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Are cats (*Felis catus*) from multi-cat households more stressed? Evidence from assessment of fecal glucocorticoid metabolite analysis

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HIGHLIGHTS

- Feline stress was investigated in terms of fecal glucocorticoid metabolites.
- Cats were selected from multi-cat and single-cat households.
- Fecal glucocorticoids did not vary as a function of feline housing condition.
- Fecal glucocorticoids did not vary as a function of feline personality.
- Young cats in multi-cat households had lower glucocorticoid metabolites.

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ABSTRACT

Given the social and territorial features described in feral cats, it is commonly assumed that life in multi-cat households is stressful for domestic cats and suggested that cats kept as single pets are likely to have better welfare. On the other hand, it has been hypothesized that under high densities cats can organize themselves socially thus preventing stress when spatial dispersion is unavailable. This study was aimed at comparing the general arousal underpinning emotional distress in single housed cats and in cats from multi-cat households (2 and 3–4 cats) on the basis of fecal glucocorticoid metabolites (GCM) measured via enzyme immunoassay (EIA). GCM did not significantly vary as a function of living style (single, double or group-housing); highly stressed individuals were equally likely in the three groups. Young cats in multi-cat households had lower GCM, and overall cats that tolerate (as opposed to dislike) petting by the owners tended to have higher GCM levels. Other environmental aspects within cat houses (e.g. relationship with humans, resource availability) may play a more important role in day to day feline arousal levels than the number of cats per se.

1. Introduction

The number of cats in ownership has increased in many countries over recent years, but this growth has seen a rise in multicat households [1]. So the density of cats in private homes is increasing and in some instances can be very high [2]. Ownership may inhibit a natural tendency to socially disperse as density increases, but this may also be partially dependent upon the availability of resources. Owners are responsible for composing and enforcing the domestic groupings which may involve cats of different backgrounds and character. There is evidence to suggest that individuals who are poorly socialized to other cats when young, will be more prone to stress when living in groups in comparison to well socialized cats [3,4]; once housed singly these cats seem to cope much better. Furthermore, without appropriate socialization to humans, cats will show stress signs in the domestic setting whether housed singly or in groups [3].

Some authors propose that domestic cats living in groups form dominance hierarchies [5,6] as a social compensatory mechanism that helps them to cope with potential competition over resources when it is not possible to organize their spatial distribution as different potential resource holders. According to Leyhausen [7], the more limiting the space for a cat group, the stricter the social hierarchy, and several studies have failed to show an increase in aggression among cats living in groups under decreased available space [8,9].

Evidence in favor of the use of a social compensatory mechanism (i.e. organizing themselves socially might compensate for the stress caused by spatial restriction) has been presented recently by Lichtsteiner & Turner [10] who did not find differences in "stress" levels (inferred from urinary cortisol) when comparing single and group (3–4) housed cats. Ramos et al. [11] found similar results in a

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comparable study in which fecal glucocorticoid metabolites were assessed [12]. However in this latter study the multi-cat groups were relatively large, consisting of 7–48 cats, and so the conclusion cannot be generalized to more typical households which are likely to consist of 2–4 cats. Indeed neither of the two aforementioned studies considered 2-cat households. It may well be that in such situations hierarchies are not established because social compensatory mechanisms are unnecessary in a less dense population [10], or the relationship remains unstable in the absence of alliances that create a clear difference in resource holding potential.

Temperament, personality or behavioral style may also play an important role in the way a domestic cat perceives its environment, as has already been demonstrated with wild species. For example, in cheetahs and clouded leopards higher levels of physiological parameters of stress were found in "nervous" types of individual compared to "calm" ones [13,14]. In the home, it may be stressful for a timid cat to attempt to adapt to a group (where it is hard to keep itself distant from others) but not to live in isolation. By contrast, living in a group may not be as challenging for a "bossy" or "easy going" cat. Thus, it seems to be of great interest to evaluate arousal levels also as a function of individual traits in the cat reflecting behavioral style, with both singly and group housed cats.

Therefore the primary aim of this study was to compare general arousal levels in cats housed singly and in multi-cat households (i.e. 2 cats per house or 3–4 cats per house) as a function of owner defined personality and a range of behavioral tendencies, by assessing fecal glucocorticoid metabolites (GCM) using an enzyme immunoassay (EIA) [12,15] in relation to these factors.

2. Material and methods

2.1. Selection of cat households

The study was advertised in several veterinary clinics within São Paulo City, including the Veterinary Teaching Hospital of the University of São Paulo. Owners of 1–4 cats contacted the main researcher either by telephone or email. If subject met the inclusion criteria (Table 1), the household was enrolled. Detailed information regarding sample collection and storage was then provided to the owners and demographic information about the cat (or cats) as well as the household was gathered.

2.2. Study groups

The sixty selected households were divided into the following groups:

- GROUP I — 23 single-cat households (n = 23 cats in total)
- GROUP II — 20 multi-cat households of 2 cats (n = 40 cats in total)
- GROUP III — 17 multi-cat households of 3–4 cats (n = 57 cats in total)

Table 1
Household selection — List of inclusion criteria.

Inclusion criteria	
- Cats at least 6 months old	- No concurrent use of medication
- Cats living in their current household for at least 4 months	- No dogs in the home
- Cats of any sex but no females in estrus during the period of sample collection	- No moving, traveling, work on the house and/or introduction of a new household member (human or animal) during the period of sample collection
- Indoor only cats or only access to enclosed yards	- No known concomitant organic disease
- No concomitant overt behavioral disorder	- No planned visit to the vet clinic during the period of sample collection (e.g. booster vaccination)

Table 2
Frequency distribution of animals according to the demographic features and group.

Variable	Category	Group						Total	p-Value
		I — 1 cat		II — 2 cats		III — 3–4 cats			
		N	%	N	%	N	%	N	
Sex	Female	12	52.2	22	55.0	34	59.7	68	0.802
	Male	11	47.8	18	45.0	23	40.3	52	
	Total	23	100.0	40	100.0	57	100.0	120	
Age	<2 years	8	34.8	11	27.5	15	26.3	34	0.741
	≥2 years	15	65.2	29	72.5	42	73.7	86	
	Total	23	100.0	40	100.0	57	100.0	120	
Breed	Pure breed	5	21.7	9	22.5	9	15.8	23	0.669
	Crossed breed	18	78.3	31	77.5	48	84.2	97	
	Total	23	100.0	40	100.0	57	100.0	120	
Neutering	No	1	4.3	5	12.5	7	12.3	13	0.538
	Yes	22	95.7	35	87.5	50	87.7	107	
	Total	23	100.0	40	100.0	57	100.0	120	

*The three groups are homogeneous regarding the frequency distribution of sex, age, breed and neutering (chi-square tests were used).

Detailed information regarding the demographic features of the groups is given in Table 2.

2.3. Collection of fecal samples

The aim was to collect four fecal samples from every cat in the household immediately after defecation. There was a total of 478 fecal samples collected since in the case of two cats, only 3 fecal samples were collected. Collections were ideally conducted on the same week day each week and immediately stored in the owner's freezer. The main researcher then went to the owner's house to pick up the samples which were brought to the university's laboratory in a thermally insulated box so that they did not defrost during transportation. In the university's laboratory, feces were placed in polypropylene tubes and stored at −20 °C until the start of the extraction.

2.4. Determination of glucocorticoid metabolites

Samples were dried and weighed, and GCM were extracted and dried for transportation. For these, 0.2 g aliquots of dry feces were placed in vials of glass (15 mL) to which 5 mL methanol (90% methanol:10% pure water) was added. A multi-vortex unit was used for shaking (15 min) followed by centrifugation (15 min; 3000 rpm). Extracts were then dried for transportation, all at once, in Eppendorf tubes. GCM measurement was then conducted at the Vienna University of Veterinary Medicine. For this, re-suspension and dilution procedures were undertaken according to the methods used at this institution [12,15]. Samples were then analyzed by an 11-oxoaetiocholanolone EIA, measuring 11,17-dioxoandrostanes, a group of cortisol metabolites (for details of the EIA see Palme and Möstl, 1997 — [16]). This method has proved to be both valid and reliable, being tested in domestic cats in two studies by the same research team that conducted the analysis [12,15].

2.5. Cat personality

Cat personality was assessed by the owners. They were asked to freely classify their cats as (a) *bossy*, (b) *timid* or (c) *easy going* — based on their observations and perceptions of these three behavioral styles (personalities). Reliability was tested by comparing the owners' answers to a second assessment, having the same three options to choose, one year after the initial classification. Owners also completed a behavior history form (especially concerning human–cat interaction — such as response to petting) about each of their cats.

2.6. Statistical analysis

Kappa coefficients were used for evaluating agreement between first and second classifications of cat personality by the owners.

Median concentrations of glucocorticoid metabolites were calculated from the respective values of the four fecal samples collected per participant cat. Then, they were used to compare general basal arousal levels as a function of the variables of interest; by using median values we mitigated against the possible interference from outliers in the four samples collected.

Given that the GCM variable presented an asymmetric distribution, a logarithm transformation was applied in order to compare groups and factors regarding the central tendency of GCM. A random effects model [17] was used as it takes into consideration the dependence of the variable for the group living cats (i.e. group living cats were from the same household). Inferences were based on the logarithm of GCM to meet the normality assumption of residuals. The analysis strategy was to consider all the variables as categorical (owner assessment of personality, sex, breed group, neuter status, age group) and evaluate possible interactions between them as well as a main effect considering all cats together. The F-approximation for the Wald test statistics was used for identifying the source of variation. When the p-value of the interaction was less than 0.15, a more detailed analysis within groups was done to explore possible differences.

All analyses were undertaken using R!® software.

3. Results

3.1. Reliability of assessment of cat personality

The reliability of owner assessment of personality types was moderate with 62.4% agreement (kappa coefficient = 0.4108; $p < 0.0001$). This value was felt adequate for inclusion of the first personality rating as a possible factor related to basal arousal levels.

3.2. GCM levels relative to variables of interest

There was no significant difference in GCM concentrations as a function of group size (GROUP 1: average = 337.71 ng/g, SE = 75.61; GROUP 2: average = 268.09 ng/g, SE = 37.14; GROUP 3: average = 314.87 ng/g, SE = 42.06; $F_{(2;60)} = 0.20$, $p = 0.816$). Groups also did not significantly differ as a function of variability of GCM measures in each cat (i.e. within subject variability) (Random Effects Model, $p = 0.771$).

There was also no significant difference in GCM between sex, neuter status, or breeds ($F_{(1;58)} = 0.66$, $p = 0.419$; $F_{(1;58)} = 0.04$, $p = 0.835$, $F_{(1;58)} = 1.38$, $p = 0.245$, respectively). Additionally, there was no interaction between sex, neuter status and breed of the cat and group size in relation to GCM (interaction — $F_{(2;58)} = 1.55$, $p = 0.220$; $F_{(2;58)} = 1.02$, $p = 0.367$; $F_{(2;58)} = 0.23$, $p = 0.794$, respectively). However there was an interaction between Age and Group ($F_{(2;58)} = 3.43$, $p = 0.039$), indicating that the difference between young (<2 years old) and adult cats (>= 2 years old) is not the same for all groups. There was no difference between young and adult cats in group I ($F_{(1;58)} = 0.35$, $p = 0.555$) and group II ($F_{(1;58)} = 0.02$, $p = 0.887$). However, in group III, adult cats had significantly higher GCM levels ($F_{(1;58)} = 8.86$, $p = 0.004$) (Fig. 1). A complementary statistical analysis also indicated that there is no difference among Single, Pair and 3–4 groups inside the category ≥2 years ($F_{(2;58)} = 0.27$, $p = 0.762$). However, there is a significant difference among groups inside the category <2 years ($F_{(2;58)} = 3.25$, $p = 0.046$): Single × Pair ($F_{(1;58)} = 0.22$, $p = 0.643$), Single × 3 or 4 ($F_{(1;58)} = 5.40$, $p = 0.024$) and Pair × 3 or 4 ($F_{(1;58)} = 3.86$, $p = 0.054$) (Fig. 1).

GCM did not show significant differences as a function of the cat being considered *bossy*, *timid* or *easy-going* ($F_{(2;54)} = 0.08$, $p = 0.922$). Furthermore, there was no evidence of an interaction between personality and group size ($F_{(4;54)} = 0.66$, $p = 0.620$).

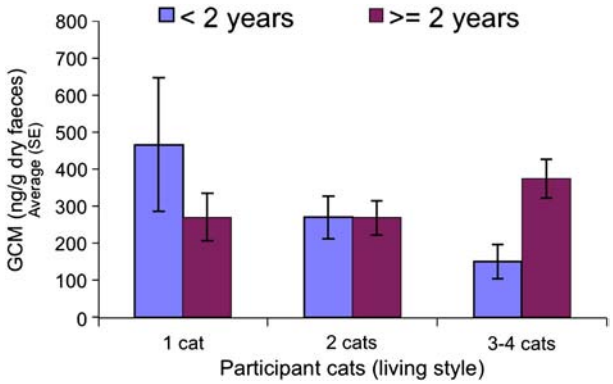

Fig. 1. GCM as a function of age and group.

The variables regarding cat–human relationship relating to "appreciation of being handled" and "appreciation of being brushed" showed no evidence of an interaction with Group size on GCM ($F_{(4;47)} = 1.19$, $p = 0.327$, $F_{(4;44)} = 1.03$, $p = 0.401$, respectively). In addition, there was no evidence of the cat's response ("enjoy", "tolerate" or "dislike") to these two activities affecting GCM levels ($F_{(2;47)} = 0.89$, $p = 0.417$, $F_{(2;44)} = 0.04$, $p = 0.965$, respectively).

For the variable "appreciation of being petted" there was no interaction with Group, but, there was some evidence of a potential main effect ($F_{(2;46)} = 2.67$, $p = 0.080$) revealing a significant difference between the categories "disliking" and "tolerating" petting ($F_{(1;46)} = 5.26$, $p = 0.027$), with subjects believed to tolerate this activity having higher GCM concentrations.

4. Discussion

In this study, levels of glucocorticoid metabolites showed great inter-individual variability to that reported in previous studies [12,18], and even within households levels may be very variable suggesting similar environmental challenges are probably perceived and dealt with in different ways depending on the individual cat. A normal reference range (i.e. expected values for non-stressed cats) has not yet been established for the species. A direct comparison between the results obtained here with those found by Schatz & Palme [12] who applied a very similar methodology is possible. Their median level of 195.8 ng/g feces representing baseline values from 10 cage adapted laboratory cats before ACTH injections was generally a bit lower than the average levels for our three groups, with some individual cats having much higher levels than this. In this regard, it is worth noting that there were some cats in each of the three groups whose baseline levels were similar to those found after ACTH injection in the cats studied by Schatz & Palme [12], i.e. a median level of 591.9 ng/g feces. This probably indicates extreme stress levels, which did not appear to be reliably related to housing conditions since significant differences in GCM levels as a function of cat housing condition (up to 3–4 cats in the house) were not found. It is worth noting that none of the cat households included in the study had any evidence of serious cat conflicts or any other overt behavioral problem among the cats. Thus, it should not be assumed that because there is no overt behavioral problem, individual cats are necessarily free of significant physiological stress. However, the occurrence of affiliative interactions between cats from the multi-cat houses (2 and 3–4 cats) reported by the owners during interviews supports the interpretation of a generally adjusted social cohabitation in most of the participant multi-cat households.

A closer look at both the behaviors presented by group versus single housed cats and the level of predictability and control afforded to individuals in their environment would add valuable information to a better understanding of their welfare state. Furthermore, it would be useful to

explore fecal levels of glucocorticoid metabolites after adding an acute stressor (e.g. a visit to the vet) in order to assess reactivity of the HPA system.

GCM did not vary significantly as a function of the cat being perceived as *bossy*, *timid* or *easy going*. Although owners' agreement in the two interviews was only moderate (62% and slightly below the preferred value of 70% — [19]) a similar result has been found recently by Iki et al. [20] when comparing adrenocortical activity (blood cortisol) with a feline temperament profile (FTP). Feline personality determines the style with which a cat behaves across situations. For instance, a *timid* cat (as opposed to an *easy-going* or even a *bossy* cat) may choose to hide every time a stranger enters the household. Such a response may help the cat to cope with the situation and so decrease arousal levels.

Young cats, when living in multi-cat houses (i.e. 3–4 cats), had significantly lower glucocorticoid levels compared to adult cats. Young cats ranged in age from 6 months to 2 years old and thus it seems surprising that they did not appear to be more aroused than the adult ones, since they might be expected to be more playful and active in other ways. As it stands, it could be argued that young cats may have their arousal and behavior inhibited when living in groups in comparison to life as a single pet. This hypothesis deserves further investigation.

Regarding sex and breed, associations with GCM were not detected which is in line with other studies [10,11]. As to neutering, a recent study demonstrated lower levels of hair cortisol in female feral cats after neutering [21]. Besides recently raised doubts about the validity of this parameter [22], a decrease in cortisol levels due to neutering might be linked to reduced social and reproductive pressures in human households where the most important sources (e.g. food, shelter) are frequently quite abundant and reproduction often strictly controlled through neutering, thus supporting our lack of differences in GCM as a function of the cat's neuter status (a similar result was found by Ramos et al. [11]).

Single-housed cats, as opposed to group living cats, may be more susceptible to some of the negative effects of human activity in the home environment [11]. Indeed, interaction with owners in the form of petting was linked to arousal levels in the studied cats. Those considered by the owners to “tolerate” petting (as opposed to “enjoying” or “disliking” it) had higher GCM concentrations. It may be that those that overtly dislike the activity are avoided or manage to avoid it, unlike those who tolerate it. Caution is warranted though with this hypothesis since there were only 4 cats in the category “disliking” while 13 in the category “tolerating” and 85 “enjoying”.

In conclusion, it seems reasonable to suggest that factors other than group size such as the degree of environmental control or the environmental composition (e.g. provision of useable 3-dimensional space, number of people, and relationship with people) may be more important in controlling stress in cats in captivity and further investigation of these individual factors is warranted. Although it seems intuitive to hypothesize that multi-cat houses are more stressful environments for cats on the basis of spatial and social restrictions, robust support for this generalization so far has not been found.

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