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Effects of stressors on the behavior and physiology of domestic cats

Judi Stella[a,*], **Candace Croney, PhD**[a], and **Tony Buffington, DVM, PhD**[b]

[a]Department of Veterinary Preventative Medicine, The Ohio State University College of Veterinary Medicine, 1920 Coffey Rd 100P Sisson Hall, Columbus, Ohio 43210

[b]Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, 601 Vernon L. Tharp Street, Columbus, Ohio 43210

Abstract

Feline interstitial cystitis (FIC) is a chronic pain syndrome of domestic cats. Cats with FIC have chronic, recurrent lower urinary tract signs (LUTS) and other comorbid disorders that are exacerbated by stressors. The aim of this study was to evaluate behavioral and physiological responses of healthy cats and cats diagnosed with FIC after exposure to a five day stressor. Ten healthy cats and 18 cats with FIC were housed at The Ohio State University Veterinary Medical Center (OSUVMC) vivarium. All cats were housed in enriched cages for at least one year prior to the experiment. Cats had daily play time and socialization outside of the cage, food treats and auditory enrichment. The daily husbandry schedule was maintained at a consistent time of day and cats were cared for by two familiar caretakers. During the test days, cats were exposed to multiple unpredictable stressors which included exposure to multiple unfamiliar caretakers, an inconsistent husbandry schedule, and discontinuation of play time, socialization, food treats, and auditory enrichment. Sickness behaviors (SB), including vomiting, diarrhea, anorexia or decreased food and water intake, fever, lethargy, somnolence, enhanced pain-like behaviors, decreased general activity, body care activities (grooming), and social interactions, were recorded daily. Blood samples were collected in the morning, before and after the stress period, for measurement of serum cortisol concentration, leukocytes, lymphocytes, neutrophils, neutrophil: lymphocyte (N:L) ratio and mRNA for the cytokines interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). Overall, the short term stressors led to a significant increase in SB in both healthy cats and cats with FIC, whereas lymphopenia and N:L changes occurred only in FIC cats. Daily monitoring of cats for SB may be a noninvasive and reliable way to assess stress responses and overall welfare of cats housed in cages.

*Corresponding author: Tel: 740.974.8418; stella.7@osu.edu.

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Keywords

Cats; sickness behavior; stress; welfare; feline interstitial cystitis (FIC)

1. Introduction

Feline interstitial cystitis (FIC) is a chronic pain syndrome diagnosed in 1-2% of domestic cats (Lund, 1999). It is the leading cause of urinating outside of the litter box and often leads to euthanasia or relinquishment to a shelter (Patronek, 1996; Salman, 2000). Typically, cats with FIC have chronic or recurrent lower urinary tract signs (LUTS) and other comorbid disorders that are exacerbated by stressors (Buffington et al., 1999; Buffington et al., 2006). For cats, such stressors include loud or unfamiliar noises, sudden movements, novel and unfamiliar places and objects, and the approach of strangers (humans, cats or other animals) into their personal space. It is therefore important to consider these factors in any assessment of the welfare of cats.

Animal welfare has been defined as the animal's state as regards its attempts to cope with its environment (Broom, 2007). Coping pertains to the process of reducing stressor-induced physiological activation by performing behaviors that either alter the stressor or reduce the emotionality associated with the stressor (Carlstead et al., 1993; Broom, 1996). Sickness behavior (SB), which refers to a group of nonspecific clinical and behavioral symptoms, is an evolutionary adaptive response that aids in survival of the individual. It is a well documented physiological and behavioral response to infection that has been found in all animal species that have been studied. These behaviors are thought to reflect a change in motivation of the animal from pursuing usual activities, such as foraging or social behaviors, to one that promotes recovery by inhibiting metabolically expensive activities and favoring ones that promote healing (Dantzer et al., 2008). Recently, psychological stress has been associated with immune activation and pro-inflammatory cytokine release (Marques-Deak et al., 2005) and a recent review has linked SB, cytokine activation, mood symptoms and pathologic pain (Raison and Miller, 2003). Cats also appear to exhibit SB in response to environmental disturbances. For example, Stella et al. (2011) reported that colony-housed cats exhibited increased SB in response to environmental stressors that occurred during routine management of the colony. These disturbances were comparable to those that typically occur in veterinary clinics, research facilities, and shelters. The most common SB exhibited in response to the stressors were vomiting of hair, food, or bile, decreased appetite, and eliminating out of the litter pan. In cats, such behaviors are often considered to be normal (vomiting), finicky (decreased appetite), or unacceptable (not using litter pan) by owners and veterinary professionals.

Physiological changes associated with stressors also can occur. The pro-inflammatory cytokines interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) are thought to play an important role in the expression of SB (Raison and Miller, 2003). Released from macrophages early in the innate immune response, they communicate the presence of peripheral inflammation to the brain, which leads to the behavioral responses of the animal (Raison and Miller, 2003; Dantzer et al., 2008). Peripheral cytokine concentrations have been shown to be elevated in patients with a variety of inflammatory

diseases (e.g. rheumatoid arthritis, cardiovascular disease, cancer) as well as in patients with depression (Raison and Miller, 2003; Miller et al., 2009).

Leukocyte profiles have also been proposed for use to assess animal stress (Davis et al., 2008). Increasing circulating glucocorticoid concentrations cause the redistribution of lymphocytes from the blood to other body compartments such as the skin, spleen, bone marrow, and lymph nodes (Dhabhar, 2002). This redistribution results in a significant decrease in peripheral blood lymphocytes. Elevated glucocorticoids also cause an increase in circulating neutrophils (heterophils in birds and reptiles), both in number and percentage of total leukocytes. This response is evolutionarily conserved and has been seen in all five vertebrate taxa. Thus, a high neutrophil to lymphocyte (N:L) ratio in a blood sample is thought to be a reliable indicator of high glucocorticoid concentrations.

Both the behavioral and physiological coping responses of cats must be better understood to ensure the well-being of cats kept in cages for various purposes. For example, animal housing for laboratories historically has been designed with practical and economical considerations in mind for human beings, often with inadequate consideration for the comfort of the animal or provision of opportunities to cope. Therefore, the welfare of laboratory housed cats can be compromised due to variable combinations of inadequate housing environment and inappropriate husbandry practices. Because predictability and control seem to be key determinants of coping ability (Broom, 1991; Morgan and Tromborg, 2007), it is important to explore ways in which it may be possible to afford cats maintained in laboratories and similar facilities these characteristics, and to investigate their responses. For example, predictability can be achieved through a temporally consistent husbandry schedule, and cats can be afforded some control over the environment by having access to a hiding space to withdraw to if a threat is perceived.

The aim of this study was to evaluate behavioral and physiological responses of healthy cats and cats diagnosed with FIC during a five day exposure to stressors. Physiologic parameters measured included serum cortisol concentrations, leukogram, and expression of the genes for the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α.

2. Material & Methods

2.1 Subjects

Ten healthy cats (4 males and 6 females) between 1 and 6 years of age and 18 cats with FIC (8 males and 10 females) between 1 and 8 years of age were studied. All cats were housed in individual stainless steel cages measuring 70× 78 × 75 cm in the Ohio State University Veterinary Medical Center (OSUVMC) vivarium (Columbus, OH, USA). A 12-hour light-dark schedule and a mean ± SD room temperature of 22 ± 1.6°C were maintained throughout the vivarium. Cats with FIC were received as donations from veterinary clients throughout the United States because of a history of severe, irresolvable, recurrent LUTS and transferred to the OSUVMC for confirmation of a diagnosis of FIC and absence of other diseases. The diagnostic evaluation consisted of a complete physical examination, complete blood count (CBC), serum biochemicalanalysis, urinalysis, urine bacteriologic culture, and cystoscopy. The diagnosis of FIC was made on the basis of variable combinations of owner-

reported chronic dysuria, stranguria, and pollakiuria; hematuria or proteinuria identified in the urinalysis; and in most cats (not all cases of FIC present with this finding) cystoscopic identification of glomerulations (submucosal petecchial hemorrhages) (Westropp and Buffington 2010).

Healthy cats were obtained from licensed vendors and determined to be free of identifiable disease on the basis of history, physical examination findings, and results of CBC, serum biochemical analysis, and urinalysis that were within established reference ranges. All cats, regardless of disease status, were neutered at least one year prior to the start of this study.

2.2 Housing and enrichment

All cats were housed at the OSUVMC vivarium in enriched cages for at least one year prior to the experiment. Cage enrichment included an elevated resting board (part of the cage, 68 × 15 cm, 42 cm above cage floor), a cardboard hiding box (approximately 15 × 10 × 15 cm recycled hospital supply shipping boxes), bedding (84 × 74 cm folded blanket), and commercial cat toys (variable). All cage items were kept in the same place consistently throughout the study. In addition, cats had daily play time outside the cage with each other and socialization with one experimenter, as well as food treats, toys and auditory enrichment. The animal care staff was trained to care for the cats at regular times and carefully observe for the presence of SB; details of cage set-up, enrichment, time out of the cage, and data collection were standardized. Diet and feeding practices, colony plan, husbandry practices, and enrichment protocols have been previously published (Stella et al., 2011).

2.3 Behavioral data collection

Sickness behavior data were collected once between 7:00 h and 10:00 h and again between 15:00 h and 17:00 h by one experimenter. Behaviors determined to be SB were based on review of the literature (Carlstead et al., 1993; Buffington, 2002; Westropp et al., 2007; Dantzer et al., 2008) and our observations of the cats during the year prior to this study (Stella et al., 2011). Sickness behaviors observed and recorded for the study included signs referable to the upper gastrointestinal tract (UGI-vomiting of hair, food, or bile) and lower gastrointestinal tract (LGI-diarrhea, soft feces, constipation), LUTS (stranguria, hematuria, pollakiuria), and skin (epilation, skin lesions, chin acne); anorexia or decreased food and water intake. Urination and defecation outside the litter box were recorded separately from other signs referable to the lower urinary and gastrointestinal tracts in an attempt to differentiate behavioral responses from organ dysfunction. Baseline data were collected for 10 days prior to the initial blood collection. Stress response data were collected for 10 days starting one week after the initial blood collection (Table 1). Stress response data were collected for five days when stressors were applied and for five days after their discontinuation. Based on our experience with these cats, there appears to be some “lag time” from the application of the stressor to the appearance of the SB. Our aim was to capture all SB related to the stressors.

2.4 Physiologic data collection

Seven days prior to the start of the stress period and again on day six of the stress period, all cats had food withheld overnight, and were transported to the laboratory the following morning in a commercial cat carrier. Cats were then anesthetized with isoflurane, and 6 ml of blood was collected from an external jugular vein. Upon recovery from anesthesia, cats were returned to their cages and provided food. Serum cortisol concentrations and CBC were analyzed at the OSUVMC clinical laboratories using standard methodology.

For cytokine analysis, venous blood was obtained from all cats as described above and peripheral blood lymphocytes (PBL) were purified by the standard Ficoll-Hypaque method. Total DNA-free RNA was isolated from the PBL using Absolutely RNA Miniprep Kit (Stratagene, Santa Clara, CA, USA). The ImPromII Reverse Transcriptase kit (Promega, Madison, WI, USA) was used for 1st strand cDNAs synthesis according to the manufacture's instructions. Relative quantitative real time RT-PCR was performed in the Mx3000p System (95°C for 10 minutes, then 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds) using QuantiTect SYBR Green PCR kit (Stratagene, Santa Clara, CA, USA). The primers to amplify IL-1!, IL-6, and TNF-! genes were designed based on published cDNA sequences for the cat. The primers for cat hypoxanthine phosphoribosyl transferase (HPRT) were made based on the sequence obtained from our laboratory. The relative quantification of IL-1! , IL-6, and TNF-! gene expression, normalized to HPRT (housekeeping control), were calculated using the LinRegPCR software, and compared using the Mann-Whitney test. The results are expressed as the ratio of the target gene *vs.* HPRT housekeeping gene.

2.5 Stressors

During all five test days the cats were exposed to multiple unpredictable stressors from 07:00 h-20:00 h. These were considered to be minor disruptions that could occur under normal vivarium, shelter, or veterinary clinic housing conditions. Stressors included introduction of multiple unfamiliar caretakers to conduct routine husbandry procedures, an inconsistent husbandry schedule, feeding delays (four hours) or food removal (removed at 20:00 h), restraint stress (placement in a commercial cat carrier for 15-30 minutes), anesthesia, and withdrawal of play time, food treats, auditory enrichment, and positive human-animal interactions (Table 1). Enriched cages, lighting schedule, diet, and room temperature were maintained throughout the study.

2.6 Statistical analysis

Sickness behaviors, 10 days of baseline data and 10 days of stress data, were averaged for each cat and analyzed using t-tests. The cytokines, IL-1β, IL-6, and TNF-α, are expressed as the ratio of the target gene *vs.* HPRT housekeeping gene for each cat and were analyzed using t-tests as were leukogram results and cortisol concentrations. Analysis consisted of paired tests for within group analysis and unpaired tests with Welch's correction for between group analyses using standard statistical software (GraphPad Prism, version 5.00 for Windows, GraphPad Software, San Diego, CA, USA). Results are presented as mean ± SD. Outliers (> 2 SD from the mean) were removed from the data sets prior to analysis.

2.7 Ethics approval

The Animal Care and Use Committee of The Ohio State University approved all experimental procedures used in this study.

3. Results

A significant increase in total SB from baseline to stress condition was observed in both the healthy cats and the cats with FIC (H cats, P = 0.02; FIC, P = 0.001). When healthy cats were compared to cats with FIC, no difference was found in either the baseline or stress condition. When looking at individual behaviors, UGI signs were significantly decreased in the stress condition from baseline in both groups (H, P = 0.05; FIC, P = 0.03). LGI (H, P = 0.34; FIC, P = 0.02), decreased appetite (H, P = 0.29; FIC, P = 0.02), and no elimination in 24 hours (H, P = 0.18; FIC, P = 0.01) were significantly increased in cats with FIC, but not in healthy cats (Table 2).

A significant decrease in lymphocytes (P = 0.009) and a significant increase in the N:L ratio (P = 0.04) from baseline to stress period was observed in cats with FIC (Table 3). When exposed to stressors, total leukocyte (P = 0.03) and lymphocyte (P = 0.03) counts were lower in cats with FIC compared to healthy cats, but were similar during baseline periods.

IL-6 in healthy cats was significantly lower (P = 0.04) from baseline when stressed (Table 3). TNF-α was higher in cats with FIC than in healthy cats, both during the baseline period (P = 0.04) and when stressed (P = 0.05).

No significant changes from baseline to stress period were observed in either group for serum cortisol concentrations, total leukocyte or neutrophil numbers, or IL-1β, or TNF-α gene expression (Table 3).

4. Discussion

This study has several noteworthy findings. First, the overall behavioral response to the environmental stressors imposed was similar for both healthy cats and cats with FIC, with both groups showing significant increases in SB. This is consistent with an earlier study (Stella et al., 2011), which found that a decrease in the number of SB was associated with an enriched environment and the absence of environmental stressors. Beneficial effects of environmental enrichment on occurrence of SB in cats with FIC also have been identified in clinical studies (Buffington et al., 2006). Carlstead et al. (1993) found that laboratory cats subjected to multiple, unpredictable caretakers and irregular feeding and husbandry schedules were chronically stressed. Also, in a study of shelter cats in four types of housing, the worst daily stress scores were found in cats housed in un-enriched cages, with unpredictable handling and husbandry practices (Gourkow and Fraser, 2006). Taken together, these results in cats agree with studies in other species that illustrate that an unpredictable, uncontrollable, poor quality environment is a potent psychological stressor (Broom, 1991; Morgan and Tromborg, 2007).

Differences in the occurrence of SB were not observed between the healthy group and the FIC group during baseline data collection or when stressed. Despite the fact that the cats

with FIC had been donated to the research colony as an alternative to euthanasia for their disease, they responded similarly to the healthy cats to an enriched environment after acclimation to the colony. This agrees with results from an earlier study that found that the decrease in SB in the cats with FIC and in healthy cats was similar (Stella et al., 2011) after implementation of environmental enrichment protocols. Signs referable to the UGI (vomiting of hair, food, or bile) decreased from baseline to stress in both groups and LGI signs (diarrhea or soft stool) decreased in cats with FIC. Additionally, cats with FIC had a significant decrease in appetite and elimination of urine and feces. The decrease in UGI, LGI, and eliminations can all be attributed to the decrease in appetite, which is a classic cytokine-induced SB. Similar results have been found in dairy cows, which express sickness behavior as reduced activity, feeding, grooming, and rumination. A study that aimed to identify cows at risk for metritis found that cows later diagnosed with severe metritis had changes in feeding behavior one week before calving. These cows had fewer aggressive interactions at the feed bunk, reduced feeding time, and reduced food intake (Huzzey et al., 2007). Monitoring feeding behavior and appetite may therefore provide animal caretakers with a measure of well-being. Lastly, there was a trend toward increased urination outside of the litter box in healthy cats ($P = 0.08$) which also agrees with earlier published results (Stella et al., 2011). The reason for this is unclear and merits further investigation. Differences in SB between the baseline and stress condition are presumed to be caused by the application of stressors, but the activation of the stress response system is dependent on individual history, the context in which the stressor occurs, and the expectation the individual has for the outcome of the event (McEwen, 2007). Additionally, the sensorium of the domestic cat is much different than humans', which may lead them to perceive threats that are not sensed by people. This may account for some of the SB seen in the baseline condition, particularly the higher incidence of vomiting.

As stated above, some of the most common SB observed were vomiting (hair, food, or bile), decreased appetite, and eliminating out of the litter pan. These behaviors are often thought to be “normal” in cats but these results, as well as earlier ones (Stella et al., 2011), suggest that veterinarians and other caregivers may need to consider the possibility that these signs might result from external as well as internal events when a cat is evaluated clinically for the cause(s) of these behaviors. These behaviors are not unique to cats; similar behaviors have been reported in other species in response to environmental disturbances (Dantzer & Kelley, 2007; McEwen, 2008).

The stressors in this study intentionally were events that occur routinely in the environments of cats housed in cages and the results highlight the need for appropriate, consistent and predictable management practices to ensure normal cat behavior and good welfare. They also reiterate the importance of careful environmental assessment when making decisions pertaining to euthanasia or culling of cats, as symptoms of impaired welfare may be mistaken for symptoms of organic disease rather than responses to environmental disturbances.

An unanticipated finding was that lymphopenia and an increase in the N:L ratio was seen in cats with FIC, but not in healthy cats. In response to an increase in glucocorticoids, a decrease in peripheral lymphocyte numbers and an increase in neutrophil numbers as well as

an increase in the N:L ratio would be expected. This is a well documented response to stressors across vertebrate species and has been proposed as a reliable method of evaluating stress (Davis et al., 2008). The reasons for the decrease in lymphocyte numbers were not determined, nor were the effects on specific lymphocyte subsets investigated in this study. Potential mechanisms for the observed differences might include increased sensitivity to circulating glucocorticoid concentrations or inhibition by increases in circulating catecholamine concentrations, both of which have been reported in cats with FIC (Buffington, 2011). Additionally, cats with FIC have a sensitized stress response system (Westropp et al., 2006) and may have perceived the stressor as more intense than the healthy cats, thus resulting in the observed difference. Although lymphopenia and an increase in N:L ratio was noted in cats with FIC, no difference was found in other measures of the leukogram of either group. One explanation is that the stressor was not intense enough to invoke this physiologic response. Another possible explanation is the healthy cats habituated to the stressors more quickly and the changes in leukogram were not captured in this study. A third explanation is that the response was missed temporally. Studies by Dhabhar (2002) have found that changes in blood leukocyte numbers occur within 30 minutes of the stressor and that this response is reversed within 3 hours of the cessation of the stressor. This suggests that the change in leukocyte profile may be in response to acute stressors, whereas the changes observed in this study occurred in response to a more chronic stress paradigm.

Also unexpectedly, a significant glucocorticoid response to the stressors was not observed in either group. One explanation is that all cats may have had elevated cortisol concentrations at the initial blood draw due to withdrawal of food, restraint stress (restrained in a carrier), transport stress (from being moved from the vivarium to the laboratory), anesthesia, or some combinations of the above factors. These stressors were superimposed upon and may have masked measurements of the effects of the environmental stressors that were imposed. Thus, it is possible that no significant difference could be seen between conditions. In fact, all values fell within our reference range of normal. Although not significant, cortisol concentration in the cats with FIC was trending downward ($P = 0.09$) from baseline in response to the stressors. A previous study of cats with FIC demonstrated an uncoupling of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. Cats with FIC had a significantly decreased cortisol response to an ACTH stimulation test along with smaller adrenal glands when compared to healthy cats (Westropp et al., 2003). Decreased glucocorticoid responses have also been reported in other medically unexplained syndromes such as posttraumatic stress disorder (PTSD) (Pace and Heim, 2011). Measurements of the glucocorticoid response to stressors have historically been challenging to assess accurately and this study may be another example. Measuring fecal or urinary cortisol may be a more reliable and less invasive method of assessing stress response in a study like this than is serum cortisol. However, since cortisol is a measure of the intensity of arousal but not its valence, and less invasive methods provide only an integrated average value over the time of collection, this method may sacrifice the ability to determine changes in the pattern of excretion.

Lastly, gene expression of the pro-inflammatory cytokines measured was not elevated between conditions. This was unexpected and is likely because this study measured changes in peripheral blood. External events, like those imposed in this study, would be expected to

lead to activation of cytokine pathways in the brain and central nervous system (Miller et al., 2009), leading to the observed increase in SB. Additionally, it has been shown that sub-effective doses of IL-1β and TNF-α, when administered simultaneously, can induce anorexia and weight lose (Bluthé et al., 2000). This synergistic effect may have led to the increase in SB but not in cytokine gene expression in this study. The decrease in the expression of IL-6 in the healthy cats after the imposed stressor is unexplained, but an error in measurement could have occurred. Future investigation of cytokines in domestic cats should aim to determine reference ranges and timing of elevation of the protein products in peripheral blood.

Collectively, our findings suggest that the SB responses could be further studied as a tool for welfare assessment of cats. The motivational state of this behavioral response has a physiologic basis and should be accorded as much weight in welfare assessments as other motivational states such as fear, hunger, and thirst. Seeking of rest, withdrawal from the environment and caring for one's self are adaptive responses to infection that are as normal as arousal and escape responses are to threat (Dantzer and Kelley, 2007). However, when this motivational state is caused by chronic environmental disturbances with which the individual is unable to cope, it is a sign of impaired welfare and should be addressed.

It should be noted that this study has limitations. The first is the small number of cats studied. Increasing the number of individuals would increase the power of the analysis. Another limitation is that the stressors examined were chosen by inference from other studies and past experience of the authors. They may not have been of the correct type or of sufficient intensity or duration to evoke the stress response system in a physiologically measurable way. A review paper (Pacák and Palkovits, 2001) that outlined the stress responses of rats to five different types of stressors concluded that the type of stressor investigated had a profound effect on the results, presumably due to the perceived threat to the individual and the level of control and predictability the animals had for coping. It is also possible that the duration of the stress period in our study may have been long enough to allow sufficient habituation so as to cause us to miss the physiologic response. Finally, the cats studied had been living in the colony for at least one year in an enriched environment. Cats recently admitted to new environments may have a different behavioral and physiologic response pattern to similar stressors.

5. Conclusions

Short term environmental stressors led to a significant increase in SB in both healthy cats and cats with FIC, whereas few differences in physiological parameters were observed. This could be because cats with FIC have some degree of immune system compromise, or they may have perceived the threat from such stressors to be more intense, and may have had less ability to cope with them. Thus, the behaviors of the cats were more reliably indicative of their level of welfare than were the physiologic data in this study. This suggests that daily monitoring of cats for SB may offer a practical, non-invasive method to assess cats' stress responses to their surroundings and thus, gauge their overall welfare. Future studies should aim to determine if different types of stressors (social, physical, psychological) cause different physiologic and behavioral responses in healthy cats and cats with FIC.

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References

Bluthé RM, Michaud B, Poli V, Dantzer R. Role of IL-6 in cytokine-induced sickness behavior: a study with IL-6 deficient mice. Physiol Behav. 2000; 70:367–373. [PubMed: 11006436]

Broom DM. Animal welfare defined in terms of attempts to cope with the environment. Acta Agri Scand A. 1996; 27(supplement):22–28.

Broom DM. Animal welfare: concepts and measurement. J Anim Sci. 1991; 69:4167–4175. [PubMed: 1778832]

Broom, DM.; F, A. Domestic Animal Behaviour and Welfare. 4th. Cambridge UK: CABS International; 2007.

Buffington CA. External and internal influences on disease risk in cats. J Am Vet Med Assoc. 2002; 220:994–1002. [PubMed: 12420776]

Buffington CA, Chew DJ, Woodworth BE. Feline interstitial cystitis. J Am Vet Med Assoc. 1999; 215:682–687. [PubMed: 10476717]

Buffington CA, Westropp JL, Chew DJ, Bolus RR. Clinical evaluation of multimodal environmental modification (MEMO) in the management of cats with idiopathic cystitis. J Feline Med Surg. 2006a; 8:261–268. [PubMed: 16616567]

Buffington CA, Westropp JL, Chew DJ, Bolus RR. Risk factors associated with clinical signs of lower urinary tract disease in indoor-housed cats. J Am Vet Med Assoc. 2006b; 228:722–725. [PubMed: 16506933]

Buffington CAT. Idiopathic Cystitis in Domestic Cats—Beyond the Lower Urinary Tract. J of Vet Intern Med. 2011; 25:784–796. [PubMed: 21564297]

Carlstead K, Brown JL, Strawn W. Behavioral and physiological correlates of stress in laboratory cats. Appl Anim Behav Sci. 1993; 38:143–158.

Dantzer R, Kelley KW. Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun. 2007; 21:153–160. [PubMed: 17088043]

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008; 9:46–56. [PubMed: 18073775]

Davis AK, Maney DL, Maerz JC. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol. 2008; 22:760–772.

Dhabhar FS. A Hassle a Day May Keep the Doctor Away: Stress and the Augmentation of Immune Function. Integr Comp Biol. 2002; 42:556–564. [PubMed: 21708751]

Dhabhar FS. Stress-induced augmentation of immune function-The role of stress hormones, leukocyte trafficking, and cytokines. Brain Behav Immun. 2002; 16:785–798. [PubMed: 12480507]

Gourkow N, Fraser D. The effect of housing and handling practices on the welfare, behaviour and selection of domestic cats (Felis sylvestris catus) by adopters in an animal shelter. Anim Welfare. 2006; 15:371–377.

Huzzey JM, Veira DM, Weary DM, von Keyserlingk MAG. Prepartum Behavior and Dry Matter Intake Identify Dairy Cows at Risk for Metritis. J Dairy Sci. 2007; 90:3220–3233. [PubMed: 17582105]

Lund EM, A P, Kirk CA, et al. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. J Am Vet Med Ass. 1999; 214:1336–1341. [PubMed: 10319174]

Marques-Deak A, Cizza G, Sternberg E. Brain-immune interactions and disease susceptibility. Mol Psychiatry. 2005; 10:239–250. [PubMed: 15685252]

McEwen BS. Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain. Physiol Rev. 2007; 87:873–904. [PubMed: 17615391]

McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. Eur J Pharmacol. 2008; 583:174–185. [PubMed: 18282566]

Miller AH, Maletic V, Raison CL. Inflammation and Its Discontents: The Role of Cytokines in the Pathophysiology of Major Depression. Biol Psychiatry. 2009; 65:732–741. [PubMed: 19150053]

Morgan KN, Tromborg CT. Sources of stress in captivity. Appl Anim Behav Sci. 2007; 102:262–302.

Pacák K, Palkovits M. Stressor Specificity of Central Neuroendocrine Responses: Implications for Stress-Related Disorders. Endocr Rev. 2001; 22:502–548. [PubMed: 11493581]

Pace TWW, Heim CM. A short review on the psychoneuroimmunology of posttraumatic stress disorder: From risk factors to medical comorbidities. Brain Behav Immun. 2011; 25:6–13. [PubMed: 20934505]

Patronek GJ, G L, Beck AM, et al. Risk factors for the relinquishment of cats to an animal shelter. J Am Vet Med Ass. 1996; 209:582–588. [PubMed: 8755976]

Raison CL, Miller AH. When Not Enough Is Too Much: The Role of Insufficient Glucocorticoid Signaling in the Pathophysiology of Stress-Related Disorders. Am J Psychiatry. 2003; 160:1554–1565. [PubMed: 12944327]

Salman MD, H J, Ruch-Gallie R, et al. Behavioral reasons for relinquishment of dogs and cats to 12 shelters. J Appl Anim Welfare Sci. 2000; 3:93–106.

Stella JL, Lord LK, Buffington CAT. Sickness behaviors in response to unusual external events in healthy cats and cats with feline interstitial cystitis. J Am Vet Med Ass. 2011; 238:67–73. [PubMed: 21194324]

Westropp, J.; Buffington, CAT. Lower Urinary Tract Disorders in Cats. In: Ettinger, SJ.; Feldman, EC., editors. Textbook of Veterinary Internal Medicine. 7. Vol. 2. Elsevier-Saunders; St. Louis: 2010. p. 2069-2086.

Westropp JL, Kass PH, Buffington CA. Evaluation of the effects of stress in cats with idiopathic cystitis. Am J Vet Res. 2006; 67:731–736. [PubMed: 16579769]

Westropp JL, Kass PH, Buffington CA. In vivo evaluation of alpha(2)-adrenoceptors in cats with idiopathic cystitis. Am J Vet Res. 2007; 68:203–207. [PubMed: 17269887]

Westropp JL, Welk KA, Buffington CA. Small adrenal glands in cats with feline interstitial cystitis. J Urol. 2003; 170:2494–2497. [PubMed: 14634458]

Abbreviations

FIC	feline interstitial cystitis
LUTS	lower urinary tract signs
SB	sickness behavior
OSUVMC	Ohio State University Veterinary Medical Center
N:L	neutrophil:lymphocyte ratio
IL-1β	interleukin-1 beta
IL-6	interleukin-6
TNF-α	tumor necrosis factor-alpha
UGI	upper gastrointestinal
LGI	lower gastrointestinal

Table 1

Time line of data collection and stressor application. All cats in the study were subjected to events on the same day.

Day 1	Day 1 baseline SB data collection
Day 10	Day 10 baseline SB data collection
Day 11	Baseline blood collection
Day 18- application of stressors begins	Day 1- no EE (removal of food treats, social interactions with JS, auditory EE, and playtime out of cage), new caretaker #1, change in husbandry and feeding time (from AM to PM)
Day 19	Day 2- no EE(removal of food treats, social interactions with JS, auditory EE, and playtime out of cage), new caretaker #2, change in husbandry and feeding time (from AM to PM)
Day 20	Day 3- no EE(removal of food treats, social interactions with JS, auditory EE, and playtime out of cage), new caretaker #3, change in husbandry and feeding time (from PM to AM)
Day 21	Day 4-no EE(removal of food treats, social interactions with JS, auditory EE, and playtime out of cage), food removed in PM
Day 22- application of stressors ends	Day 5- no EE(removal of food treats, social interactions with JS, auditory EE, and playtime out of cage), restraint in carrier, anesthesia, blood draw
Day 29	End of stress period data collection

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Table 2

Results of paired t-tests of sickness behaviors. Bold type indicates significant results at P = 0.05 level. FIC=feline interstitial cystitis; B=baseline; S=stress; SD=standard deviation; N=number of cats analyzed

	Healthy	Healthy			FIC	FIC		
SB	**Baseline Mean +/- SD**	**Stress Mean +/-SD**	**B v S P-value**	**N**	**Baseline Mean +/-SD**	**Stress Mean +/- SD**	**B v S P-value**	**N**
Total	0.08±0.24	0.95±0.8	**0.02**	9**	0.13±0.28	1.3±1.1	**0.0001**	17**
UGI	0.09±0.1	0±0	**0.05***	10	0.25±0.17	0.13±0.28	**0.03***	17**
LGI	0.003±0.009	0±0	0.34	10	0.02±0.03	0.0±0.0	**0.02***	17**
↓ App	0.09±0.12	0.21±0.34	0.29	10	0.12±0.1	0.54±0.71	**0.02**	16**
No Elim	0.01±0.02	0.28±0.6	0.18	10	0.04±0.04	0.28±0.36	**0.01**	18
U out	0.004±0.01	0.24±0.36	0.08	9**	0.06±0.16	0±0	0.17	17**
BM out	0.0±0.0	0.0±0.0	N/A	9**	0.002±0.007	0±0	0.16	17**

* indicates a statistically significant decrease in the behavior from baseline.

** outliers removed from data set (Healthy N=10, FIC N=18)

Table 3

Results of paired t-tests of physiologic measures. Bold type indicates significant results at P = 0.05 level. FIC=feline interstitial cystitis; B=baseline; S=stress; SD=standard deviation; N=number of cats analyzed

			Healthy				FIC			
Measure	**Units**	**Reference Range**	**Baseline Mean ± SD**	**Stress Mean ± SD**	**B v S P-value**	**N**	**Baseline Mean ± SD**	**Stress Mean ± SD**	**B v S P-value**	**N**
leukocyte	(x 10^9/L)	4.0-14.5	8.1±1.7	7.84±1.49	0.62	10	6.87±1.99	6.38±1.7[a]	0.24	18
lymphocyte	(x 10^9/L)	3.0-9.2	2.9±0.74	2.86±1.02	0.98	10	2.48±0.92	1.96± 0.77[a]	**0.009**	18
neutrophil	(x 10^9/L)	0.9-3.9	4.25±1.79	4.24±1.18	0.98	10	3.35±1.18	3.79±1.47	0.40	17**
N:L			1.71±1.25	1.71±0.94	0.99	10	1.61±0.96	2.19±1.22	**0.04**	17**
IL-1β			0.8±0.28	0.67±0.46	0.28	10	0.55±0.34	0.59±0.64	0.74	17**
IL-6			1.51±0.86	0.85±0.49	**0.04***	9***	0.89±0.48	0.68±0.55	0.43	15***
TNF-α			0.6±0.48	0.62±0.52	0.47	10	1.24±0.92[a]	1.10±0.71[a]	0.24	18
cortisol	(ng/ml)	1.0-13.5	5.6±3.3	5.26±2.87	0.72	10	4.52±2.25	3.98±2.14	0.09	18

* indicates a statistically significant decrease from baseline

** outliers removed from data set (Healthy N=10, FIC N=18)

*** removed for missing data

[a] Significantly different from Healthy group under this condition