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Evaluation of environment and a feline facial pheromone analogue on physiologic and behavioral measures in cats

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Abstract

Objectives This study assessed behavioral and physiologic stress parameters in cats placed in two environments: home and the veterinary hospital. With a widely used scale, several parameters were assessed, including respiratory rate (RR), heart rate (HR), systolic blood pressure (SBP), vagosympathetic responses using calculated intervals (heart rate variability [HRV]10, HRV20 and vasovagal tonus index [VVTI]) and 'stress attitude', such as struggling, vocalization and agitation during handling. In addition, we evaluated whether a feline facial pheromone analogue (FFPA) had an effect on any of these measures in either environment.

Methods Using a placebo and a pheromone substance, we evaluated 30 adult and healthy cats at home and in veterinary hospitals. Statistical analyses were performed using the Shapiro–Wilk, Kruskal–Wallis, and Dunn or ANOVA and Tukey tests, as well as Spearman's correlation ($P < 0.05$).

Results We found that exposure to FFPA did not reduce the effects of stress. Some parameters presented differences with regard to environment: the RR was 45 and 70 breaths/min and stress attitude score was 1.3 and 0.0 for cats evaluated at home and at the hospital, respectively. The HR and two vagosympathetic responses were also different between the two environments, with a HR of 160 and 187 beats/min, HRV10 of 14.24 and 14.00, and HRV20 of 14.89 and 14.65 in cats at home and the hospital, respectively. There was no variation in SBP and VVTI parameters between the environments.

Conclusions and relevance Exposure to FFPA does not reduce the physiologic and behavioral changes measured in this study. Furthermore, environmental change, physical restraint and manipulation during the physical examination alter RR, HR, HRV and behavior but not SBP and VVTI. This study is relevant because physiologic and behavioral stress can affect the quality and interpretation of physical examination results. This study presents detailed data that show the effects of environment and manipulation on such parameters. Furthermore, this study shows a lack of effect of FFPA on any of these parameters.

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Introduction

The balance of the sympathetic and parasympathetic nervous system can alter various physiologic parameters, depending on the stimuli imposed upon the organism. Sympathetic discharge increases during stressful events and leads to elevation of the respiratory rate (RR), heart rate (HR) and systolic blood pressure (SBP), as well as a reduction of heart rate variability (HRV) and vasovagal tonus index (VVTI).[1–5]

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Cats are extremely sensitive to manipulations, as well as to changes in environment and normal routine.[6] During a veterinary consultation, cats can react with fear and hostility to stress, such as physical restraint with a strong grip, abrupt movements and loud conversations.[7] As a consequence, the physiologic parameters can change,[6] known as the 'white-coat effect'.[8] These changes can include hypertension, tachycardia, tachypnea and hyperglycemia;[5,9–12] however, they can also be indicative of common diseases, such as hyperthyroidism, diabetes mellitus and chronic kidney disease.[13]

Protocols for reducing the effects of stress on cats during veterinary evaluation have been recommended,[7] such as acclimating the animal to the environment before introducing manipulations,[8,9] using a quiet office that is separated from the normal environment of the veterinary clinic,[7] and reducing or eliminating the odors of other animals and disinfecting products in the area.[8] Other recommendations include the use of a feline facial pheromone analogue (FFPA) on the area or material where the cat will be placed.[7] FFPA manufacturers hypothesize that it simulates the effect of a feline facial pheromone on territorial demarcation and provides a familiar smell and odor to the environment.[14] Therefore, a putative calming effect in stressful environments has been attributed to FFPA, which could reduce anxiety, fear and aggressiveness in cats.[15–17]

This study assessed behavioral (struggling, vocalization and agitation) and physiologic stress parameters in cats based in two environments (home and the veterinary hospital) with the intention of minimizing interference of the clinic during clinical evaluation. We also evaluated whether FFPA has any effect on these parameters in either environment.

Materials and methods

A minimum of 23 cats were required for this study in order to achieve an alpha(α) = 0.05 confidence level at 95% power (1 – beta[β])[18] based on previous studies assessing SBP and HR.[19–21] This allowed us to detect differences of 15 mmHg for SBP and 35 beats/min for HR between the two groups. Therefore, we used 30 cats in this study.

All cats in the study were determined to be healthy, based on physical, electrocardiography, echocardiography and full laboratory evaluations. All owners agreed to participate in this project and signed an agreement and authorization form. The institutional ethics committee approved this study (protocol #240-2012).

The study used a single observer and was placebo controlled and double blinded. The observer, a resident in small animal internal medicine, is a veterinarian with advanced training (MS in Animal Science) and 5 years' experience, and was trained (a pilot study with 60 cats) before the beginning of the study evaluation. In addition to the observer, two other veterinarians with advanced training (MS in Animal Science) assisted with all the manipulations and observations of the cats.

The cats were randomized with respect to the treatment group. Each animal was evaluated in all four groups as follows: home with placebo (HP), home with FFPA (HFFPA), veterinary hospital with placebo (VHP), and veterinary hospital with FFPA (VHFFPA). Evaluations were conducted over 6 days in each environment every other day. Placebo and pheromone were used on alternating days for a total of 3 days each. The Feliway spray product (Ceva) was used as the source of FFPA and 70% ethanol was used as the placebo, as in Griffith et al.[15]

For this study, only houses with a maximum of two cats living together in harmony and without changes in their routine were selected. When evaluations were performed at homes that had two cats, they were evaluated without the presence of each other and in different rooms. Furthermore, all owners confirmed that their cats returned to normal activity the same day after the evaluation, even when stress attitude – struggling, vocalization and agitation – was exhibited.

A standard clinic office (on average, around 9 m^2) was used for veterinary hospital evaluations, and for home evaluations the room in the home (also, on average, around 9 m^2) that the animal liked best was chosen. Although the office was previously used by other animals, it was thoroughly cleaned between appointments to minimize other animal odors, as well as to eliminate the previous substance applied (FFPA or placebo). Furthermore, when two cats were evaluated in the veterinary hospital on the same day, two different offices, but with the same architecture, were used, taking a minimum of 24 h between introducing another cat in the study into the office. Prior to starting the evaluation, the room was prepared with the randomly selected substance each day, based on the methodology described by Gunn-Moore and Cameron,[16] applying the substance on every protuberant object and on sites with a depression, always keeping the distance of the spray nozzle from the object (10 cm) and from the floor (20 cm). Fifteen minutes later the cat was introduced to the environment so that the alcohol had already evaporated. During the first 10 mins the animal was acclimated to the environment and no manipulations were performed. Evaluations were then initiated beginning with the least stressful (with minimal contact and physical restraint) and ending with the most stressful. All evaluations were performed in the same order: RR, HR, SBP and electrocardiography, followed by behavioral classification during all manipulations, as in Quimby et al.[5]

RR was measured without manipulations and HR was assessed while using minimal physical restraint of the cat. For the SBP measurement, the cat was positioned in right lateral recumbency and an indirect method using

Table 1 Respiratory rate (RR), systolic blood pressure (SBP) and behavioral score evaluated in different environments and with exposure to a feline facial pheromone analogue, expressed as median (25% percentile – 75% percentile)

Parameters	Home placebo	Home pheromone	Hospital placebo	Hospital pheromone	*P*
RR (breaths/min)	45 (35–50)[a]	43 (37–52)[a]	70 (46–105)[b]	73 (47–85)[b]	<0.0001
SBP (mmHg)	134 (120–151)[a]	136 (123–147)[a]	133 (122–142)[a]	131 (117–138)[a]	0.6090
Behavioral score (0–3)	1.3 (0.9–1.7)[a]	1.3 (1.0–1.7)[a]	0.0 (0.0–0.7)[b]	0.0 (0.0–0.7)[b]	<0.0001

Data are median (25th–75th percentile).Values followed by distinct letters in the same line significantly differ from each other ($P < 0.05$), based on Dunn's test

Table 2 Heart rate (HR), heart rate variability with 10 R-R intervals (HRV10), heart rate variability with 20 R-R intervals (HRV20) and vasovagal tonus index (VVTI) in cats in home and veterinary hospital environments with exposure to placebo or a feline facial pheromone analogue

Parameters	Home placebo	Home pheromone	Hospital placebo	Hospital pheromone	*P*
HR (beats/min)	161 ± 22[a]	158 ± 21[a]	189 ± 26[b]	185 ± 20[b]	<0.0001
HRV10	14.17 ± 0.34[ab]	14.24 ± 0.34[a]	14.05 ± 0.29[ab]	14.00 ± 0.35[b]	0.0450
HRV20	14.82 ± 0.33[ab]	14.89 ± 0.33[a]	14.69 ± 0.29[ab]	14.65 ± 0.33[b]	<0.0414
VVTI	4.90 ± 1.24[a]	5.04 ± 1.04[a]	4.64 ± 1.00[a]	4.57 ± 1.32[a]	0.5122

Data are mean ± SD. Values followed by distinct letters in the same line significantly differ from each other ($P < 0.05$), based on Tukey's test

a vascular Doppler (Parks model 841 with infant flat probe) connected to headphones was used. For electrocardiography, the animal was restrained in right lateral recumbency and the electrodes of a multichannel digital electrocardiograph system (TEB ECGPC VET) were positioned according to the recommendations of Tilley.[22] The values obtained from consecutive R-R intervals were analyzed using three different methods to evaluate vagosymphathetic balance. To evaluate HRV, a formula described by Carareto et al was used,[3] with either 10 consecutive R-R intervals (HRV10) or 20 consecutive R-R intervals (HRV20) as follows:

$$HRV = \log_e \left(\frac{n\sum_{i=1}^{n} X_i^2 - \left(\sum_{i=1}^{n} X_i\right)}{n(n-1)} \right)$$

where $\log_e$ = natural logarithm (naperian); n = number of R-R intervals evaluated; and x = R-R intervals (in ms). To obtain VVTI, we adopted the formula described by Doxey and Boswood,[1] using values from 20 consecutive R-R intervals:

$$VVTI = \log_e \left(sd_{R\text{-}R} \right)^2$$

where $\log_e$ = natural logarithm (neperian) and $sd_{R\text{-}R}$ = SD of R-R intervals.

All data were evaluated for normality using the Shapiro–Wilk test. For data with non-parametric distribution the Kruskal–Wallis and Dunn's tests were used; for data with normal distribution ANOVA and Tukey's tests were used. Spearman's correlation was used to determine a correlation between SBP and HR, as well as SBP and behavior. For all tests, the results were considered significant when $P < 0.05$. Descriptive statistics were used to evaluate qualitative data.

Results

Thirty adult cats were evaluated (15 male, 15 female; mean age 3.5± 2.8 years; mean weight 4.6 ± 0.9 kg), of which 27 (90%) were domestic shorthair and three (10%) were Persian.

The median, 25th and 75th percentile values for RR, SBP and behavioral scores obtained in each group are described in Table 1, and the mean and SD of HR, HRV10, HRV20 and VVTI are described in Table 2.

The measured RR was 25 breaths/min higher in cats evaluated in the clinical environment compared with those at home, which was a statistically significant difference. Moreover, tachypnea was observed in all groups. HR differed according to the environment, with cats in the hospital exhibiting a HR that was 28 beats/min greater than in those at home. However, despite the elevated HR, no animal presented with tachycardia during the evaluation. Importantly, no differences in either parameter were found when animals were exposed to FFPA.

SBP did not differ between environments or with FFPA exposure. In both environments, some animals presented elevated blood pressure; however, the medians of each group remained within a normal range. Systemic hypertension could be found if evaluating cats' blood pressure alone in this study. Using the assessment of risk of end-organ lesions secondary to systemic arterial hypertension,[9] 23 animals at home and 27 animals at the hospital were classified as minimum risk, three animals at home and one at the hospital were classified as mild risk, three animals at home and one at the hospital were classified as moderate risk, and one animal at home and one at the hospital were classified as severe risk. In addition, because of day-to-day blood pressure variation, the recommended 3 day evaluation was performed,[8] and we found no significant differences in SBP in cats from the HP ($P = 0.9843$), HFFPA ($P = 0.9914$), VHP ($P = 0.9292$) and VHFFPA ($P = 0.9623$) groups on any of the days for each group.

The scores for exhibited stress behavior only differed between environments, with higher scores observed in cats at home. We found that 29 cats at home and 13 cats at the veterinary hospital exhibited one of the stress behaviors analyzed. Struggling and vocalization were the most frequently observed stress behaviors during the evaluations. Twenty-five animals at home and eight at the hospital exhibited struggling, whereas 22 animals at home and six at the hospital exhibited vocalization. Agitated behavior was observed in 15 animals at home and in six at the hospital.

There was no correlation between HR and SBP ($r = –0.15$; $P = 0.0942$), but there was a positive correlation between SBP and stress behavior ($r = 0.39$; $P < 0.0001$; $Y = 0.01332*X – 0.9465$).

A difference was observed in the HRV between groups, with a higher HRV being observed in cats at home compared with those in the hospital environment. However, the environment and FFPA exposure did not change the VVTI.

Discussion

During hospitalization, several procedures are often used to reduce environmental stress for a cat, such as reducing strange odors and applying FFPA to the cage. FFPA is postulated to promote assurance of a comfortable place with a familiar odor for the animal and reduce anxiety, as this substance is hypothesized to mimic a cat's natural pheromones.[15] In this study, we used FFPA before evaluating several physiologic parameters of cats in both a home and hospital environment. However, the use of FFPA did not have an effect on RR, HR and SBP, or stress behavior during manipulation, HRV and VVTI in this study.

In a study by Kronen et al,[17] FFPA combined with preanesthetic medication helped to promote calmness in cats, but during manipulation and physical restraint for catheterization, struggling was not reduced. The findings of our study indicate that the use of FFPA had no effect on any measure of stress-related behavior or physiology, which corroborates previous findings.[17]

An FFPA has been used in many situations where cats were in stressful situations and developed behavioral and physiologic changes.[15,16] However, the use of FFPA alone as an exclusive stress reducer is not recommended, and current best practices include removing or minimizing other environmental stress factors.[7] In this study, noises and odors near the veterinary office, as well as the movements of employees, were not reduced. Although FFPA was the only method used to reduce stress in the hospital environment, we found it to be ineffective.

The increased RR and HR in cats placed in a hospital environment in our study is thought to be related to exposure to stressful situations, such as different odors, noises and people, as an increase in physiologic parameters can be influenced by norepinephrine and epinephrine action in response to acute stress.[6]

Similar to observations made by Quimby et al,[5] routine changes in both environments could have influenced the increase and maintenance of RR above normal levels,[23] and changes to a cat's routine have been shown to be associated with stress development.[16] Furthermore, the higher RR in cats in the hospital compared with home suggests that changes in routine in the home environment promoted less stress potential and consequently less sympathetic discharge. Another factor that could have influenced the increased RR was a bad experience in previous exposure to the veterinary hospital, which may have been recorded in the animal's memory regarding the environment.

Higher HR in the hospital environment has also been observed by Abbott and Quimby et al when comparing the HR of healthy cats in home and hospital environments.[2,5] Exposure of cats to different environments and objects could induce tachycardia as a fear response.[10] Therefore, for cats in this study, although the HR elevation did not exceed reference intervals,[23] the difference observed between the two environments indicates higher stress potential for cats in the hospital relative to home.

The acclimation period in both environments could have contributed to the HR value remaining within normal limits during the evaluation. Belew et al found that 10 mins of acclimation substantially reduced cats' HR by reducing the white coat effect syndrome.[8] Therefore, although tachycardia may have presented as a stress response initially, the acclimation period before the evaluation likely reduced the HR to normal values.

A previous study by Schenberg et al found that although a fight or flight reaction in rats elevates HR at

the beginning of a stress situation, the HR progressively reduces to normal values over a short time frame, even if the stress factor is maintained.[24] Therefore, despite being a different species, the previous study in rats suggests that fear of change can initially promote a higher HR, followed by a decrease thereafter, similar to what was observed in cats by Belew et al.[8]

Based on the study by Belew et al it was possible to identify a 30 mmHg elevation in SBP in cats introduced to a new environment;[8] however, after 10 mins of acclimation the SBP decreased by 20 mmHg. Furthermore, the white coat effect syndrome can lead to individual variation between days, suggesting that serial measurements using the same protocol must be performed in order to obtain a more accurate estimate of SBP in cats. In this study an acclimation time of 10 mins was utilized and we conducted evaluations on three different days for each group. Using this method we found no differences in SBP between days in each group.

Similar to Quimby et al,[5] we found that the SBP in all groups was in the minimum risk classification of end-organ lesions.[9] However, we did not observe differences in SBP between environments, which was in contrast to observations described by Quimby et al,[5] where higher values were obtained in the hospital setting. Despite these differences, no clinical relevance was observed, as the mean difference of 6 mmHg in SBP as measured by the indirect vascular Doppler method was below the known variation of ± 10 mmHg when using this method.[9]

Moreover, individual analyses found that some cats had SBP elevations that were considered to be a mild risk, moderate risk or severe risk in both environments,[9] suggesting that hypertension can be induced by stressful situations in healthy cats.

Animal manipulation required for obtaining SBP measurements using an indirect vascular Doppler method can induce stress, which consequently increases blood pressure.[5,8,25] In this study, the SBP obtained in cats from both environments was similar, indicating that the physical restraint and manipulation procedure only had a moderate influence on the SBP of cats in both environments.

Stress behaviors observed during manipulation occurred more frequently in cats in the home environment, which corroborates the findings by Quimby et al,[5] showing that cats have less tolerance to manipulation in their own environment. Struggling and vocalization behaviors are associated with sympathetic activation due to stress, which increases epinephrine, norepinephrine, glucose and cortisol levels.[12,26] In this study the most frequently observed stress behaviors were struggling and vocalization. Moreover, there was a positive correlation between stress behavior and SBP, suggesting that stress behavior has some influence on sympathetic system activation.

It was reported that stimulation of some specific brain sites can promote behavioral changes and that constant stimuli can promote habituation with progressively decreasing behavior or can produce an experience for the animal that could demonstrate defensive behavior towards the unexpected. After brain stimulation, the behavioral changes can persist for hours or even days.[27] Besides the several days of evaluation, in our study cats did not present habituation to the stress stimuli imposed and, as all cat owners reported that their cats returned to normal activity on the same day of the evaluation, the stress behavior persisted for only a few hours. Based on that, we do not believe that multiple days of evaluations promoted a persistent stress behavior in these cats.

In a study by Abbott,[2] higher HRV was observed when cats were at home compared with the hospital. Moreover, Hanas et al found with Holter monitoring that cats at home commonly presented a respiratory sinus arrhythmia,[21] demonstrating higher vagal influence at home. In this study, the HRV was different between environments, with greater variation observed in cats at home. The higher HRV observed in cats at home suggests a higher tendency for the parasympathetic system to overlap with the sympathetic system, thus corroborating the results of Abbott and Hanas et al.[2,21]

Although the stress behaviors observed in cats at home should have had a higher sympathetic influence, the HRV was evaluated 1 min and 30 s after the electrocardiograph recording was initiated to reduce the influence of manipulation stress. This allowed for a better evaluation of the environment's influence on vasovagal balance, even though the stress from permanent physical restraint during the examination still occurred, as observed by Abbott.[2]

The VVTI in the cats from this study did not differ between environments, although higher variations were observed in animals at home, demonstrating that this technique is less sensitive than HRV for detecting small changes in the vasovagal balance of cats. According to Doxey and Boswood,[1] VVTI provides a good representation of HRV due to respiratory influence, in which longer periods of inspiration result in higher values of VVTI. In both environments, the RR remained above normal values for the species; therefore, the tachypnea that presented in all groups may have been less influential on VVTI.

Exposure to FFPA during a situation that mimicked a veterinary consultation both at home and at the hospital had no influence on RR, HR, SBP, HRV, VVTI, or stress behavior in cats in this study. In the hospital environment, there was an increase in RR and HR, while at home we observed higher HRV and higher scores of stress behavior. Moreover, SBP and VVTI were not altered by the stress of environment change.

One limitation of this study was the lack of cortisol concentration measurements, which could have provided an expanded profile of sympathetic activation.

Conclusions

Based on data collected in this study, we conclude that exposure to FFPA does not reduce physiologic and behavioral changes caused by stress in these specific situations. Furthermore, environmental change, physical restraint and manipulation during a physical examination alter some physiologic parameters, such as inducing higher RR and HR as well as lower HRV. These stress stimuli were also found to increase stress behaviors, such as vocalizing, struggling and agitation.

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