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

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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.

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

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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 sabaeus*) (3–18 years 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 the 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 to 117 m^2 of ground area (mean = 61 m^2), 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 and 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 1 m of the object, and the number of 1-minute intervals that each animal was observed within 1 m 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 m for each interval. The area within 1 m 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 8–10 mg/kg Ketamine hydrochloride. Using electric hair clippers, a 4×4 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 1 ml 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 m of the novel object were inversely correlated (snake, $r = -0.42$; tarantula, $r = -0.48$). A composite score combining these measures