Development and Validation of a Behavioral Acclimation Protocol for Cats to Respiration Chambers Used for Indirect Calorimetry Studies

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Published online: 29 Mar 2012.


To link to this article: http://dx.doi.org/10.1080/10888705.2012.658332
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Cats exposed to novel environments initiate stress responses by behavioral and physiological changes that modify metabolism and lead to the collection of unreliable data. Fourteen cats (10 ± 2 months) were subjected to an 11-week acclimation procedure to adapt to restriction within chambers used for indirect calorimetry studies. Cats were acclimated to chambers in their home environments, to chambers in the study room, and to increasing periods of restriction within chambers. Ten additional cats (11 ± 1 month), used as controls, were subjected to a single 5-hr restriction without any prior exposure. Stress level, feed intake, fearfulness, and eliminations were recorded. Latencies to approach a novel object peaked on Weeks 4 and 8 (p < .05). Cat-Stress-Scores (CSS) declined with exposure and on Week 11, stress levels were low and consistent (p < .05). CSS was greater in unacclimated versus acclimated cats (p < .05). In conclusion, acclimation protocols prepare cats for repeated, temporary restriction within chambers, whereas short acclimations do not. A step-up acclimation procedure with behavioral indices of stress should be utilized to prepare cats for research that necessitates restriction.

Training nonhuman animals to different environments or tasks ensures that they are accustomed to them and do not experience stress when encountering them.

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Acclimation and training procedures employed on a variety of captive species, including nyala (Grandin et al., 1995), bongo (Phillips, Grandin, Graffam, Irlbeck, & Cambre, 1998), snow leopards (Broder, MacFadden, Cosens, Rosenstein, & Harrison, 2008), baboons (O’Brien, Heffernan, Thomson, & McGreevy, 2008), and chimpanzees (Lambeth, Hau, Perlman, Martino, & Schapiro, 2006) have resulted in reduced serological and behavioral indicators of acute stress responses. Furthermore, white blood cell count, neutrophils, hematocrit, glucose, cortisol, escape behaviors, and the need for anesthesia during routine husbandry practices have been minimized in response to successful training and acclimation.

Appropriate housing conditions and low stress levels are important to obtain optimal welfare for research cats. Stress-related behavioral and neuroendocrine responses modify energy requirements and macronutrient metabolism, potentially increasing animal variability and reducing the reliability and validity of results from nutritional trials (Meunier, 2006). Cats usually respond to novel and stressful environments such as animal shelters, boarding catteries, and restriction within cages by inhibiting normal activity patterns and/or reducing overall activity. The domestic cat is very unusual in that many also show a reduced or inhibited behavioral repertoire in situations of chronic stress, generally showing less active stereotypical behaviors such as pacing in order to cope with stressful situations (Casey & Bradshaw, 2007; Shepherdson, Carlstead, Mellen, & Seidensticker, 1993). Normal behavior patterns inhibited in response to chronic stress include self-maintenance, exploratory, and play behavior (McCune, 1992; Rochlitz, 1999). Acute stress has received much less attention than chronic stress responses in cats; however, there are behavioral and postural indicators of acute stress, like activity, body and tail position, which may decline after short-term exposure to a novel environment (McCune, 1992).

Stress response and fearfulness of cats can be measured using a variety of behavioral observations such as the Cat-Stress-Score (CSS) and Novel Object Test (NOT). The CSS is a noninvasive behavior score to test for fearfulness that has been utilized to measure acclimation of cats to novel environments (Kessler & Turner, 1997; McCune, 1994) and ranges from “fully relaxed” (score 1) to “extremely stressed” (score 7) based on posture and behavioral elements. The NOT is a test for fear response to, or the perception of threat from, novel stimuli (Boissy, 1995).

Behavioral acclimation procedures used to accustom cats (*Felis Silvestris Catus*) to temporary restriction within chambers used in indirect calorimetry studies range from no acclimation to an acclimation of 1 week, with restriction periods of 2, 4, and 6 hr; to our knowledge, only one study used behavioral measurements of feeding, drinking, and movement to determine successful acclimation (Lester, Czarnecki-Maulden, & Lewis, 1999). Lester et al. (1999) concluded that cats were acclimated to respiration chambers when normal behavior patterns were observed; however, it is unclear what the researchers defined as normal,
and because cats can be uncommunicative when stressed, further explanation and definition of the term normal are warranted. Kessler and Turner (1997) demonstrated that two thirds \((n = 140)\) of the cats in their study adjusted to a novel environment (boarding cattery) after 2 weeks of exposure as indicated by measurement of stress behavior. When cats were exposed to a novel environment (a quarantine situation or group/individual cage restriction), the stress response declined within the first 24 hr, continued to decline after several days of exposure, and further declined with prolonged exposure periods up to 5 weeks (McCune, 1992; Rochlitz, 1995; Smith, Durman, Roy, & Bradshaw, 1990). Therefore, it can be assumed that the acclimation protocols used in previous oxidation studies were insufficient for complete acclimation to a novel environment such as a respiratory chamber. Based on the evidence presented, regarding length of adaptation to novel environments (~2 weeks) and restriction (~5 weeks), an 11-week acclimation protocol was hypothesized to be a conservative estimate of a sufficient amount of time to produce full acclimation.

The objectives of this study were to design an acclimation procedure for cats to respiratory chambers and to demonstrate that cats successfully adapt to the chambers. Acclimation was measured by the performance of normal behavior, including feed intake, elimination, low fear response, and a low incidence of behavior indicative of stress (Carlstead, Brown, & Strawn, 1993; Loveridge, Horrocks, & Hawthorne, 1995). We hypothesized that, over the 11-week acclimation period, the cats would successfully acclimate to restriction within the respiratory chambers and the associated environment. Acclimation would be indicated by low CSS and fear responses, the demonstration of elimination, and by normal feed intake as determined during baseline observations.

ANIMALS, DIET, AND HOUSING

A total of 24 domestic, shorthaired cats (10 months ± 2; 3 kg ± 1.5) were selected from the Pet Health and Nutrition Center at Proctor and Gamble-Pet Care, Lewisburg, Ohio. Standard veterinarian evaluation of overall health was completed prior to the initiation of the study, and all cats entered the study healthy.

Cats were fed 60 g/d of Iams® Multi-cat dry Chicken diet; each cat was provided approximately 232 ME kcal/d. Cats were fed 30 g per feeding, and feed was provided at 7:30 a.m. and 1:00 p.m. Cats were fed individually, and each cat was permitted 60 min to eat during both food offerings.

Cats were group housed in a free-living environment with inside/outside access during the day but kept inside at night. The room was outfitted with environmental enrichment including perches, beds, toy houses, scratching posts, toys, and climbing apparatuses. All cats had daily social interaction for a minimum
of 60 min. Socialization entailed human petting, grooming, and interaction time with restricted-access toys. Cats were maintained on a 12-hr lighting schedule with the lights turning on at 6:30 a.m. and turning off at 6:30 p.m. The room temperature was maintained at 22°C and relative humidity >50%. Rooms were cleaned daily and disinfected weekly. Water was provided ad libitum from automatic waterers.

Respiration calorimetry chambers, made of Plexiglas, were 53.3 x 53.3 x 76.2 cm. The chambers were outfitted with a shelf, feeder, water bowl, hammock, litter box, toy, and a free area with a fleece bed. Water was provided ad libitum from water bowls. The chamber was designed to allow separation of feeding, sleeping, and elimination areas. Chambers and water bowls were disinfected; litter, litter boxes, toys, hammocks, and fleece beds were removed, cleaned, and replaced after each use. Calorimetry machinery was provided by Qubit Systems®, Kingston, Ontario, Canada.

MATERIALS AND METHODS

All procedures were reviewed and approved by The Institutional Animal Care and Use Committee established at The Iams Company, Procter and Gamble-Pet Care and were in accordance with The Iams International Animal Welfare Advisory Board standards.

Acclimation Procedure

Fourteen cats (n = 14) were randomly selected and subjected to an 11-week acclimation procedure to respiratory chambers. Fourteen cats were used because oxidation techniques require at least 10 to 12 cats to provide an adequate number of data points for determining nutrient requirements. Based on body weight as determined prior to the initiation of the study, the cats were allocated to one of three groups. Cats were acclimated to the primary researcher prior to the study; normal behavior was observed during these social interaction periods, and all cats were observed to willingly approach and interact in a friendly manner. Once the cats were determined to be acclimated to the primary researcher, baseline behavioral observations were taken.

**Weeks 1 to 3.** For the first 3 weeks of acclimation, the cats had free access (24 hr) to the respiratory chambers within the room in which the cats were permanently housed. One chamber was closed so cats could become acclimated to the metal door, and the other chamber was open to allow free access to the chamber and materials inside. Chambers were outfitted as if prepared for temporary restriction as described earlier.
Weeks 4 to 6. During Weeks 4 to 6, cats were introduced to the study room. With each week of exposure to the study room, cats were introduced, as the installation process was completed, to increasing amounts of calorimetry machinery. During Weeks 4, 5, and 6 of the acclimation period, cats were exposed to the study room for 20 min, 40 min, and 1 hr, respectively. Cats were exposed to the study room five times each week; therefore, each cat was exposed to the study room 15 times prior to restriction within the chamber. The primary researcher was present to offer positive reinforcement through talking to, playing with, and petting the cats.

Weeks 7 to 11. During week 7, the cats were restricted within the respiratory chamber three times for 1 hr/restriction. Following restriction, cats were permitted 20 min of free access within the study room. During Week 8, individual cats were restricted within the respiration chamber for 5 hr with 20 min of free access to the study room after restriction. During Weeks 9, 10, and 11, cats were housed in the respiration chamber for 10 hr, 15 hr, and 20 hr, respectively. Each cat was restricted only one time each week throughout Weeks 8 to 11. At the end of each confinement period, cats were provided with positive reinforcement in the form of petting, playing, and talking. Food was offered to the cats if restriction coincided with regular feeding times.

Behavioral Observations

The CSS was utilized to rank the level of stress of cats based on postural and behavioral indicators. The CSS system used has been described at length in Kessler and Turner (1997). Briefly, a score of 1 was given to cats demonstrating fully relaxed behavior, and a score of 7 was administered to cats demonstrating terrorized behavior; if cats demonstrated behavior split between two levels, then a half score was assigned. Once acclimated to the primary researcher and prior to the introduction of novel stimuli, five baseline CSSs were collected over a 5-day period. During the 3 weeks of free access to the chambers (Weeks 1–3), 5 CSS observations were conducted per week for a total of 15 observations. Throughout Weeks 4–11, cats were assessed at 10%, 50%, and 80% time points for 2 min, after initiation of each acclimation period, excepting the 20-min study room acclimation period on Week 4 in which the behavioral measurements were completed at 5, 10, and 15 min. Time intervals are represented as a percentage of the total time for a particular acclimation period for each study day. To calculate a particular time point for behavioral assessment, the following equation was used:

\[ \text{Observation Time (min)} = \% \text{ Time} \times \text{Total Duration of Exposure (min)}. \]
Cats exhibiting abnormal behavior or stress levels above, or equal to, a CSS of 6 were removed from the study. One observer was responsible for all CSS measurements.

NOTs were conducted to determine the cats’ latency to approach within a 5-cm radius of the novel object, as measured in seconds, to assess level of fearfulness and response to novelty. The duration of each observation was 5 min; if a particular cat did not approach during the 5-min observation period, the cat was classified as “no approach.” Cats were assessed at the 85% time point once each week on the 1st day of exposure (Day 1) for the entire acclimation period. NOTs completed in the study room (Weeks 4–6), without restriction within the chamber, were initiated upon the placement of the novel object in the center of the study room. The NOTs completed while the cats were maintained in their feeding cages and chambers started when the experimenter placed a novel object in the front left corner. The novel object was changed for each test to maintain novelty, and each novel object that was selected differed from those to which cats had been routinely exposed. Order of exposure was consistent for all cats.

After each twice-daily feeding, the remaining feed was weighed, and daily feed intake was calculated for each cat. After each restriction period, eliminations were recorded for each cat.

Control Group: Unacclimated Cats

Two groups of 5 cats (n = 10) of age, weight, and genetics similar to the cats undergoing acclimation to the respiratory chambers were randomly selected to act as a control group that had no previous experience of the study room or respiration chambers. The 10 cats were restricted one time within the chambers without acclimation and for a total of 5 hr. During the 5-hr restriction, behavior was monitored, including stress level and fearfulness. Because the restriction duration was only 5 hr total, elimination was not measured in the control group of unacclimated cats. Three CSS values were measured during restriction at the 10%, 50%, and 80% time points. If cat stress scores exceeded or equaled a CSS of 6, then the cats were immediately removed from the chambers. A NOT was performed to measure level of fearfulness of the cats. The NOT was performed at the 85% time point. The control group of cats was subjected to similar housing and daily management routines as the acclimated group of cats.

Statistical Analysis

CSS, feed intake, and NOT data analysis for the acclimated group of cats were completed using repeated measures ANOVA. CSS data were analyzed within week, within day, and between weeks; NOT and feed intakes were analyzed...
between week using the proc mixed function of Statistical Analysis System (SAS 9.1; SAS Institute Inc., 2002–2003). NOT data were further analyzed using the life-test procedure of SAS to test homogeneity of survival curves for latency to approach the NOT, which yielded product-limit survival estimates for the acclimated group. Correlation between CSS and NOT during acclimation was tested using Pearson correlation coefficients and the corr procedure of SAS. CSS and NOT of acclimated versus unacclimated cats was completed using repeated measures ANOVA and was further analyzed using the proc glm function of SAS. Differences were compared using the Wilcoxon signed-rank test and the Kruskal–Wallis one-way analysis of variance to ensure consistency of results. Differences were considered significant when \( p < .05 \).

RESULTS

Cat-Stress-Scores

Cats displayed between-week, within-week, and within-day variation in CSS. During Weeks 4 and 7 of the acclimation procedure, the cats displayed significantly higher stress scores than for all other weeks (\( p < .05 \)). Between Weeks 4–6 and Weeks 7–11 of the acclimation procedure, CSS decreased. On Weeks 4, 5, 7, and 8, CSS at the 10% time point within each study day was significantly higher than the CSS obtained at the 50% and 80% time points (Figure 1; \( p < .05 \)). By Week 11 of the acclimation procedure, CSS was consistent throughout the entire exposure period and did not change significantly within day (Figure 1; \( p < .05 \)). Overall, CSSs at the end of the acclimation procedure during Week 11 were lower than the CSSs obtained during baseline observations (Figure 1; \( p > .05 \)). No cat subjected to the acclimation procedure displayed significantly higher stress scores when compared with another cat undergoing acclimation.

CSS declined with day of exposure within Weeks 4–7 as there was a significant decline in CSS from Day 1 to Day 5 (Week 4–6) or Day 3 (Week 7). CSS did not decline significantly during Weeks 1–3; for Week 3, there was a small decline on Day 3; however, CSS did not differ between Day 1 and Day 5 (Figure 2; \( p > .05 \)).

Novel Object Test

One cat consistently exhibited a higher latency to approach the novel object (seconds) and was therefore removed from analysis as a statistical outlier (\( p > .05 \)). One cat did not approach the novel object within the 300-s time limit on Week 5 of the acclimation procedure and was classified as a “No Approach.”
FIGURE 1  Least squares means of Cat-Stress-Scores (mean ± Standard Error of the Mean) of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment on Day 1 of each week of the acclimation procedure at the 10%, 50%, and 80% time points. Letters identify relative differences among Cat-Stress-Scores for time within day over the 11 acclimation weeks; means not sharing a superscript letter are significantly different; “a” represents the highest value and “f” the lowest (p < .05).

FIGURE 2  Least squares means of Cat-Stress-Scores (mean ± Standard Error of the Mean) of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment on each sampling day within a particular week with multiple observations per week. Letters identify relative differences among Cat-Stress-Scores within week; means not sharing a superscript letter are significantly different; “a” represents the highest value and “d” the lowest value (p < .05). Days within week without superscripts do not differ (p > .05). Weeks 8–11 are not represented as there were no repeated day measures within these weeks.
Latencies to approach the novel object were significantly different on different weeks of the acclimation procedure (Figure 3; \( p < .05 \)). Increased latency to approach the novel object appeared to be correlated to the additional introduction of a novel event (study room or restriction within the chamber) as latency to approach was high on both Weeks 4 and 8 when cats were exposed to the study room and restriction within the chamber.

During Weeks 1–3 and 9–11, the cats displayed a similar approach pattern to the novel object (Figure 4). During these weeks, cats approached the novel object almost immediately, and the probability that all cats approached the novel object within 15 s of exposure was 100%. All cats did not approach the novel object immediately following exposure; thus, during Weeks 4–8, survival distribution and the pattern of approach to the novel object were variable. Approximately 20% of cats subjected to the NOT displayed longer latencies, greater than 25 s, to approach the novel object on Weeks 4–8. During Weeks 9–11, the pattern of approach was uniform because all cats approached the novel object within 10 s of exposure.

Feed Intake

Feed intake did not significantly differ between individual cats (\( p > .05 \)). There were differences observed in average weekly feed intake (Figure 5; \( p < .05 \)); however, these differences were <3.57 g or 6% of total intake, and therefore we do not feel this is physiologically significant.
FIGURE 4  Product-limit survival estimates for latency to approach the novel object within a 5-cm radius of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment (color figure available online).  

(continued)
FIGURE 5  Least squares means of weekly feed refusal (ORTs) with a 60-g daily feed offering (mean ± Standard Error of the Mean) of 14 cats undergoing acclimation to indirect calorimetry equipment and associated environment. Letters identify relative differences among feed refusal; means not sharing a superscript letter are significantly different; “a” represents the highest value and “c” the lowest value ($p < .05$).
Elimination Behavior

All cats subjected to the acclimation protocol showed some form of elimination behavior during restriction within the respiration chamber. There were no quantitative measurements of elimination; the evidence was simply that the litter box had been used.

Correlation Between Cat-Stress-Score and Novel Object Test

There was no consistent correlation between CSS at the 80% time point and the latency to approach the novel object on the specific test day ($p > .05$). The associated Pearson correlation $r$ and $p$ values were as follows: Week 4 ($r = -0.02, p = .9$), Week 5 ($r = -0.02, p = .6$), Week 6 ($r = 0.18, p = .5$), Week 7 ($r = 0.53, p = .05$), Week 8 ($r = 0.51, p = .4$), Week 9 ($r = 0.02, p = .5$), Week 10 ($r = 0.3, p = .3$), Week 11 ($r = 0.03, p = .9$). CSS and latency to approach were only correlated on Week 7 of the acclimation procedure.

Comparison of Unacclimated and Acclimated Cats

Three unacclimated cats were removed from the chambers because these cats exhibited a CSS of at least 6. Average CSS for the unacclimated cats (excluding the cats who were removed) was significantly greater ($p < .05$) compared with the average CSS for the acclimated cats.

CSS at the 10% time point was higher than the CSS at the 50% and 80% time points demonstrating that time (10%, 50%, and 80%) within test day had an effect on the CSS of both unacclimated and acclimated groups of cats. The reduction in CSS was significant for the acclimated group of cats from the 10% to the 50% and 80% time point; however, the CSS of the unacclimated cats did not decrease significantly during the same time frame (Figure 6; $p < .05$).

Unacclimated cats had a mean latency to approach the novel object as measured in seconds of $13.21 \pm 13.36$; the acclimated cats exhibited a mean latency to approach of $9.89 \pm 13.36$ s. Latency to approach the novel object in seconds was not significantly different between the unacclimated and acclimated group of cats with one- or two-sided $t$-tests used to analyze these data.

DISCUSSION

All cats successfully acclimated to the respiration chambers when exposed to the acclimation procedure; cats who were not exposed to an acclimation procedure demonstrated greater levels of stress-related behavior during restriction. CSS
increased when cats were introduced to a novel environment but declined over time (within day, week, and between weeks) with exposure. During the last weeks of acclimation, cats demonstrated low fear responses and rapid approaches to a novel object. All acclimated cats demonstrated some form of elimination behavior, and feeding patterns were consistent with those observed during baseline observations.

At the start of the last week of acclimation (Week 11), CSS had declined to levels below those observed during baseline observations but were similar to those observed during Weeks 1, 2, and 3, when cats had unrestricted access to chambers located within the group living room (Figure 1). Elevated stress levels during baseline observations were likely a consequence of a disturbed room environment. One of the cats required veterinary care, which disrupted the normal housing routine. For cats, such disruption (as has been demonstrated) can act as a stressor (Carlsted et al., 1993).

Cats did not display behavior indicative of stress when subjected to 24-hr free access to respiration chambers. This lack of disruption was likely achieved by placing the chambers in a familiar environment. This procedure provided cats with an opportunity to control the situation by facilitating behavioral choices and adoption of various flexible strategies, decreasing the perceived degree of novelty (Broom & Johnson, 1993; Rochlitz, 2000).
The largest increase in stress response was observed during Weeks 4 and 7 when cats were initially exposed to the study room and to chamber confinement. The change in stress level could be attributable to the following factors:

1. Novelty of the environments as a consequence of spatial modification,
2. Changes in scent and auditory and visual stimuli,
3. Sudden changes in the predictability of the environment, and
4. Degree of social isolation during chamber confinement (Carlstead et al., 1993).

CSS levels at the 10% time point on Week 6 (3rd week of exposure to the study room) and on Week 8 (2nd week of restriction) were similar to baseline levels. CSS at 50% and 80% in Week 8 declined to levels observed in Weeks 1–3, suggesting that cats adapted more rapidly to enclosure restriction than to the study room environment (Figure 1). The study room indeed contained higher levels of novel stimuli that would tend to stimulate curiosity, vigilance, and responsiveness of cats (Weipkema & Koolhass, 1992). Cats (including the control group) were acclimated to enclosure restriction as kittens and had been routinely exposed to all devices employed within the chambers in an attempt to minimize associated stress response. Research indicates that previous exposure to a cattery or shelter reduced the time required to fully acclimate cats to the environment upon return (Kessler & Turner, 1997; McCune, 1994, 1995). Cats require between 2 and 5 weeks of acclimation when exposed to a novel environment. To some extent, this depends on previous exposure (Kessler & Turner, 1997; Rochlitz, Podberscek, & Broom, 1998). In the present study, cats with previous experience of restriction and exposure to the chamber and associated environment required approximately 2 weeks to fully adapt to restriction and, without previous exposure, more than 3 weeks to adapt to the study room environment. Rochlitz et al. (1998) and Smith et al. (1990) found that cats continued to show reduction in stress characteristics when exposed to a shelter or quarantine environment for greater than 1 month; however, we did not observe any similar reduction in CSS beyond 1 month of exposure. McCobb, Patronek, Marder, Dinnage, & Stone (2005) observed a consistent decline in CSS with increased exposure to a shelter environment. However, others have suggested that some cats may not have fully adapted to an extended period of restriction. Rather, the cats may have lapsed into a state of listlessness and depression that results in increased time spent sleeping—typical of adoption of a passive defensive system commonly observed in felids and other carnivores in captivity (Carlstead, Brown, Monfort, Killens, & Wildt, 1992).

The CSS attempts to distinguish between stressful, feigned sleep and true, relaxed sleep; however, the difference is often difficult to determine. Occasionally, disturbed cats who exhibit apparently normal sleep behavior may be incorrectly
classified as having a low CSS. The CSS was selected for this study because
the scale has successfully been proved, validated, and classified as a noninvasive
behavioral measure of stress in cats in response to novel environments (Kakuma
& Bradshaw, 2001; Kessler & Turner, 1997, 1999), people (Kessler & Turner,
1999), and enrichment tools (Kry & Casey, 2007). We felt that the CSS, when
used in conjunction with other established behavioral measures, would facilitate
accurate assessment of noninvasive behavioral characteristics fundamental to
proper determination of the true status of cat acclimation.

CSS status did not significantly change during Weeks 1 and 2; changes
were observed during Week 3 of the acclimation program. Average CSS during
Day 1 was not different from average CSS on Day 5 for Weeks 1, 2, and 3
as, because no novel experience or object was introduced, would be expected.
Conversely, within-week CSS significantly decreased from the first day to the
last day that cats were exposed to the room or chamber environment (Figure 2)
during Weeks 4, 5, 6, and 7. Smith et al. (1990) found that behavioral indicators
of stress in cats exposed to a novel environment declined quickly after the first
4 days of exposure to the novel environment. The present results are similar to
those of previous studies where cats exposed to a novel environment displayed
the highest stress responses during the first few days of the experience (Kessler
& Turner, 1997; McCune 1992, 1994; Rochlitz et al., 1998; Smith et al., 1990).

During Weeks 4, 5, and 7–10, CSSs at the 10% time point of exposur,
within each study day, were observed to be significantly higher than the CSS
obtained at the 50% and 80% time point. During Weeks 11 and 6, CSSs between
percentage time points were not different (Figure 1). McCune (1992) observed
that the greatest reduction in stress levels occurred during the first 10 min of
exposure and between the first and second hour after restriction within a novel
caged environment. Similarly, our data suggest that observed stress levels decline
with increasing exposure, and the largest reduction in stress response occurs
between the 10% and 50% time point.

All cats subjected to the acclimation procedure successfully adapted to the
respiration chambers; no cat subjected to the acclimation procedure displayed
significantly higher CSS at any time point. Kessler and Turner (1997) observed
some variability among cats; approximately 4% of their cats did not demonstrate
any behavioral signs of acclimation after 2 weeks in the cattery. McCobb
et al. (2005) also observed some variability between cats; however, as cats
spent more time in the shelter environment, variability between cats declined.
Behavioral variability is attributed to differences in genetics, maternal care,
previous experience, and features of the current situation (Mendl & Harcourt,
2000). The similarity in genetics, maternal care, and previous experience of the
cats in our particular study was likely the reason for the observed similarity in
their response to novelty and restriction.

During the NOT, cats demonstrated neophobic tendencies during Weeks 4–
8 as latency to approach the novel object was highest during these weeks
(Figure 3). Cats in stressful environments will frequently exhibit neophobic behavior as a consequence of their perception of threat due to the unknown controllability and predictability of the environment (Boissay, 1998). Although cats demonstrated higher levels of stress during exposure to the study room and during the first 2 weeks of restriction, there was no correlation between CSS at the 80% time point and the latency to approach the novel object on the specific test day. The delayed approach to the novel object on Weeks 4–6 may have been a consequence of a lack of interest as cats often continued interacting/playing with another object within the study room. The higher latencies to approach the novel object during exposure to the study room may have been a direct consequence of the environment and the distance that the cats had to travel, as the NOTs were completed during restriction for all weeks except Weeks 4–6. Latency to approach the NOT on Weeks 7 and 8, the first 2 weeks of restriction, differed with the higher approach latency being associated with Week 8. The delay in responsiveness to novel stimuli during restriction may have been a consequence of the feeding regime imposed on these cats as they are individually restricted for an hour within an assigned cage daily for feeding; therefore, the hour restriction in the chambers may not have been entirely novel. Latency to approach the NOT on Weeks 1–3 and 9–11 were rapid (less than 25 s) and consistent between cats (Figure 4). The low level of avoidance, fearfulness, and the consistency of time to approach the object at the end of the acclimation period would suggest that cats were successfully adapted to such restriction.

All cats subjected to the acclimation demonstrated elimination behavior. Contributors to inhibition of elimination behavior include environmental and social stress; household disruptions/changes; medical problems; and, in particular, feline urological syndrome and a cats’ idiosyncratic inclination for specific elimination locations (Olm & Houpt, 1988). Because study cats demonstrated normal elimination patterns, it can be reasonably concluded that restriction within the chamber did not cause any significant stress response that may have inhibited or modified normal urination or defecation patterns.

In stressful environments, cats have been observed to inhibit feed intake, modify feed selection habits, and generally demonstrate anorexia and/or neophobic tendencies when a food source is presented under stressful conditions (Mugford & Thorne, 1980). In the present study, cats maintained consistent feed consumption patterns during the entire acclimation procedure; this suggests that the cats were not stressed to a level that inhibited or modified feed intake. The small variation in feed intake observed during Weeks 1–3 of the study may have been attributed to the change in feeding process put in place for the study; once the feeding protocol was regimented, feed intake did not change. The decrease in feed intake during Week 11 was ~2 g and likely not physiologically indicative of stress as all other signs of stress were low during this week (Figure 5).

There were significant differences noted between stress levels in cats undergoing acclimation and the control cats who were subjected to a one-time, 5-hr
restriction. The cats who were subject to unscheduled removal from the chambers displayed a variety of stress-related characteristics, including strong escape behavior, extreme vigilance, increased respiratory rates, fearful immobility, and destructive behavior (Casey & Bradshaw, 2007); no such characteristics were observed in the acclimated group of cats. Further, in contrast to the acclimated group (Figure 6), unacclimated cats did not show a significant reduction in CSS with exposure to the respiratory chambers. Such differences were determined to be a consequence of previous experience within the study room and with restriction within the respiration chambers. These factors caused differing perceptions of the degree of novelty of the environment and level of perceived threat to homeostasis, thus initiating a proportional stress response. Unacclimated cats took longer to approach the novel object; this is associated with display of strong neophobic behavior and supports the conclusion that unacclimated cats had significantly higher stress responses to restriction within respiration chambers than acclimated cats.

CONCLUSION

The 11-week acclimation procedure led to successful acclimation of young cats to the novel respiration chamber and study environment. This acclimation procedure may be used for cats being trained for indirect calorimetry studies. It is expected that almost all candidate cats will become fully and successfully acclimated; thus, with a high success rate, fewer animals may need to be selected for training. It should be noted that this acclimation procedure utilized young, spayed, and neutered cats; the acclimation procedure may require adaptation if applied to older or intact cats. Furthermore, as there was an increase in stress score with each introduction to a novel environment, it may be beneficial to increase the length of acclimation to minimize the significant increase in stress response with each new exposure. Following full acclimation, it is imperative that normal study cats demonstrate low levels of stress during confinement in the respiration chambers to achieve robust, accurate, and repeatable data collection derived from indirect calorimetry studies. High stress responses and presumably cortisol, typical of the unacclimatized cat, may have an adverse impact on macronutrient metabolism, energy requirements, and the overall well being of the research animals.

ACKNOWLEDGMENT

This study was supported by Procter and Gamble-Pet Care, 8700 Mason-Montgomery Road, Mason, OH 45040–9462.
Author Disclosure: I. J. H. Duncan and J. L. Atkinson have no conflicts of interest. A. K. Shoveller and M. A. Gooding have a financial and personal interest in Procter and Gamble-Pet Care due to employment with the funding company.

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