The effects of operant training on blood collection for domestic cats

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A B S T R A C T
The impact of operantly training domestic cats to accept jugular blood collections in a dor-sally recumbent (novel) position was assessed. Cats were assigned to one of three groups: Group 1 (N = 14): no training, traditional jugular blood collection; Group 2 (N = 17): trained in novel position but traditional blood collection method used; Group 3 (N = 15): trained in novel position and new blood collection method used. The impact of handler was assessed by testing each cat twice, once with a familiar and once with an unfamiliar person, one week apart. For each test, cats received two venipunctures 20 min apart. Blood samples were analyzed for cortisol levels with collection one serving as the initial stressing event/baseline and collection two serving as test/change from baseline. All instances were filmed and coded for behavioral signs of stress. Cats displayed significantly more escape attempts with the unfamiliar than the familiar handler (P < 0.01). Paired comparison with Bonferroni adjustments showed that Group 3 took significantly longer to position than Group 2 (P < 0.01) and Group 1 (P < 0.04), but overall took the same amount of time to complete blood collections. There was a significant difference between heart rates (beats per min; bpm) at release between groups (P < 0.01). Group 3 had lower heart rates when released than Group 2 (P < 0.01) and Group 1 (P < 0.01). This suggests that the trained/recumbent cats showed the least physio-logical reaction to the blood collection. Trained cats, despite method or familiarity with handler, showed lower cortisol levels (μg/dL) when the procedure was repeated (P < 0.02). Cortisol levels did not differ significantly at baseline on either day or between groups on day one. There was a significant difference in cortisol levels between groups on day 2 where Group 1 had significantly higher mean test cortisol concentrations when compared with Group 2 (P < 0.01) or Group 3 (P < 0.02). Operant training to blood collections appears to have a positive impact on the cat’s experience whether a traditional or novel position is used. These results support the use of operant training to improve the overall blood collection experience for domestic cats.

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1. Introduction

High levels of acute stress can significantly affect blood parameters in otherwise healthy cats (Rand et al., 2002). These stress-induced hormonal changes can compromise the quality and validity of the blood sample as a reliable health marker. When used in research settings, it is vitally important that blood samples reflect the true status of the animal in order to make appropriate conclusions. Domestic cats tend to show higher levels of reactivity to this procedure, specifically due to restraint, than other companion animals used in research. These reactions can include behaviors such as loud vocalizations, clawing, and attempting to escape from restraint. These behaviors have been shown to indicate that cats are experiencing high levels of stress (Archer, 1979; Rand et al., 2002). In addition, an animal that is attempting to escape during a blood collection puts itself and staff at risk of injury.

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Quinby et al. (2011) were able to show that the veterinary visit in and of itself caused significant physiological changes due to stress. These changes included increased blood pressure, rectal temperature, heart rate, and respiratory rate. When these measures were performed in home, the results were in keeping with a cat under lower stress (i.e., all parameters were lower when taken in home versus the clinic setting). This indicated that the environment in which the tests were performed had a significant impact on stress; thus the more familiar the environment the lower the cat stress levels during health exams.

In 2005, the American Association of Feline Practitioners (AAFP) established the Panel on Feline Behavior Guidelines and released a special report on the appropriate care and handling of cats in order to prevent or eliminate potential behavior issues (Overall et al., 2005). These guidelines specifically call out the healthcare experience for young cats and strongly suggest acclimating young cats to standard practices. In addition, these guidelines discuss the stress effects of unpredictable and unfamiliar handling on the development of behavior issues in cats.

The socialization period for kittens to humans is most sensitive from three to seven weeks of age. Socialization that occurs before nine weeks of age results in lasting changes to behavior. Kittens that are handled in positive ways by humans during this developmental window will persist in being highly social and friendly to humans into adulthood (Karsh, 1983; Karsh and Turner, 1988; Lowe and Bradshaw, 2001). This window of opportunity allows handlers to provide training that has a significant impact on the overall behavior of the adult cat. Kittens are very trusting and impressionable during this developmental stage and can form lifelong reactions to events based on experiences during this period.

Knowing that high levels of stress can negatively and significantly impact the quality of a blood sample collected from cats, a blood collection method that specifically sought to reduce the stress associated with this procedure was developed. Given that the actual event of a routine health exam can be minimized when performed in a familiar setting and that early exposure to standard healthcare practices can decrease the stress associated with those procedures, we designed a blood collection technique that took advantage of familiar settings and incorporated early exposure to typical handling during healthcare exams. We hypothesized that training kittens to remain in a technician’s lap while the blood collection is performed using systematic desensitization and operant conditioning would reduce stress and improve welfare. We expected the impact of this early experience to last well into adulthood for each animal because the training was started during the kittens’ socialization period.

2. Methods

This study was approved by the Institutional Animal Care and Use Committee at Proctor & Gamble Pet Care Division. All work took place at the Proctor & Gamble Pet Health and Nutrition Center, Lewisburg, OH, USA.

2.1. Animals and housing

A total of 46 cats (18 females, 28 males) aged 1–6 years was included in the study. Two cats were removed from the study. One female cat was removed from Group 1 on the first day of collections due to aggression during restraint. One male cat was removed from Group 3 on the first day of collections due to an unrelated health issue. This resulted in a total of 44 cats (17 females, 27 males) completing the study.

Cats were maintained in a cage-free environment in mixed-sex groups of 10–12 individuals. All cats had been surgically sterilized between the ages of 6–8 months. Cats were provided with water ad libitum and fed once a day. Food bowls remained accessible for 24 h but were only filled in the morning. Cattery rooms offered windows to hallways, neighboring rooms, and outdoors. In addition, cats had access to covered, fenced-in, outdoor porches for 8 h each day. The ambient indoor temperature was maintained at 20 °C. Although windows provided natural light cycles, the indoor fluorescent lights came on at 0630 and were on a 12 h light/dark cycle.

Regular patterns of enrichment and socialization were maintained throughout the study. Each cat room contained enrichment items including mounted shelves on walls and by windows, mounted ‘mail boxes’ on walls, hiding spaces on the floor, hammocks outside, and cat swings inside. In addition, a number of non-supervised toys were scattered through-out the room. These items included durable balls and tops. A minimum of three scratching posts were placed in each room. All cats received daily interaction with people for a minimum of 20 min each day. During this time, cats had access to supervised toys. These items included feather cat dancers, crinkle sacks, crinkle balls, and small toy ‘mice’.

2.2. Blood collection procedures

Cats were separated into three groups based on training experience and type of blood collection performed. Group 1 (Untrained, Table method) consisted of 14 cats (seven females, seven males) with an age range of 3–6 years (median age 4). Group 1 cats had never received any training in the new blood collection procedure and had their blood collections performed on the table. Group 2 (Trained, Table method) consisted of 17 cats (five females, 12 males) with an age range of 1–2 years (median age 2). Group 2 cats had been trained in the new method but had their blood collections performed on the table. Group 3 (Trained, Lap method) consisted of 15 cats (six females, nine males) with an age range of 1–2 years (median age 2). Group 3 cats had been trained in the new method and had their blood collections performed in the lap.

This is a very novel blood sampling technique and requires a large amount of upfront work. The risk to both personnel and animal in attempting to use this method in an untrained cat is significant. If the cat was to react negatively while in the technician’s lap the animal could scratch/bite the technician or the needle could ‘slip’ and cause damage to the cat. There was no safe way to attempt this work with untrained animals in the novel position.
Two different blood collection procedures were used in this study. The traditional (table) method required one technician to perform the blood collection while one or two other technicians restrained the cat. During restraint, the technician would place the cat on a treatment table, extend the cat’s front legs down over the side, and stretch the cat’s head upwards to expose the cat’s jugular furrow for collection. If the cat showed high levels of resistance, a third technician would assist in restraining the body and back leg portion of the cat. This method requires a minimum of two technicians: the first to fully restrain the cat and the second to perform the actual blood collection. The trained (lap) method required a single technician to position the cat on its back and perform the blood collection. The technician would use one hand to lift the cat’s head and expose the jugular furrow while taking the blood sample with the other hand. This method required minimal restraint and only one technician to position the cat and collect the blood sample.

2.3. Technicians

The familiar technician was the person responsible for conducting the initial training with all the cats. She was also the daily veterinary technologist in the area where these cats were housed. The unfamiliar technician was a licensed veterinarian and responsible for performing required veterinary care for the cats housed in this area. Both people performing the blood collections had an equal amount of time in practice and would be considered experts at blood collections in cats.

2.4. Training

All cats selected for this study, except for those in Group 1, had previously completed training. Cats were selected for the trained groups (Group 2 and Group 3) based on successful completion of the training program. The training program started when the cats were 3 weeks of age. All kittens were exposed to the smell and feel of alcohol, the sound and feel of the clippers, the experience of lying on their backs, and the feel of a small needle stick. These steps were followed in progressive order once a week for approximately 6–12 weeks. During this time kittens were monitored for signs of stress or reactivity to any portion of the blood collection experience. If they appeared stressed the training was altered to allow the kitten time to adjust to the procedure.

Once kittens were considered trained (a veterinary technician successfully performed a jugular blood collection on the cat in the new position) then training was reduced to once a month. Once cats reached 1 year of age, training was again reduced to once a quarter. The reinforcement training sessions typically did not involve shaving of the fur, but bladeless clippers were used to perform a sham neck shave. This exposure was designed to maintain the trained behavior.

2.5. Procedure

On the day of collections cats were placed in large rolling ProSelect® Foldable Cat Cages (Pet Edge, Beverly, MA, USA) measuring 90.17 cm L × 60.96 cm W × 121.92 cm H (35.5 in L × 24 in W × 48 in H). No more than four cats were placed in a single cage and all cats were familiar with their cage mates (i.e., only cats from the same room were placed together in the temporary enclosures). These enclosures were a routine part of husbandry and were used to temporarily house cats while animal care technicians cleaned the rooms. These cages were kept in hallways which allowed cats increased exposure and interaction with staff.

Cats had blood collections performed based on the order in which they could be removed from the cage. All blood collections were performed in a separate treatment room familiar to all cats. All cats had pre- and post-sample heart rates taken for each blood collection via auscultation. Each cat received an initial blood collection using the designated method. This served as both the baseline and stressing event for cortisol measures. The cats were then returned to the holding cage. After 20 min the blood collection was repeated and the resulting sample was compared back to baseline to assess the impact of the blood collection. Cats were then returned to their primary enclosures after the second sample was collected. After 7 days the procedure was repeated with the opposite handler. All cats were counterbalanced between a familiar and unfamiliar handler.

2.6. Measurements

Blood samples were taken using 22 ga Vacutainer Needles VT7210 (VWR International, LLC, Batavia, IL, USA) and collected in 3.5 mL SST Vacutainer Tubes BD367983 (VWR International, LLC, Batavia, IL, USA). All samples were spun in 2.0 mL Conical Upright Centrifuge 20170–710 (Bulter Schein Animal Health Supply, Dublin, OH, USA) with BioPlas Caps 20170–770 (Bulter Schein Animal Health Supply, Dublin, OH, USA) at 2200 rpm for 30 min. All samples were immediately frozen until ready for analysis.

Frozen blood serum samples were thawed and analyzed for cortisol content as a batch using an ELISA analysis Cortisol Assay Kit (KGE008, R and D Systems Inc, Minneapolis, MN, USA). Each sample was run neat and, if results were outside of the range of the assay, they were diluted and reanalyzed.

All blood collections were videotaped in real time at 30 frames/s using a high definition digital camera (Sony HD Handycam model HDR-UX1, Tokyo, Japan) and recorded to disks (Sony DVD-RW 1.4 GB single sided minidisks model DMW30L2, Taiwan). All videos were later scored for the following items: time to first needle insertion, total time of blood collection, number of attempted bites, number of contact bites (that did not break skin), number of escapes, number of scratches, presence or absence of negative vocalization (e.g., growling) (Crowell-Davis, 2005), presence or absence of positive vocalization (e.g., meowing) (Crowell-Davis, 2005), number of restrainers, change of restrainers, number of needle insertions, number of attempts to ‘search’ for veins post needle insertion, accepts shaving (yes/no), the presence of tail flicks (yes/no), and quality of demeanor overall and at release (5 point Likert scale; 1 = positive, 5 = negative). A composite “escape behavior” score was created as the sum of attempted bites, contact bites, escapes,
Table 1
The effect of technician familiarity on cat behavior response and heart rate to blood draws.

<table>
<thead>
<tr>
<th>Technician</th>
<th>Escape behavior*</th>
<th>Demeanor*</th>
<th>Release demeanor*</th>
<th>Heart rate (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LSM</td>
<td>SE</td>
<td>LSM</td>
<td>SE</td>
</tr>
<tr>
<td>Familiar</td>
<td>0.49*</td>
<td>0.20</td>
<td>2.22*</td>
<td>0.16</td>
</tr>
<tr>
<td>Unfamiliar</td>
<td>1.23*</td>
<td>0.20</td>
<td>2.75*</td>
<td>0.17</td>
</tr>
</tbody>
</table>

* Escape behavior was based on a total sum of attempted bites, contact bites, escapes, and scratches; demeanor and release demeanor were scored on a five point Likert scale.

and scratches and used in data analysis. A trained scorer that was familiar with cat behavior coded the videos.

The demeanor scores for overall and at release were generated by a single scorer based on review of the recorded sessions. For the overall demeanor score, the observer was to take into account the level of comfort the cat demonstrated when it entered the room, the ease with which the technician could handle the cat, how readily the cat was placed in position, the level of compliance demonstrated by the cat, and the absence (or presence) of signs of tension in the cat’s body language. For the demeanor at time of release, the observer was to take into account the reaction of the cat when released from restraint. Lower scores were given to cats that demonstrated distance seeking behaviors, displayed tense body positions, and demonstrated signs of discomfort.

Cats were eliminated from the study if they posed a risk to personnel safety (e.g., bit hard enough to break skin) during blood collections or for any health reasons that made them ineligible to participate.

2.7. Statistical analysis

All data were analyzed using SPSS MIXED procedure. This procedure uses a Satterthwaite estimate for error degrees of freedom. A mixed linear model ANOVA using compound symmetry as the covariance matrix and repeated measures within technician and collection number was used to analyze continuous variables. Data were tested for differences between technicians, between blood collections, and among the three treatment groups. All pairwise comparisons used Bonferroni adjustments.

The heart rate and cortisol data were analyzed using a mixed linear model ANOVA using compound symmetry as the covariance matrix and repeating measures within technician. Initial heart rate was used as a covariate in the statistical model.

Normality of residuals was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. An alpha level of 0.05 or below was required to determine significance. Unless stated otherwise, means are reported as least square means with associated standard error (LSM ± SE).

3. Results

3.1. Technician effects

There were no significant differences between technicians for number of attempts to perform a blood collection or total time for collecting a blood sample (P > 0.05 for all comparisons). Escape behavior was defined as the total sum of the number of attempted bites, contact bites (that did not break skin), escapes, and scratches.

There was a significant effect of cat’s familiarity with the technician for escape behaviors (F₁₉₹.₈₇ = 14.64, P < 0.01), demeanor (F₁₉₀.₈₅ = 9.03, P < 0.01), and release demeanor (F₁₉₄.₁₂ = 4.62, P < 0.03, Table 1). In general, greater familiarity with the handler resulted in fewer escape behaviors, more positive overall demeanor, and a more positive demeanor when released from either form of restraint (Table 1). There was no significant difference in cat heart rates between the two technicians (P > 0.05, Table 1).

3.2. Training effects

There was a significant effect of group on escape behaviors (F₂₆.₂₇ = 4.49, P < 0.02). When compared between groups, Group 2 (0.196 ± 0.27) had significantly lower scores than either Group 1 (1.03 ± 0.31, P < 0.05) or Group 3 (1.36 ± 0.31, P < 0.007). There was no significant difference in scores between Group 3 and Group 1. However, this may be due in large part to the fact that cats in Group 3 were free to move away from the technician until the blood collection was actually taking place. Only Group 2 (trained/table) and Group 3 (untrained/table) had blood sampled in a similar fashion making any difference between these two groups due to training.

There was a significant difference between groups for general demeanor (F₂₆.₄₄ = 4.17, P < 0.02) and release demeanor scores (F₂₆.₃₅ = 4.28, P < 0.02) respectively. Group 2 cats showed more favorable demeanor (1.95 ± 0.22) and release demeanor (1.75 ± 0.24) than Group 1 (2.70 ± 0.25, P < 0.03; 2.50 ± 0.28, P < 0.03) or Group 3 (2.81 ± 0.25, P < 0.01; 2.77 ± 0.28, P < 0.01), respectively.

There was a significant effect for treatment group for time to first stick (F₂₆.₉₇ = 6.76, P < 0.01). It took significantly longer to perform the initial needle insertion for Group 3 (46.53 ± 4.66 s) than Group 1 (33.26 ± 4.12 s, P < 0.04) or Group 2 (24.75 ± 3.67 s, P < 0.01); however, the overall time required to collect a blood sample was not significantly different between any groups (P > 0.05). In other words, the overall amount of time to position cat, draw sample, and release cat from restraint was no different between methods despite the differing initial time requirement to first insert the needle.
3.3. Heart rate and cortisol levels

There was a significant difference in heart rates based on group ($F_2, 17.66 = 8.99$, $P < 0.01$, Fig. 1). Heart rates were significantly lower for Group 3 (178.79 ± 7.22 bpm) than Group 1 (208.39 ± 4.99 bpm, $P < 0.01$) or Group 2 (217.29 ± 4.91 bpm, $P < 0.01$).

On the first day of collection, there were no significant differences between baseline and test cortisol levels for the three groups ($P > 0.05$). When the procedure was repeated 7 days later, there was a significant difference in test cortisol levels based on group ($F_2, 15.07 = 4.703, P < 0.02$, Fig. 2). When compared to Group 1 (Untrained/Table: 2.66 ± 0.63 μg/dl), both Group 2 (Trained/Table: 0.35 ± 0.54, $P < 0.01$) and Group 3 (Trained/Lap: 0.13 ± 0.80, $P < 0.02$) had significantly lower spikes in cortisol when undergoing the blood collection procedure.

4. Discussion

The purpose of this study was to determine if the training process for the blood collection procedure actually resulted in a reduction in stress surrounding this event and any improved efficiencies in conducting blood collections for routine health care. In the current study, both behavioral and physiological measures were used to assess the level of stress experienced during the blood collection procedure. In addition, all blood collections were timed to first needle insertion and overall time to collect a blood sample. Our results show that the training has a long-reaching positive effect on the blood collection experience both to the cats directly and to the technicians in terms of efficiency.

Reichard et al. (1993) previously demonstrated that training for veterinary procedures resulted in decreased stress and increased efficiencies in collecting samples in non-human primates and other zoo animals. Our results seem to parallel this finding in that trained cats demonstrated lower cortisol spikes on the second round of collections. In addition, the cats that were sampled using the new method required the same amount of time to sample, but only one person to perform the venipuncture. This resulted in a savings of 50% in time budget regarding staff.

Due to the fact that all kittens on-site received training in the new method, we had to use older cats for the non-trained/traditional group (Group 1). Anecdotally, we have had limited success training older cats to the new procedure which means that it is vital that all kittens start training at a young age. Our facility has chosen not to delay training, which means that the only ‘control’ group we could create would be older than our test groups. However, the control group had had previous experience with venipuncture (due to routine health care) which meant that the procedure itself was not novel. Any changes in reaction to venipuncture both behaviorally or physiologically would be assumed to be the result of systematic training. In addition, we selected cats for Groups 2 and 3 based on current housing which prevented selection based on sex distribution. However, the trained cats were randomly assigned to Groups 2 and 3. In other words, two cats could live in the same room but had equal chances of being placed in Group 2 versus Group 3. The social housing rooms on-site are not sex-balanced due to the fact that we stabilize animals prior to sexual maturity; rather cats are selected based on temperament and goodness of fit with the other members in the room.

The level of familiarity the cats had with the technicians had a significant impact on the level of stress displayed by the cats. The fact that the familiar technician experienced fewer escape attempts and over-all more positive demeanor from the cats seems to be due to the level of familiarity the cats had with this person. Both the familiar and the unfamiliar technician were highly experienced in handling cats, and were equally skilled at blood sampling (there was no significant difference between the familiar or unfamiliar technician in number of attempts to collect samples). The resulting differences between the technicians appear to be based on the cats’ reactions to the individuals rather than a difference in skill sets between individuals. It is assumed that there was a higher level of ‘trust’ between the cats and the familiar technician which led to a decrease in attempting to escape and an overall more positive attitude during handling.

Although the amount of escape behaviors was highest for Group 3 (Trained/Lap), this may be confounded by the fact that this was the only group that received minimal restraint. One of the components of escape behaviors was number of times the cat escaped or moved free of the...
restraint/placement. The cats in this group were in a position to freely move out of the technician’s lap until the blood collection was actually performed.

A more direct comparison of the impact of training is between Group 1 (Untrained/Table) and Group 2 (Trained/Table). Both of these groups experienced the same amount of restraint and a blood collection in a similar fashion. Group 2 (Trained/Table) had the lowest score for escape behaviors of the three groups. This group had been trained to receive blood collections in the technician’s lap, and, prior to this study, had never received a blood collection in the traditional manner. The fact that this group had the lowest score for escape behaviors indicates that the training had a significant generalized impact on reducing the overall stress experienced during a blood collection. When compared to Group 1 (Untrained/Table) it is clear that the trained group was more accepting of the procedure even though the actual procedure was unfamiliar. The cats in Group 2 had been desensitized to all components of the blood collection process (sights, smells, sounds, etc.). This desensitization appears to have generalized to blood collection performed using alternate methods (i.e., table versus lap).

The results from the physiological measures also indicate a significant reduction in stress. The heart rate data indicate that Group 3 (Trained/Lap) had the lowest stress reaction to the process as compared to the other two groups. One possible reason for the increased heart rates in Group 2 (Trained/Table) is that this was the first time they had their blood sampled in this method. This group of cats had never been restrained in this manner for a blood collection. Given that Group 2 (Trained/Table) had significantly fewer Escape Behaviors and that they maintained a generally positive demeanor during and after the blood collection indicates that the novelty to the procedure may have been the stress inducing element. However, when the cortisol results are taken into account, it appears that both trained groups were experiencing significantly less stress than the untrained group especially when the procedure was repeated.

The current study design used a control group of cats that received similar handling and sample collections, but did not receive any of the training. All cats were sampled at the same time of day on the same day of the week in order to help control for natural circadian shifts in cortisol levels. Based on these precautions, total changes in cortisol from baseline to test were assumed to reflect the amount of stress dissipated by training for venipuncture samples (Hart, 2012).

The cortisol results showed no significant differences on the first day, but when the process was repeated a week later, both trained groups showed a significant decrease in test cortisol levels. This same decrease was not seen in the untrained group. This indicates that the stress of the event had been tempered by training. If the blood collection method itself was the reason for reduced stress effects, then the two groups sampled using the table method would have had similar cortisol levels on all occasions. However, the reduced cortisol response on the second day of collections for the two trained groups (sampled in two different fashions) indicates that the training had prepared the cats for the blood collection procedures despite sampling method. This training may have increased the predictability of the procedure thus, when the process was repeated, the significant impact of training was evident in the cortisol measures.

Currently, the use of operant conditioning to train non-human animals to voluntarily participate in husbandry and veterinary procedures is considered a humane process that reduces stress and improves welfare (Owen and Amory, 2011). However, one of the primary reasons for resistance to implementing a training program, such as the one used in this study is cost/time investment. Most training programs do require staff to invest additional time up-front to receive benefits in the future. The majority of training for this method is conducted as part of the routine socialization practice for raising kittens on-site. The socialization practices have been increased to include this training and now require an additional time investment for each kitten; however, the results regarding time required to collect the blood samples indicate that there is an overall savings for performing this work. There was no significant difference in amount of time to collect the blood sample between methods, but only one person is required to collect the sample in the trained method. The traditional method requires a minimum of two people (and on occasion up to three) to collect the same sample. The new method actually reduces staff requirements by 50% without increasing the overall time needed to collect blood samples. From a pure efficiency standpoint, this training appears to provide an opportunity to reduce the number of work hours dedicated to routine healthcare.

In addition to the time savings, the threat of harm to staff was reduced as well based on the Escape Behavior results. If cats are more accepting of the procedures they struggle less and thus reduce the threat of injury to personnel assisting in collections. It does appear that the time invested in training does offer benefits into adulthood for these animals. This benefit is seen even if the blood sample is collected in a traditional fashion.

5. Conclusion

The use of systematic desensitization and operant training have a significant impact in reducing the stress associated with routine healthcare in cats. Even when blood was sampled on a table, in a traditional fashion, the trained cats still responded more positively than the untrained cats based on fewer escape behaviors, more positive demeanor, and lower spikes in cortisol on second test day.

In addition, familiarity with the technician collecting the samples had a significantly positive impact on stress levels during the procedure based on fewer escape behaviors and more positive demeanor. Overall the amount of time required to sample blood using the new method was equal to that of the traditional method; however, only one technician is required for the new method thus reducing the staff-time required to complete routine healthcare procedures. Given the positive impact on reduction of stress for blood collection we recommend the incorporation of a
systematic desensitization and operant training program into the socialization protocols for young kittens.

**Conflict of interest**

None.

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**References**


