three months before or after sample collection did not differ from the remaining females in mean level ($t = 0.09$, $df = 228$, ns).

Hair cortisol level was inversely related to Novelty seeking score for both test years (snake, $r = -0.26$, $df = 228$, $p < .001$; tarantula $r = -0.18$, $df = 228$, $p < .01$; mean Novelty Seeking score $r = -0.25$, $df = 228$, $p < .001$). Controlling the effects of age did not change the relationship between hair cortisol levels and Novelty Seeking scores (snake, partial $r = -.25$, $df = 227$, $p < .001$; tarantula, partial $r = -.17$, $df = 227$, $p = 0.01$).

3.3. Relation of Novelty Seeking and Hair Cortisol

In order to distinguish subjects with overall enhanced or blunted cortisol activity (19), subjects were divided into three subgroups based on the upper and lower standard deviations (SD) of the distribution. The lowest group ($n = 28$) ranged from 22.4 to 40 pg/mg, the midrange group ($n = 169$) ranged from 40.5 – 64 pg/mg, and the highest group ($n = 33$) ranged from 65–101.2 pg/mg.

Mean (+SE) Novelty Seeking scores for the Low, Middle and High hair cortisol groups are shown in Figure 3 for the two stimulus objects. There was a significant main effect of hair cortisol group for both novel stimuli (snake: $F_{(2,227)} = 5.11$, $p < 0.01$; tarantula: $F_{(2,227)} = 3.31$, $p = 0.04$), and the unweighted linear term was also significant for both (snake: $F_{(1,227)} = 6.72$, $p = 0.01$; tarantula: $F_{(1,227)} = 5.88$, $p = 0.02$). Females in the high hair cortisol group falling in the upper SD of the cortisol distribution (>65 pg/mg), had significantly lower Novelty Seeking scores than the females from the low hair cortisol groups for both tests (Tukey test, $p < 0.05$). The middle and low groups did not differ significantly from each other for either test. Controlling age did not alter the significance of the linear relationship between Novelty Seeking and hair cortisol for either year ($p < 0.05$). Removing the seven

females who delivered an infant within two to three months of sample collection also did not alter the significance of the above results.

4. Discussion

The present results add further support to a growing literature regarding the utility of hair cortisol as a marker of underlying long term HPA activity (23, 26, 27, 32–40). In the present study, reduced Novelty Seeking behavior in female vervet monkeys (indicated by fewer approaches and less time spent in proximity to a novel object introduced near the home enclosures) was associated with higher hair cortisol levels. In vervet monkeys, we have also shown that hair cortisol increases following a chronic stressor, is trait-like, and is heritable (55). In other nonhuman primate studies, hair cortisol levels of adult rhesus monkeys increased in response to the challenge of housing relocation, which was modulated by a history of behavioral pathologies (26, 27). Hair cortisol level of young rhesus monkeys predicts performance on cognitive tasks. Elevated hair cortisol level was associated with delayed response acquisition on a cognitive task (32).

Higher levels of novelty seeking behavior are associated with less HPA activation. For example the cortisol response to the combined dexamethasone/CRH challenge is reduced among individuals scoring high on novelty seeking assessments (41). In response to an anxiety provoking public speaking challenge in humans, the Trier Social Stress Test (TSST), individuals with a high novelty seeking temperament have a lower overall cortisol response to the TSST challenge (42). Mobbing behavior in the common marmoset (e.g., vocalization response in the presence of a predator) has been associated with lower salivary and hair cortisol levels (43,44). These results suggest that reduced HPA activity promotes a bold and fearless response to challenging circumstances.

In the present study, vervet females were sampled during the mating season and only a few were detectably pregnant. When hair was collected during the birth season in another environment, vervet females sampled within one month of delivery had significantly higher hair cortisol levels compared to females sampled earlier in pregnancy (55). The duration of pregnancy for vervet females is approximately 5.5 months, and the period of cortisol accumulation in hair for the females who were sampled within one month before or after delivery probably included most or all of the final third of pregnancy. The results of that study (55) supported the sensitivity of hair cortisol to increases in circulating cortisol in late pregnancy also shown in human studies (23,56).

Results from the present study, as well as another from our group (55), suggest that hair cortisol reflects a trait-like dimension of individual differences in HPA activity. Hair cortisol levels are responsive to environmental change, but individual differences within environments are heritable and trait-like. Here we demonstrate that hair cortisol is associated with a relatively stable behavioral measure of novelty seeking temperament. We do not have a precise estimate of hair growth rates or the time course of cortisol accumulation in vervet hair, but this study demonstrates that under stable conditions hair cortisol can be used to measure individual differences in HPA activity that have relevance for behavioral health and welfare.

What are potential contributions to individual differences in response to novelty that may underlie activation of the HPA? Experiences with mildly challenging situations early in development inoculate squirrel monkeys against overwhelming stress responses to challenges at a later age (45). Early maternal experiences affect novelty seeking behaviors in adolescent rhesus and vervet monkeys (14,46, 47) and environmental challenges that impact the maternal relationship lead to offspring that become independent of their mothers at an

earlier age (48). Similar observations have been long known in rodents (49,50). Maternal patterns of interactions with offspring affect gene expression via epigenetic mechanisms (51). Finally there are a number of genetic contributions to variations in the responsivity of the HPA axis (52–54). Thus multiple pathways, including early stressor exposure, maternal environment, and genetic background, affect responsivity to the environment and contribute to individual differences in novelty seeking as well as HPA activation.

5. Conclusions

The present observations demonstrate that novelty seeking is a persistent dimension of temperament for female vervet monkeys. In this study, female vervet monkeys with high hair cortisol levels were consistently lower in novelty seeking compared to females with low hair cortisol levels. Our observations add further support for the use of cortisol measured in hair as a marker of integrated cortisol release over a retrospective period. These results suggest that persistently high levels of HPA activity may inhibit novelty seeking and increase risk for pathology.

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Figure 1. Novelty Seeking scores for the snake (2006) and tarantula (2007) home group novelty tests. Individual differences were consistent across tests ($ICC = 0.68$) suggesting a trait-like temperamental characteristic.

Figure 2.

Frequency distribution of hair cortisol observed in 230 vervet monkeys. The group was divided into three groups based on the upper and lower SD, lowest SD (n=28) ranges from 22.4 to 40 pg/mg, the midrange group (n=169) ranges 40.5 – 64 pg/mg, and the highest SD (n=33) ranges from 65–101.2 pg/mg.

Figure 3.

Mean (+SEM) Novelty Seeking Score for snake and tarantula home group novelty tests by hair cortisol level subgroups indicated in Figure 2. The low cortisol group differed significantly from the high cortisol group in both years ($p < 0.05$).