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Animal Welfare 2012, 21: 285-291
doi: 10.7120/09627286.21.2.285
ISSN 0962-7286

Factors affecting faecal glucocorticoid levels in domestic cats (Felis catus): a pilot study with single and large multi-cat households

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Abstract

*Domestic cats (*Felis catus*) are widely believed to be highly sensitive to the effects of social stress, especially when living in high density populations. Cats are capable of adapting to living in a group, but this will often require opportunities for escaping and hiding. In this pilot study, adrenocortical activity, as a valuable physiological indicator of arousal underpinning potential emotional stress, was evaluated through the measurement of mean faecal glucocorticoid metabolites (mGCM) in fourteen singly and sixteen group-housed cats. Living conditions and ratings of the owners' quality of life (evaluated from self-reported questionnaires) were used as factors associated with faecal glucocorticoid levels of the cats. A direct association between the scores of owners'* social *dimension of quality of life and the cats' mGCM was found for single cats only, with higher owner social scores associated with higher cat mGCM. No significant differences in mGCM were found between singly versus group-living cats. This suggests that the under-explored factor of owner lifestyle could play an important role in domestic cats' day-to-day levels of arousal, especially when kept as single pets.*

Keywords: *animal welfare, cat, faecal glucocorticoids, feline, life quality, stress*

Introduction

Domestic cats (*Felis catus*) are considered a social species (Crowell-Davis 2004). However, despite several examples of affiliative behaviour among conspecifics (McDonald *et al* 1987; Crowell-Davis 2004), as well as bonding with heterospecifics (Edwards *et al* 2007), they still retain independency, spending a great proportion of their time in isolation (Barry & Crowell-Davis 1999). Indeed, functional space occupation, together with distance communication, using both visual and olfactory signals (Beaver 2005), reflect a tendency to organise themselves spatially, controlling when and where contact with others occurs.

Depending on several conditions imposed by the environment in which cats are maintained, support for their social and spatial needs may not be possible, leading to increased arousal and distress. Unfortunately, most domestic cats are not chosen with consideration of their adaptability to live in confinement and near people (Jongman 2007). Densities of cats in private houses are commonly very high (Bernstein & Strack 1996), which does not permit dispersion. In addition, owners are responsible for composing the groups, which generally involves bringing together cats of different backgrounds, character traits and social status, which may be problematic. Some authors suggest that once living in groups, domestic cats form dominance hierarchies (van den Bos & de Cock Buning 1994; van den Bos 1998), a social mechanism that helps them cope with the impossibility of organising themselves spatially when confined. According to Leyhausen (1953), the more limiting the space for a cat group, the stricter the social hierarchy. The argument in favour of a social compensatory mechanism (ie organising themselves socially to compensate for the stress caused by spatial restriction) has been proposed recently by Lichtsteiner and Turner (2008) to explain the lack of increased stress levels (measured by urinary cortisol) among group-living cats compared with singly housed cats.

Domestic cats have great behavioural plasticity, adapting to a variety of environmental conditions either in complete isolation or as part of large groups (Kerby & Macdonald 1988). However, although there is a range of individual living styles, clearly not all cats are able to adapt to a given environment equally well. Evidence of this is the high incidence of behavioural problems in multi-cat households — eg urine-marking behaviour (Pryor *et al* 2001). Thus, despite the possibility of social stratification to prevent stress, higher arousal levels (measured by faecal glucocorticoids) may be seen in multi-cat households.

In terms of people's influence on cat behaviour and global levels of arousal and distress, little has been investigated scientifically. However, there is some evidence that cat behaviour is more dependent upon some owner/house-related factors (eg owner age, presence of children) than to some cat ones (eg cat's sociability with people; Adamelli *et al* 2005). Frenetic environments (as opposed to calm ones) were identified as a risk factor for human-directed feline aggression (Ramos & Mills 2009).

Analysis of faecal glucocorticoid metabolites (Schatz & Palme 2001; Young *et al* 2004; Brown 2006) have proven to be both practical and appropriate for use with felines, as cats excrete glucocorticoids mostly via faeces (Schatz & Palme 2001).

This study aimed at investigating faecal glucocorticoid metabolites in cats from single- and multi-cat households in order to compare their arousal levels. In addition, a human factor (owner's subjective life quality) was used to be contrasted with cats' glucocorticoid metabolites to explore a possible influence of owners on the cats' arousal states. We therefore tested the following hypotheses: (i) owners' perceived quality of life is negatively correlated to cats' mean glucocorticoid metabolites concentrations (mGCM); (ii) social group size (single versus large-group housing) is positively correlated to mGCM in cats.

Materials and methods

Study animals and data collection

The subjects of this study were 30 domestic cats (14 housed singly; 16 housed in groups — the latter coming from six different households); some owned by staff members of the School of Veterinary Medicine and Animal Science (University of São Paulo, Brazil) and others owned by clients drawn from a veterinary clinic specialising in feline medicine, whose owner was a member of the research team. A total of 120 faecal samples (four weekly samples per cat, in order to calculate individual mGCM concentrations; Palme 2005) were collected by the owners, who were advised to choose the same weekday to perform collections. In the case of group-living cats, owners were instructed to select the cat (or, in the case of five households, cats) whose defaecation act was easily witnessed, thus making it possible to identify its faeces during the whole sampling period, ensuring weekly sampling from the same selected cats. The samples were stored at –20°C until radioimmunoassay analysis.

Among the 14 cats housed singly, there were six males and eight females; nine mixed-breeds, two Ragdolls, one Siamese and one Mainecoon. Ages ranged from 8 months to 13 years (mean: 55.5 [± 53] months). All were neutered. Among the 16 group-housed cats, there were five males and eleven females; eight mixed-breed, four Persians, two Mainecoons, one Bengal and one Norwegian Forest. Their age ranged from 2 to 14 years (75.37 [± 37. 31] months). Nine of them were neutered whilst seven were entire cats. The total number of cats in each of the six households was as follows: household one = 9 cats; household two = 14 cats; household three = 9 cats; household four = 24 cats; household five = 48 cats; and household six = 7 cats.

A self-reported questionnaire regarding the owner's perception of their own life quality — the Portuguese-translated version of the WHOQOL-bref (THE WHOQOL GROUP 1998, which is a revised version of the original WHOQOL-100, 1994) — was used. The questionnaire has been developed by the WHO (World Health Organisation) and allows the assessment of both global and specific dimensions (ie 'physical', 'psychological', 'social' and 'environmental') of subjective human well-being. This Portuguese version of the WHOQOL-bref has been validated with the Brazilian populations (Fleck *et al* 1999, 2000) — under coordination of the 'WHOQOL for Brazil' group.

Sample processing

All laboratory procedures were performed at the Laboratory of Hormone Dosages, Department of Animal Reproduction, School of Veterinary Medicine and Animal Science, University of São Paulo, Brazil.

The samples were dried in a speed vac rotator device (SC110 Speed Vac, SC 110 Savant, Thermo Scientific, USA).

For the glucocorticoid metabolites extraction stage, 0.2 g aliquots of dry faeces were placed in vials of glass (15 ml) to which 5 ml ethanol (80% ethanol: 20% water type 1; Ethanol, PA, Merck, USA) were added. A multi-vortex unit (VWR Scientific Products, VX, 2500, VWR Scientific Products, USA) was used for a 15-min homogenisation of the sample followed by 15 min 3,000 rpm centrifugation (Universal Hittich Zentrifuge 320, Hettich Zentrifugen, Germany). The obtained layers were diluted in buffer steroids using a commercial kit (MP Biomedicals, Irvine, CA, USA) following the 1/20 proportion (950 µl buffer and 50 µl sample) and stored at –20°C until the next step.

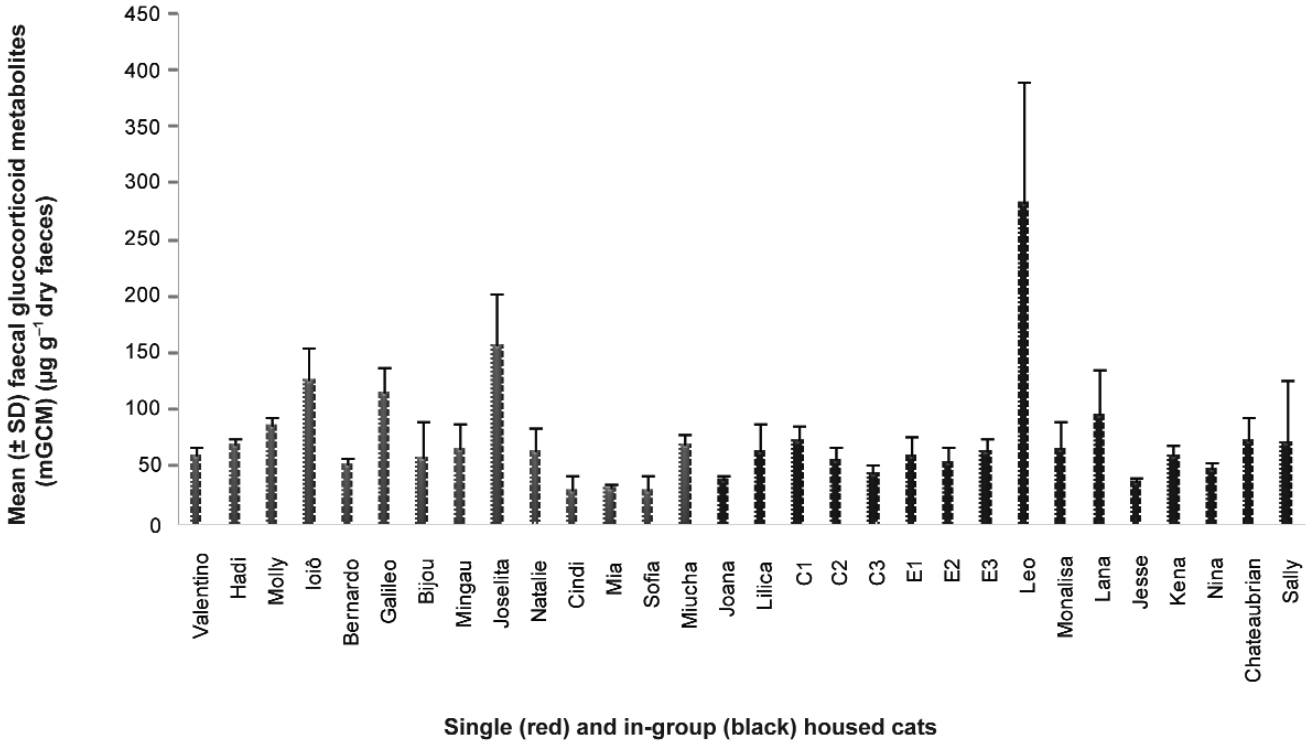
Radioimmunoassay (RIA) was performed by using a commercial kit (Double Antibody Corticosterone ImmuChemTM, MP Biomedicals), previously validated for domestic cats (Graham & Brown 1996).

Statistical analysis

Initially, a random effect model was considered to incorporate the dependence of the mGCM measures of the group-housed cats that lived together (Verbeke & Molenberghs 2009). However, since this dependency resulted as unimportant (ie the cats in the same house did not tend to have more similar glucocorticoids measures than cats from different houses), independence methods were chosen. To compare the mGCM as a function of cats living style (ie singly or group housing), a Student's *t*-test was applied; to investigate the joined effect of age (in months), gender, breed and neutering status in mGCM, a multiple regression was used (Neter *et al* 1996). Furthermore, to evaluate the relationship between the owners' subjective life quality and mGCM, and its interaction with the living style of the cats, a multiple regression was performed. The R® software was used for all analyses.

https://doi.org/10.7120/09627286.21.2.285 Published online by Cambridge University Press

Figure 1



Mean (± SD) mGCM for each of the singly and group-housed cats. Household 1: Joana and Lilica; household 2: C1, C2 and C3; household 3: E1, E2 and E3; household 4: Leo; household 5: Monalisa, Lana and Jesse; household 6: Kena, Nina, Sally and Chateaubrian.

Results

As can be seen in Figure 1, mGCM for the cat, Leo, is clearly distinct from the rest of the data, which led us to consider it as an *outlier*, thus excluding it from the data set for the following statistical analysis.

The mGCM concentration was 71.9 (± 37.6) µg g^{-1} for singly housed cats and 59.9 (± 15.2) µg g^{-1} for those group housed. There was no significant difference in mGCM regarding cat living style (ie singly vs group housing, Student's *t*-test, $P = 0.262$, 95% confidence interval = 54.88; 76.51).

As for owners' subjective life quality, most had high scores both globally and on all four dimensions composing the WHOQOL-bref (ie physical, social, psychological, environmental; see Tables 1 and 2). For single cats only, there was a positive association between mGCM and social life quality ($P = 0.0003$), which suggests that for each unit increase in the owners' social life quality, the mGCM increases by 1.76 µg g^{-1} (the slope of the straight line estimated).

The mGCM did not show any association with sex ($P = 0.5622$), neutering status ($P = 0.0874$), breed ($P = 0.0613$) or age ($P = 0.2236$).

Discussion

The normal reference range of mGCM (ie expected values for non-stressed cats) is yet to be established for the species, and may not prove to be possible. However, a direct comparison between the results obtained here with those found by Graham and Brown (1996), who applied an identical methodology (ie a commercially available corticosterone RIA) is possible. In their study, 1–15 faecal samples were collected from 14 apparently 'non stressed' adult cats and results ranged from 16.6 to 165.3 µg g^{-1}. Our results ranged from 29.3 to 156.17 µg g^{-1}, with the exception of one cat, Leo (household 4), whose concentration was 283.25 µg g^{-1} and was therefore considered an *outlier*. If the cats used in Graham and Brown's study (1996) were, in fact, free of stress, as the authors concluded (although living in cages) we could argue that the cats in our study were likely to hold a low stress level as well. However, considering the difference in housing conditions, this comparison should be considered carefully.

Differences in glucocorticoid metabolites as a function of cat living style were not detected in our study, which leads us to reject our second hypothesis. This is consistent with the results of Lichtsteiner and Turner (2008), who proposed a social compensatory mechanism as explanation. As, in our

Table 1 Owners' subjective life quality (global, physical, psychological, social and environmental dimensions) in owners of singly housed cats.

Cat name	WHOQOL-bref Global (1–5)†	WHOQOL-bref Physical (%)	WHOQOL-bref Psychological (%)	WHOQOL-bref Social (%)	WHOQOL-bref Environmental (%)
Valentino	4	78	62	58	75
Hadi	5	82	79	67	78
Molly	4	50	67	75	69
Ioiô	*	*	*	*	*
Bernardo	4	71	50	58	59
Galileo	4	89	71	92	84
Bijou	4	75	58	58	81
Mingau	4	68	58	58	53
Joselita	4	82	79	100	66
Natalie	4	82	67	58	56
Cindi	4	71	67	67	66
Mia	4	89	75	83	62
Sofia	4	78	75	58	78
Miucha	3	68	67	75	62

† 1: very bad; 2: bad; 3: not bad, not good; 4: good; 5: very good. * Owner was a child thus the questionnaire was not applicable.

Table 2 Owners' subjective life quality (global, physical, psychological, social and environmental dimensions) in owners of group-housed cats.

Cat name	WHOQOL-bref Global†	WHOQOL-bref Physical	WHOQOL-bref Psychological	WHOQOL-bref Social	WHOQOL-bref Environmental
Joana	5	78	71	58	75
Lilica					
C1	4	53	46	42	50
C2					
C3					
E1	3	78	71	75	53
E2					
E3					
Leo	4	36	42	50	41
Monalisa	4	93	92	92	69
Lana					
Jesse					
Kena	4	78	75	75	62
Nina					
Chateaubrian					
Sally					

† 1: very bad; 2: bad; 3: not bad, not good; 4: good; 5: very good.

https://doi.org/10.7120/09627286.21.2.285 Published online by Cambridge University Press

study, group-cat owners were allowed to choose the cat (or cats) whose faeces were collected (this was the only way they found it possible to perform individual collections in such large cat groups), it is possible that owners have favoured the selection of preferred, more close and friendly, non-stressed cats. However, their commentaries on the different behavioural styles of their participant cats suggest such a possibility to be unlikely. Therefore, even though rank order was not determined in the present study, anecdotal evidence on the apparent ability of the owners of the group-living cats to easily identify their cats' social status, and their statements on their different cats' profiles may support the social compensatory mechanism as an explanation for the lack of difference between singly and group-housed cats.

Lichtsteiner and Turner (2008) studied single-cat households in comparison with households of 3–4 cats and used social organisation as a key argument for the lack of differences in stress levels between them. However, even though rank-order tests revealed alpha-omega pairs of cats in most of the 3–4 multi-cat households, their low densities (average: 57.1 m^2 per cat) may suggest a rather spatial mechanism of avoiding conflict (and stress reactions) by dispersion. As stated by Barry and Crowell-Davis (1999), living in households with few cats creates the possibility for individuals to avoid each other, thus being able to regulate aggression through spatial organisation. In our case, available space for the cats was not assessed formally but from what we could see when going to the households to pick up the samples we can say that average densities were certainly higher than those found by Lichtsteiner and Turner (2008). Thus, spatial dispersion does not appear to be a reasonable argument in our case.

Another important point for consideration relates to the activity of the HPA axis under prolonged periods of stress. A number of authors argue that chronic stress can be detected through increased levels of excreted glucocorticoid metabolites (Beerda *et al* 1999), while others even go as far as stating that in chronically stressed animals we see an over-reactive HPA axis under an ACTH stimulation or the introduction of an acute stressor (Carlstead & Brown 2005). However, Beerda *et al* (1999) found the opposite result (ie HPA hypo-responsiveness as an indicator of chronic stress), but stated that their five weeks of social and spatial restriction may not have been sufficiently stressful to determine HPA hyper-responsiveness, and still concluded that increased cortisol levels are a strong indication of chronic stress, although the lack of it may not exclude the possibility of stressed animals. Given this, and considering that group living may represent a possible source of chronic stress for cats, it would be interesting to explore faecal levels of glucocorticoids metabolites after adding an acute stressor (eg a visit to the veterinarian) in group- versus singly housed cats.

For single cats, there was a positive correlation between scores for owners' subjective *social* life quality and levels of mGCM, thus rejecting our first hypothesis. The *social* dimension was composed of three questions regarding how well the person feels in relation to the support received by friends, sexual life satisfaction and relationship with family and friends. Owners who considered themselves to be doing very well socially have apparently more aroused (potentially stressed) cats. This is probably not due to a relatively more distant and/or limited interaction with the cat, as owners of multiple cats may not have enough time to maintain a close relationship with each of the cats. Such an association may be related to a more frequent/intrusive presence of people in the owners' surroundings (ie frequent visitors or more residents in the house). Some of these (eg number of people in the house) have already proved to be associated with stress responses in cats (Lichtsteiner & Turner 2008). And, as argued previously, such human aspects of the home environment apparently affect single cats more.

Other life-quality dimensions (ie physical, psychological and environmental) were not found to be associated with mGCM. It may be that, apart from how well the person feels themselves socially (ie social dimension), other aspects related to the owner may influence the cats' behaviour (eg occurrence of undesired behaviours; Adamelli *et al* 2005; Ramos & Mills 2009) but not their basal levels of stress. In other words, an owners' behaviour or emotional status may trigger a behavioural response in the cat, but it does not necessarily result in prolonged arousal, measurable through cortisol secretion.

Similarly to other studies (Cauvin *et al* 2003), associations between mGCM and age, sex, and breed were not detected. As to neutering, a recent study demonstrated decreased levels of faecal glucocorticoids' metabolites as a function of neutering in female feral cats (Finkler & Terkel 2010). However, as stated by the authors, the decrease in cortisol levels in feral cats due to neutering is probably linked to reduced social and reproductive pressures; such elements may be diminished in human households where the most important sources (eg food, shelter) are frequently quite abundant — thus not finding lower levels of mGCM in neutered cats was not unexpected. In fact, if neutering was associated with lower glucocorticoid levels, as seen with feral cats, we would expect higher mGCM among group-living cats (since we had nine entire group-housed cats versus none singly housed), which was not the case.

Animal welfare implications

Results obtained in this pilot study point to the importance of considering both physical and human aspects of the home environment when keeping cats as pets. Regarding group living, no changes to the traditional recommendations regarding keeping cat densities low should be made as yet. However, regarding owning a single cat, a closer examination of the quality of the environment (eg number of people in the house, frequency of visitors coming into the house) should be taken: busy homes may lead to aroused cats. Resources should be provided to enable cats in such households to manage their stress levels by, for example, the provision of multiple resting places, some of which should be secluded.

Conclusion

The results of this pilot study suggest the following:

• Cat living style (single versus large-group housing) does not seem to significantly affect arousal underpinning potential emotional stress. However, further studies are still required in order to check for chronic stress in small-group housing (ie 3–4 cats from various backgrounds and under high densities);

• Owners' perceived social life quality affects the global levels of stress in cats only when living as a single pet;

• Singly housed cats may be more prone to the negative effects of the human and/or environmental features of the home environment, probably due to a major exposure to them.

Acknowledgements

We would like to thank São Paulo Research Foundation, FAPESP which gave an undergraduate scholarship to one of the authors as well as financially supported the research. We are also very thankful to Carine Savali Redigollo for helping with statistics, to Professor Daniel S Mills for reading and commenting on our initial manuscript, and to all the cat owners who were so dedicated, committed and enthusiastic during the entire study.

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