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Adrenal activity and anxiety-like behavior in fur-chewing chinchillas (*Chinchilla lanigera*)

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ABSTRACT

Due to its complexity, in combination with a lack of scientific reports, fur-chewing became one of the most challenging behavioral problems common to captive chinchillas. In the last years, the hypothesis that fur-chewing is an abnormal repetitive behavior and that stress plays a role in its development and performance has arisen.

Here, we investigated whether a relationship existed between the expression and intensity of fur-chewing behavior, elevated urinary cortisol excretion and anxiety-related behaviors. Specifically, we evaluated the following parameters in behaviorally normal and fur-chewing animals of both sexes: 1) mean concentrations of urinary cortisol metabolites and 2) anxiety-like behavior in an elevated plus-maze test. Urinary cortisol metabolites were higher only in females that expressed the most severe form of the fur-chewing behavior ($P \leq 0.05$). Likewise, only fur-chewing females exhibited increased ($P \leq 0.05$) anxiety-like behaviors associated with the elevated plus-maze test. Overall, these data provided additional evidence to support the concept that fur-chewing is a manifestation of physiological stress in chinchilla, and that a female sex bias exists in the development of this abnormal behavior.

Introduction

Adaptive behavioral responses are essential for an animal to maintain homeostasis, especially by allowing the animal to control and modify its environment (Garner, 2005). Abnormal repetitive behaviors can arise when an animal is housed in an environment with lack of sensory or cognitive stimulation and/or where it is exposed to chronic aversive stimuli, and can be especially pronounced when the animal is unable to express behaviors that would normally be essential for survival in the wild, or when it cannot perform behaviors that would correct the homeostatic imbalance it is experiencing. Indeed, abnormal behavior serves not only as an indicator of poor welfare, but often also as an indicator of stress (Garner, 2005; Mason, 1993).

The chinchilla, a South American hystricomorph rodent, possesses one of the most valuable pelts in the world and has been domesticated, bred and selected for fur quality, color and growth rate for more than 80 years (Grau, 1986). However, the establishment and maintenance of intensive captive breeding programs led to the description of an abnormal repetitive behavior known as "fur-chewing", an exaggerated form of autogrooming in which the fur is chewed rather than simply groomed (Ponzio et al., 2007). Some anecdotal observations have suggested that fur-chewers will chew their own fur either constantly, or in some cases at intervals, usually at their hips and sides about half-way down the length of the hair (Rancher's Handbook, 1987). However, to our experience, the behavior tends to be stable within individual chinchillas (Ponzio MF, personal observation). A recent survey study revealed that the mean percentage of animals that perform the behavior in commercial farms was $4.32 \pm 0.37\%$ (range 0–16.7%) (Ponzio et al., 2007).

Although little scientific work has been published on this condition, a few studies have supported the hypothesis that fur-chewing is a stress-related behavior. For example, the behavior has been associated with increased adrenocortical activity (increased plasma corticosterone and adrenocortical hyperplasia) (Tisljar et al., 2002; Vanjonack and Johnson, 1973). Recently, we described a number of environmental/management factors that may be contributing to the development of the abnormal behavior in domestic chinchillas (i.e. crowding, number of wood shaving changes per week, dustbathing, etc.) and hypothesized that fur-chewing behavior in the chinchilla is caused by management/environmental stressful factors and/or lack of naturalistic stimuli in the caging conditions (Ponzio et al., 2007). Extrapolating from this hypothesis, we can make two predictions. First, there may be a positive relationship between the occurrence of fur-chewing behavior, elevated glucocorticoid production and anxiety-related behaviors. Second, animals that

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display an increased severity of the abnormal behavior may exhibit relatively higher levels of adrenal activation.

In this study we tested these predictions in normal chinchillas and in those displaying different severities of fur-chewing behavior by evaluating: 1) mean concentrations of urinary cortisol metabolites and 2) anxiety-like behavior using an elevated plus-maze test.

Materials and methods

Animals, housing and management

Sexually mature domestic chinchillas (*Chinchilla lanigera*) used in this study exhibited either normal behavior or different categories of fur-chewing behavior as follows: (1) slight (only a few tufts of hair are chewed); (2) moderate (one of the sides or hips is extensively chewed); (3) severe (both sides of the body or hips are chewed); and (4) very severe (all the fur in regions of the body the animal can reach are chewed) (Ponzio et al., 2007).

The animals were obtained at least 1 month before the study onset from different local commercial breeding farms where the fur-chewing and the behaviorally normal animals were part of a larger population and taken to our chinchilla breeding facility. During that time, the animals were observed and assessed by an experienced researcher to determine the fur-chewing severity scoring. Animals were housed in individual stainless steel cages (0.32 m wide × 0.30 m height × 0.50 m length) with wood shavings as substrate, exposed to naturalistic fluctuations in photoperiod (spring and summer: 14.2 ± 0.0 and 14.0 ± 0.0 h respectively) and controlled temperature (20–25 °C). Pelleted chinchilla food (GEPSA Feeds, Córdoba, Argentina) and water were provided ad libitum, and a cube of compressed alfalfa was fed once weekly. A tablespoon of marble powder was added to the substrate of each cage on a regular basis so that animals could perform a "dust bath" to keep the fur dry and uncompressed. These housing, environmental and management conditions are the same as those used in any commercial breeding farm in the world.

All experiments were conducted in accordance with the National Institutes of Health, Guide for the Care and Use of Laboratory Animals.

Urine collection

To separate urine and feces at the time of excretion, metabolic cages were constructed by making slight modifications to a regular housing cage. Cage bottoms were triple-layered steel litter pans. The top pan had transversal steel rods that provided a supportive surface for the animal. The middle pan consisted of a steel mesh (1.0 mm diameter openings) that permitted urine, but not feces, to pass through to the lower pan, which forms a steel funnel that directed urine into a collection tube containing 500 μL of ethanol 70% as preservative. To avoid discomfort of the animals because of the grid floor in the metabolic cages, a platform was added in the upper back of the cage to provide a solid resting area (32 × 14 cm). This element is provided to the animals in the regular housing cages also, both in the laboratory and in commercial breeding conditions.

Animals were placed in the metabolic cages 2 days before the onset of sample collection, to allow habituation. Samples were then collected for 10–12 consecutive days to establish a baseline of endocrine activity. Each urine sample were collected overnight (chinchillas usually urinate only during nighttime hours; 18:00–09:00 h), centrifuged to separate detritus and stored at −20 °C until processing. The animals were then placed back into their regular housing cages.

Cortisol metabolite assay

Immunoreactive forms of conjugated cortisol metabolites were assessed in unprocessed urine samples by enzyme immunoassay (EIA) as previously validated and described (Ponzio et al., 2004). Briefly, horseradish peroxidase ligands and polyclonal antisera (cortisol-R4866) were provided by C. Munro (University of California, Davis, CA). The antiserum crossreacts with prednisolone (9.9%), prednisone (6.3%), cortisone (5.0%), corticosterone (0.7%), 21-deoxycortisone (0.5%), deoxycorticosterone (0.3%), progesterone (0.2%), 11–desoxycortisol (0.2%), 17a-hydroxyprogesterone (0.2%), and (0.1%) with all other steroids tested. Parallel displacement curves were obtained by comparing serial dilutions of pooled chinchilla urine with standard hormone preparations ($r^2 = 0.97$ and 0.96 for the standard and diluted pool, respectively). Recovery of known amounts of cortisol added to a pool of diluted urine was 86.3 ± 3.7% ($y = 2.12 - 0.84x$, $r^2 = 0.99$).

Before assay, unprocessed urine samples were diluted 1:500 with PBS. All samples were assayed in duplicate, and to account for day-to-day fluctuations in fluid balance, hormone concentrations were expressed as hormone mass per mg of creatinine (Monfort et al., 1990; Taussky, 1954) (creatinine standards 925–11, Sigma Diagnostics Inc, St. Louis, USA). Inter-assay coefficient of variation (CV) for two separate internal controls were 16.5 and 19.3% (n = 15). Intra-assay CV was <10% and assay sensitivity was 0.078 ng/mL.

Experiment 1: baseline cortisol metabolites excretion

For Experiment 1, chinchillas with (n = 45; 25 males and 20 females) and without (n = 14; six males and eight females) fur-chewing behavior were used. After 2 days of habituation to the metabolic cage, baseline adrenal activity for each undisturbed individual was evaluated by assessing urinary cortisol metabolites excretion for 10–12 consecutive days.

Experiment 2: anxiety-like behavior assessment (elevated plus maze test (EPM))

For this experiment a different group of animals were used; based on the results obtained in Experiment 1, only fur-chewers categorized as #3 and #4 where evaluated. The experimental group was composed of 18 animals with fur-chewing behavior (nine males and nine females) and 15 behaviorally normal ones (six males and nine females).

The elevated plus maze (EPM) test was a modification of the apparatus described by Pellow et al. (1985) for rats and by Lister (1987) for mice, adjusted by proportion to accommodate adult chinchillas (500–700 g body weight). The apparatus was constructed from medium density, black painted fibreboard that consisted of two open arms (80 × 23 cm), and two closed arms of the same dimensions (50 cm height) that extended from a central platform (23 × 23 cm), arranged so that both open and closed arms opposed one another. The maze was elevated 60 cm above the floor, placed in a testing room without windows but illuminated with a red light mounted vertically above the center of the maze.

Chinchillas were acclimatized to the testing room for at least 1 h before test onset. As in other rodents, animals were tested between 10:00–12:00 a.m. For the testing procedure (a single trial per animal), animals were placed into the center area of the maze facing an open arm. An observer, situated at approximately 1 m from the maze (in the back of the left closed arm), recorded the following behavioral occurrences observed during a 5 min test session: the number of entries through the open arms; the number of entries into closed arms, and time spent in closed arms; the number of rearing, grooming and risk assessment behaviors (head dipping); and the number of fecal boli shed. Chinchillas that jumped off the EPM were excluded from the study (n = 2). The maze was thoroughly cleaned with dilute alcohol solution between each test occasion.

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Statistical analysis

All values are expressed as mean ± standard error of mean (SEM). Data analysis was performed using the Infostat statistical software package (Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. InfoStat version 2010. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar). The normality test procedure included in the software was applied to check for normal distribution (modified Shapiro–Wilks test). Homogeneity of variances was evaluated with an ANOVA of the absolute residuals of each group. Nonconforming data were Ln transformed before further analysis.

Because within-animal variation in daily urinary cortisol excretion was significant ($P<0.05$), median hormone concentrations were used to dampen the impact of extremely high or low values (Dukes and Sullivan, 2007). The median of 10–12 urinary hormone metabolite values was used to establish baseline hormone excretion for each individual animal. Results from Experiment 1 (cortisol metabolites) were analyzed using a two way ANOVA, and where appropriate, post-hoc analyses were carried out using the DGC test (Di Rienzo et al., 2002). Results from Experiment 2 were subjected to multiple variance analysis (MANOVA) with two factors: normal–fur-chewing and female–male, followed by Hotelling's T^2 test as post-hoc test. The accepted level of significance for all statistical tests was $P\leq 0.05$.

Results

Experiment 1: baseline cortisol metabolites excretion

A total of 604 urine samples from 31 males and 28 females were collected. Mean concentration of creatinine in the samples was 1.14 ± 0.02 mg/mL. The group averages of median individual animal cortisol metabolite excretion for each category of fur-chewing behavior are depicted in Table 1. The analysis of variance indicated a significant interaction between sex of the individual and severity of the fur-chewing behavior. The DGC post-hoc test indicated that urinary cortisol metabolite concentrations were significantly higher only in females showing a very severe (Category 4) level of the fur-chewing behavior ($F=2.60$; $df=4$; $P=0.04$); no significant sex-based differences in urinary cortisol excretion were detected among all other categories.

Experiment 2: anxiety-like behavior assessments

Fig. 1 shows the behavioral profile (relative indexes of anxiety and locomotor activity) performed by fur-chewing and normal chinchillas of both sexes during the 5 min observation periods in the EPM. The MANOVA test revealed a significant effect of the more severe fur-chewing condition relative to normal animals (*Wilks* = 0.37; $df=12$; $P=0.03$). Only the fur-chewing females group showed a significant decrease in the parameters inversely related to anxiety (percent open arms entries, and percent time spent in open arms; $df=29$, $P=0.005$ and $P=0.04$ respectively; Fig. 1, panels A and B) indicating an increase in anxiety-like behavior. Freezing also increased in the fur-chewing females group (40 ± 19.7 vs. 0 ± 0 s; $df=29$, $P=0.01$).

No significant differences were observed in the total number of entries in the EPM (a measure of overall locomotors activity) (Fig. 1, panel C) and other parameters measured (i.e., grooming, rearing and risk assessment behaviors, number of fecal boli).

Discussion

Excessive grooming is one of the most common displacement activities exhibited by animals in response to conflict (Eibl-Eibelsfeldt, 1970). Captivity may promote chronic conflict if the threshold for performance of certain species-specific behaviors is altered, and what may begin as a displacement activity can develop into a grooming abnormality with serious physical/physiological consequences (Mon-Fanelli et al., 1999). Fur-chewing is one of the most challenging behavioral problems common to captive chinchillas. Although it has been repeatedly suggested that stress plays a role in the development and performance of this behavior (Ponzio et al., 2007; Tisljar et al., 2002; Vanjonack and Johnson, 1973), our work represents the first study designed to systematically explore this relationship, using objective measures of the physiological stress response in affected animals. Females that exhibited the most severe form of fur-chewing excreted significantly elevated concentrations of urinary cortisol, which suggested that the expression of this behavior was mediated, at least in part, by physiological stress. However, our prediction that we would find a relationship between increasing severity of the fur-chewing behavior and cortisol metabolites excretion was not supported by our data.

The relationship between plasma cortisol and the expression of abnormal repetitive behaviors (ARB's) has been investigated in several species, yet conflicting findings are described in the published literature (for a review see Mason and Latham, 2004). This should not be surprising given the complexity of factors (i.e., seasonality, reproductive status, social and metabolic variables) that can confound the interpretation of variability in cortisol excretion, including the inter-individual differences in the ability to utilize behavioral strategies to cope with stressors. Indeed, in our study we observed a high degree of inter-individual variation in mean urinary cortisol excretion.

At this point, one question that remains unanswered about our results is: why was cortisol excretion elevated only in females with the most severe forms of fur-chewing behavior? Just as in any population of animals only some proportion of individuals might be predisposed to the stress of captivity (and subsequently exhibit fur-chewing behavior) it is possible that coping mechanisms in some of those animals may be insufficient to fully ameliorate an aversive situation. Female fur-chewing chinchillas seem to represent this most susceptible group. Moreover, we previously demonstrated a female sex bias whereby 58.7% of fur-chewers were females (Ponzio et al., 2007). A similar sex-biased expression of other forms of ARBs has been reported in several species, including trichotillomania in humans (a compulsive urge to pull out the hair) (Christenson, 1995), barbering in mice (Garner et al., 2004), self-injuring in clouded leopards (Wielebnowski et al., 2002), and psychogenic alopecia in cats (Mon-Fanelli et al., 1999) and rhesus macaques (Steinmetz et al., 2006).

Several reports that studied similar forms of ARB's pointed out that life events related to the female hormonal cycle may trigger or exacerbate the obsessive compulsive disorder (OCD) and trichotillomania in women

Table 1
Urinary cortisol metabolites concentration in domestic *Chinchilla lanigera* showing different levels of fur-chewing behavior.

	Cortisol metabolites (μg/mg creatinine)				
	Normal	Slight	Moderate	Severe	Very severe
Females	635.6 ± 66.9 (8) a	493.5 ± 114.9 (4) a	682.4 ± 195.6 (5) a	969.2 ± 188.7 (6) a	1652.6 ± 623.8 (5) b
Males	981.8 ± 172.2 (6) a	594.2 ± 83.9 (5) a	1156.0 ± 155.4 (6) a	619.4 ± 80.4 (5) a	904.3 ± 200.6 (9) a

Data are expressed as the mean of the medians obtained for each group ± SEM. In parenthesis, number of animals in each group. a vs b (in rows): $P=0.04$.

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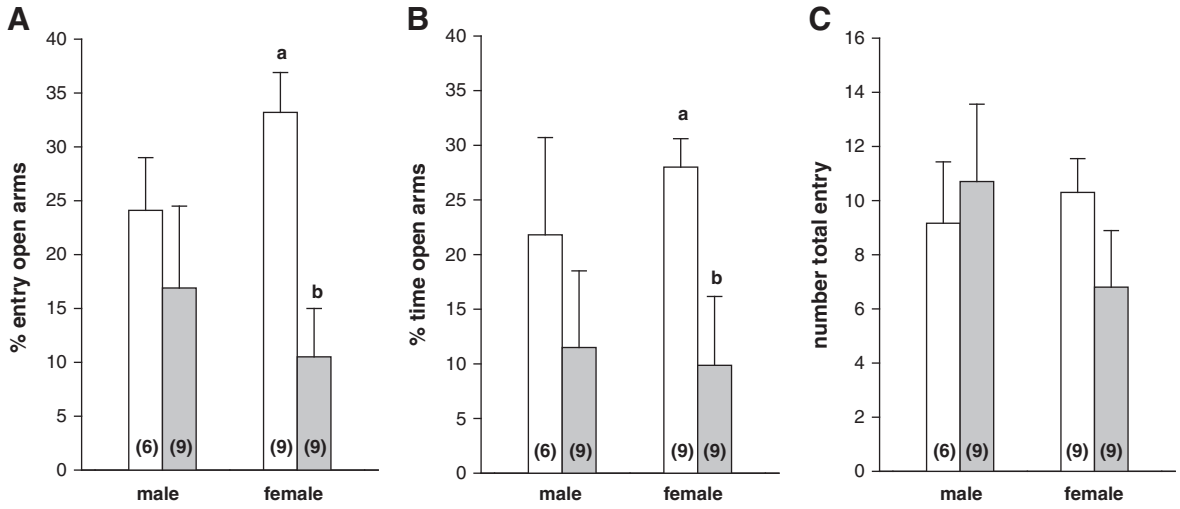

Fig. 1. Behavior of normal or fur-chewing *Chinchilla lanigera* males and females exposed to the elevated plus maze test. Values are expressed as mean ± S.E.M. The number of animals is indicated at the bottom of each column. a vs. b: panel A: $P = 0.005$; panel B: $P = 0.04$.

patients, suggesting that ovarian hormones play a modulatory role in the course of those disorders (see reviews Albelda and Joel, 2012, in press). Indeed premenstrual, pregnancy and post-partum periods were associated with an increased risk of onset and exacerbation of OCD (Abramowitz et al., 2003; Labad et al., 2005; Vulink et al., 2006) and trichotillomania (Chamberlain et al., 2009; Duke et al., 2010). This is in line with evidence implicating the modulation of compulsive behavior by ovarian hormones in three animal models of OCD: 8-OHDPAT-induced decreased alternation in rats, marble burying in mice and rats and signal attenuation in rats. Those studies revealed that an estradiol increase exerts an anti-compulsive effect and that the withdrawal from repeated estradiol administration has an opposite effect, namely to increase compulsive responding (Albelda and Joel, 2012, in press; Flaisher-Grinberg et al., 2009; Hill et al., 2007). In mice, females were one and a half times more likely to barber than males and breeding mice were approximately five and a half times more likely to barber than nonbreeders (Garner et al., 2004).

With respect to the results of our second experiment (EPM test), even thought the use of the test was not pharmacologically validated for chinchillas, normal chinchillas appear to respond in a manner that is similar to that reported in the rat, with comparable amounts of open arm time, open arm entries and number of total entries (e.g. Pellow et al., 1985). In addition, no changes in locomotor activity were observed (see number of total entries). More importantly, our results provide clear evidence that females that exhibited severe fur-chewing also demonstrated increased anxiety-like behaviors associated with the elevated plus-maze test (e.g., decrease in the percentage of entries and time spent in open arms, increase in freezing behavior).

Conclusion

In summary, we provided additional evidence to support the concept that fur-chewing is a stress related behavior in the chinchilla, and that a female sex bias exists in the expression of this behavior. Which behaviors and/or housing conditions are restricted to captive chinchillas is a key issue that should be fully addressed in future studies, as this may have a direct impact in breeding management, housing and animal welfare of captive chinchillas.

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