Accepted Manuscript

Title: Effect of cognitive enrichment on behavior, mucosal immunity and upper respiratory disease of shelter cats rated as frustrated on arrival

Author: Nadine Gourkow Clive J.C. Phillips

PII: S0167-5877(16)30217-3
DOI: http://dx.doi.org/doi:10.1016/j.prevetmed.2016.07.012
Reference: PREVET 4068

To appear in: PREVET

Received date: 28-7-2015
Revised date: 12-7-2016
Accepted date: 24-7-2016

Please cite this article as: Gourkow, Nadine, Phillips, Clive J.C., Effect of cognitive enrichment on behavior, mucosal immunity and upper respiratory disease of shelter cats rated as frustrated on arrival. Preventive Veterinary Medicine http://dx.doi.org/10.1016/j.prevetmed.2016.07.012

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Effect of cognitive enrichment on behavior, mucosal immunity and upper respiratory disease of shelter cats rated as frustrated on arrival

Nadine Gourkow a and Clive J.C. Phillips a*

 a Centre for Animal Welfare and Ethics, School of Veterinary Medicine, University of Queensland, Gatton, Queensland. Australia 4343.

*Corresponding author: E-mail: c.phillips@uq.edu.au. Tel.: +61 7 5460 1158; mobile: 0406340133 (C.J.C. Phillips)

Highlights

- Regular cognitive enrichment of frustrated cats - a human teaching them a task - improved their welfare
- Treated cats were more content than cats not taught the task
- Treated cats had increased concentrations of immunoglobulin A in their feces
- Cats that responded well to the treatment had the biggest increase in concentrations of immunoglobulin A in their feces
- Treated cats had less respiratory disease
ABSTRACT

Acquisition of resources and opportunity to engage in natural behaviors has been shown to reduce frustration-related behaviors and enhance health in nondomestic felids kept in zoos, but little is known about whether there are similar effects in domestic cats living in confinement in animal shelters. Fifteen cats rated as Frustrated during the first hour of confinement to a cage at an animal shelter were assigned to either a Treatment (n=7) or Control (n=8) group. Treatment cats were taken from their cages to a separate room four times daily for 10 min each time over a 10 d period, where they took part in training sessions to learn a novel behavior (paw-hand contact with a researcher). Changes in emotional states and mucosal immune response were evaluated over 10 days. Infectious status was determined upon admission and incidence of upper respiratory was determined up to day 40 based on clinical signs. Treated cats were more likely to be rated as Content than Control cats and had greater concentrations of S-IgA (537 µg/g) in feces than Control cats (101 µg/g). Within the Treatment group, cats that responded positively had greater concentrations of S-IgA (925 µg/g) than those that responded negatively (399 µg/g). Control cats were more likely to develop respiratory disease over time compared to cats that received treatment (Hazard Ratio: 2.37, Confidence Interval: 1.35–4.15). It is concluded that there is prima facie evidence that cognitive enrichment of cats exhibiting frustration-related behaviors can elicit positive affect (contentment), stimulate secretion of IgA and reduce incidence of respiratory disease, which is worthy of further study.

Keywords: Emotions, Enrichment, Frustration, Human interaction, Secretory Immunoglobulin A, Respiratory disease, Shelter cats
Introduction

Animals respond differently to captivity according to their behavioral motivation (Mason, 2010). Non-domestic felids are driven to roam over a wide territory to hunt (Carlstead et al., 1993; Lyons et al., 1997), which when thwarted in captivity makes them prone to pacing (Clubb and Vickery, 2005) in response to lack of stimulation and frustration (Mohapatra et al., 2014).

Despite at least 4000 years of domestication (Serpell 2000) and even when socialized and fed by humans, some domestic cats have retained the drive to hunt and roam (Montague et al., 2014) and when living outdoors have a range which exceeds 1.7 km (Bradshaw, 1992). Although some cats adapt well to confinement to the home (Jongman, 2007), it is also clear that domestic cats can experience frustration in confinement, particularly in cages in animal shelters and laboratories. Studies of household cats indicate that thwarted access to outdoors can exacerbate elimination problems, psychogenic dermatitis (skin irritation due to over grooming), pica (eating non edible things) (Amat et al., 2009), inter-cat aggression (Leyhausen, 1979), redirected and irritable aggression towards people and other pets (Beaver, 2004). Confinement has also been proposed as a risk factor for health issues such as cystitis (Cooper et al., 1983; Jones et al., 1997; Buffington, 2002; Sævik et al., 2011), type 2 diabetes mellitus (Slingerland et al., 2009) and inflammatory bowel disease (Zoran and Buffington, 2011). Although sparse, there is evidence of frustration-related behaviors in shelter cats, expressed as pacing, destructive behavior, persistent vocalization, bar biting and redirected aggression (McCune, 1992; Kessler and Turner, 1997; Gourkow et al., 2014c).
Frustration has important implications for the mental well-being and health of shelter cats. In relation to this, it has been associated with a decrease in secretion of immunoglobulin A (s-IgA) in shelter cats (Gourkow et al. 2014a). s-IgA is the most abundant mucosal antibody and is necessary for protection against pathogens that are inhaled or ingested (Stokes and Waly, 2006), particularly in multi-cat environments where such pathogens are found in high concentration (Pedersen et al., 2004). When induced by electrostimulation of the anteromedial hypothalamus, frustration (labelled as restlessness by the authors) had important pathophysiological and immunological effects such as elevated cortisol and cathecolamine, arrhythmia, increased responses of lymphocytes to phytohemagglutinin (Mori et al., 2001), and gastric and cardiac lesions (Kojima et al., 1996).

Enrichment has been the focus of much research aimed at helping domestic and non-domestic felids cope with confinement. In zoos, olfactory enrichment has successfully reduced pacing (a behavior associated with frustration) in Oncilla cats (Letícia de et al., 2011), lions, ocelots, and tigers (Skibiel et al., 2007) and similar benefits have been found with the use of artificial prey in Serval cats (Markowitz and LaForse, 1987) and moving bait in cheetahs (Williams et al., 1996). Visual stimuli, such as presentation of videos combining prey animals and linear movement (Ellis and Wells, 2008), and olfactory enrichment using cloth infused with catnip have been tested with shelter cats (Ellis and Wells, 2010). However subjects were not specifically identified as frustrated cats and thus the benefit of such enrichment for that population remains unknown. Operant training has successfully reduced frustration-related behavior in various species in zoos and on farms (Putman, 2005; Meyer et al., 2010).
Learning a complex behavior with the use of a clicker device (employing a click sound as a secondary reinforcer) is promoted by the Humane Society of the United States, and courses designed for the shelter environment are available (Pryor, 2009; Johnson, 2011). However, the potential benefits of clicker training, specifically for frustrated cats, have not been experimentally tested in domestic cats. One study (Tami et al., 2011) reported that laboratory cats tended to show quieter postures while waiting for a reward during clicker training sessions. However, quiet behavior, e.g. sit and wait, may simply reflect the natural food-search behavior typical of cats (Bos et al., 2003).

In this study, we conducted a preliminary investigation of the effects of cognitive enrichment on the behavior and health status of cats identified as frustrated on entry to a shelter.

2. Material and methods

This study was approved by the University of Queensland Animal Ethics Committee (CAWE/231/10).

2.1 The shelter and experimental ward
The study took place at the Vancouver Branch of the British Columbia Society for the Prevention of Cruelty to Animals (BC SPCA, Vancouver, Canada). The shelter had six separate housing areas, with a maximum capacity to house 120 cats. The facility also included an isolation area for sick cats and an on-site veterinary hospital. A small room adjacent to the reception area was used for examination and vaccination of incoming cats.

A housing unit located on the second floor of the shelter was used as the experimental ward. This room was maintained at a constant temperature of 20 ± 2 °C, and was naturally lit but with the provision of additional (artificial) light for 4 h each day. Visitors were discouraged from entering the experimental ward; however, approximately 24 people over the course of the study were provided entry to look for their stray cats. Apart from this, the only people entering the ward were shelter staff and two research staff. In common with most shelter environments, some sounds of dogs barking, and people walking and talking nearby, were audible to the human ear. The experimental ward included a food preparation area out of sight of the cats. Feed was provided twice daily at 0700 and 1700 h and comprised 70 g of age-appropriate pellets and approximately 30 g of wet food (Science Diet, Hill's Pet Nutrition, Inc. ®/™ Topeka, KS, U.S.A.). Fresh water was provided ad libitum. Feeding was undertaken by the experimenter (NG), shelter staff or volunteers.

The cat housing in the experimental ward consisted of 20 stainless steel cages (76 x 76 x 71 cm). Each was furnished with a litter box and non-absorbent cat litter (Veterinary Concepts, Wisconsin, U.S.A.), a stainless steel food and water bowl, and a towel for bedding. Each cage was fitted with an infrared camera (Sony CCD25M crystal-View Super Hi-Res ICR IR Camera
SLED w/9-22mm Variable Focal Lens, Microtech Advanced Technologies Ltd, Vancouver, Canada) mounted at cage height on a rod suspended from the ceiling at 1 m from the cage door. Footage was available for real-time viewing in an adjacent room, and was stored for subsequent analysis.

2.2 Biosecurity

Shelter staff cleaned cages daily by removing all waste, changing bedding, and wiping walls with a clean cloth soaked in water. Cages were disinfected between cats with a 1% disinfectant solution (Virkon®, Du Pont, Mississauga, Ontario, Canada). Staff and the experimenter sanitized their hands (Microsan™ Antiseptic instant hand sanitizer, DEB Worldwide Healthcare Inc. Ontario, Canada) following each contact with a cat.

2.3 Animals

This study was part of a research project designed to examine the effects of behavioral interventions on emotions, mucosal immunity and incidence of respiratory infections on 250 cats rated as Frustrated (n=15), Anxious (n=139), or Content (n=96) upon admission to the shelter. Between May and November 2010, cats that had been surrendered by their owner or brought in as strays by a humane officer, that were over 6 months old and free of clinical signs of upper respiratory disease (URD) and injury formed the pool from which cats were obtained for this study. Of the 15 cats in this study, all but one was adopted. The only cat not adopted, from the control group, was sent to isolation upon being diagnosed with URD and subsequently euthanized after 2 unsuccessful completed courses of antibiotic treatment. At the end of the 10-day experimental study, the health and fate of all study cats continued to be monitored for up to 40 days.
2.3.1 Physical examination, viral and bacterial cultures

Upon admission, a certified animal health technician (AHT) examined the cats to determine the presence of clinical signs of upper respiratory disease and injuries. They were vaccinated (Fel-O-Guard+3 Boehringer Ingelheim Ltd., Burlington, Ontario, Canada) and dewormed (Strongid® T. Pfizer, Quebec, Canada). The AHT also examined cats daily. Those with clinical signs of URD such as mucopurulent nasal and/or ocular discharge; conjunctivitis (uni or bi-lateral) sneezing and / or coughing were removed from the study and sent to a medical isolation ward for treatment. The prevalence of different bacteria and viruses was determined from pharyngeal swabs in this study has been reported previously (Gourkow et al., 2013).

2.4 Behavioral assessment

2.4.1 Observation on admission (day 0)

Following examination, each cat was placed in a small wire cage covered with a towel and transported by staff to the experimental ward on the second floor of the shelter. The journey of approximately 2 min did not require passing through any other cat housing units or dog areas. Cats were allocated to cages as available, which produced an approximately random distribution to the 20 cages with cameras. Upon entering the room, staff lifted each cat into their cage (covered with a towel prior to lifting if they were growling or hissing) and immediately exited the room.

A 1 h real-time video observation (from an adjacent room) commenced as soon as a cat was placed in a cage. This was followed by the experimenter entering the room and conducting a
Human Approach Test, adapted from a previous test (Kessler and Turner, 1999) as follows: Step 1: the experimenter stood in front of the cage without interaction, no eye contact or verbal greeting (2 min); Step 2: the experimenter talked to the cat using a high-pitched gentle tone, and had some eye contact, with eyes half closed (1 min); Step 3: the same procedure was repeated with the door open, followed by an approach of one hand so that it was near the cat (2 min). However, if cats responded aggressively (growling, hissing, attempts to scratch or bite), the door was closed immediately. If they responded with aggression, escape attempts or defensive retreat they were deemed to have responded negatively, otherwise they were deemed to have given a positive response.

Following the observation period and the Human Approach Test, cats were assigned an emotional rating of Content, Anxious or Frustrated based on their overall response (Tables 1 and 2). Of the 250 cats assessed upon admission (day 0), 15 were rated as Frustrated, 139 cats were rated as Anxious and 96 were rated as Content. Specifically, cats were rated as Frustrated if they met the criteria for Frustration listed in Table 1 throughout the 1h observation period (Day 0). These behavioral indicators had been previously validated with physiological correlates, S-IgA and cortisol, in 34 cats during their first week at an animal shelter (Gourkow et al., 2014a). In that study, 37 behaviors used in other studies for the assessment of welfare in shelter and household cats were initially selected as candidates for an index of emotions. Following observations, some behaviors with seemingly similar motivation and significant Spearman rank correlations were amalgamated and infrequently observed behaviors were removed. The 24 remaining behavior variables were subjected to a principal component analysis producing a three dimensional model which was interpreted according to biplot methodology (Gabriel, 1971). The
resulting multidimensional model represented two contrasting emotions, anxiety and contentment, indicative of high and low arousal of the emotional defense system respectively. A third dimension represented an emotion elicited by low arousal of the reward system that was consistent with frustration.

This paper reports the results of behavioral treatment of the cats rated as Frustrated upon admission (day 0). The effect of gentling on Anxious cats (Gourkow et al, 2014b) and human interaction on Contented cats are reported separately (Gourkow et al., submitted). The Frustrated cats were alternately allocated to either a Treatment (n = 7) or Control group (n = 8) immediately after the emotional rating (day 0), in order of admission to the study.

2.4.2 Rating of moods

To examine changes in cats’ moods over the ten days, their behavior was assessed daily using focal sampling from the video records (10 min per hour for the entire period). A Frustrated mood score was assigned if target behaviors were observed > 10% of awake time, and if there was no behavior observed from the Anxiety category. The results amalgamated over 24 h gave a total of 55 cat days for the treatment group and 67 cat days for the Control group. Moods were rated using the same emotion indicators as for the initial behavior assessment (Table 1).

2.5 Treatment procedure

Interventions were provided four times per day by one experimenter (NG), thus not all cats received treatment at the same time. Treatments started at 0600, 1100, 1600 and 2000h. Sessions took place in a 4m² treatment room adjacent to the housing unit. If necessary, cats were held
under the experimenter’s arm and carried to the room; but after a few sessions most cats jumped out of the cage and walked to the treatment room unassisted. Sessions started with 2 min of free exploration. Shaping of the desired behavior (high five: contact between the cat’s paw and the experimenter’s hand) was initially started by conditioning the cat to the clicker sound (The Clicker Company, Payson, Arizona, USA) immediately followed by dropping a food reward close to the cat (Whiskas Temptations, tuna flavor, www.whiskas.ca/en). Cats were considered conditioned to the marker signal (click sound) when they looked in the area where the treat was last dropped immediately upon hearing the click sound. Subsequently the click sound was used to mark the desired behavior and was immediately followed by the dropping of a treat next to the cat each time.

The behavioral sequence for the shaping of high five behavior was for the cat to sit, raise paw to shoulder height, touch handler’s hand, and finally sustain contact with handler’s hand for a few seconds. Each approximation and successful completion of these behaviors was reinforced with the click sound followed immediately by a treat dropped next to the cat. The sit behavior could occur naturally or the cat could be lured into position using a target stick. Luring cats into a sit was achieved by holding the stick near the cat’s nose and rewarding nose to stick contact and subsequently moving it backwards until the cat was in a sit position. Any movement of the right or left paw was rewarded. Paw lift could occur naturally or the cat could be lured into lifting the paw by holding a treat in the hand close to the cat (most cats touched the hand briefly in response). When the paw lift reached the cat’s shoulder height, the handler placed her hand in the path of the movement and rewarded contact. Subsequently, the click and food reward were delayed briefly to extend contact time of the cat’s paw with the handler’s hand. Once the
sequence was learned, the behavior was requested verbally ("give me five") while placing the hand near the cat. At the end the training session, the cat was picked up and placed back in the cage. Control cats were ignored and not interacted with by either staff or the experimenter during routine care or when in the room tending to other cats.

2.6 Rating of response to treatment
Response to treatment was noted immediately after each session. A cat received a Negative Rating if while in the treatment room, it paced at the door or window, avoided the handler or displayed aggressive behavior (growling and hissing). A cat received a Positive Rating if it interacted with the experimenter in a friendly manner (whether they learned the task or not), remained in close proximity and expressed friendly behaviors (i.e. tail up, purr, rub, head butt, chirp, or attempt to climb on lap). No neutral responses were observed.

2.7 Feces collection and S-IgA assays
Stools were collected whenever produced, and were weighed and immediately frozen at -40°C. Samples were analyzed for IgA concentrations, using the method described in Gourkow et al. (2014a). In brief, samples were extracted and vortexed until homogenized. Following centrifugation, addition of a protease inhibitor and placement in ELISA plates, IgA values were obtained in a multilabel plate reader. Coefficients of variability were 5.4% and 9.1% for intra and inter assays, respectively, within the accepted limits of 10 and 15%, respectively (Anon, 2014)

2.8 Statistical analysis
Results were considered significant at alpha ≤ 0.05.
2.8.1 Effects of treatment on daily mood (days 1-10)

A Cox-proportional hazards model determined if Treated cats were more likely to remain content from the onset of contentment to end of the study than Control cats; hazard ratios (HR), confidence intervals and the associated p-values are reported.

2.8.2 Influence of daily mood and cognitive enrichment on S-IgA levels (days 1-10)

A t-test was used to determine if there was a difference in the mean number of stools between Treated and Control groups of cats. S-IgA values were loge transformed to achieve a normal distribution. A GEE (Hardin and Hilbe, 2003) was used to determine if there was a significant difference in S-IGA levels depending upon the day. GEE were used to determine if there were associations with the factors because there were correlations between observations, i.e. days for each of the cats. The GEE was then used to determine differences in S-IgA levels between the Treated and Control groups.

2.8.3 The effect of cognitive enrichment on incidence of viral and bacterial shedding and URD

A Cox-Proportional Hazards model was used to compare the incidence of URD between Treated and Control groups over time.

3.0 Results

3.1 Baseline assessment (day 0)
Of the 250 cats assessed upon admission, 15 (6%) were rated as Frustrated. None responded negatively (defensive aggression or retreat) to the Human-Approach Test during the initial observation (day 0).

3.2 Effect of treatment on daily mood (days 1-10)

Treatment cats were more likely to be rated as Content than Control cats (Fig.1). Within the Control group, 49 of the 67 days of observation (73%) were rated as negatively valenced, more specifically 41 days (61.2%) were rated as Frustrated and 8 days (11.9%) as Apathetic (mean time to first rating of Apathetic = 6 days). Within the Treatment group, 28 of the 55 days of observation (51%) were rated Frustrated and 0% as Apathetic (Table 2). There was a trend for Treatment cats to be rated as Content sooner. Mean days to first rating as Content for Treated cats were 3.2 (±1.3 days) versus 4.4 days (±0.3) for Control cats, and there was a trend for Treated cats to be 3.6 times more likely than Control cats to remain Content from onset of contentment to end of study (HR: 3.64, CI: 0.85-15.67, \( P = 0.08 \)).

3.3 Influence of baseline mood and cat characteristics on response to treatment

Proportionately, more cats had a positive response to treatment on days when they had been rated as Content, compared to days when they had been rated as Frustrated (Table 3). Males and stray cats were significantly more likely to respond positively to treatment than females and owner-surrendered cats, but age did not appear to affect response to treatment (Table 4).

3.4 Influence of mood and response to treatment on S-IgA levels (Days 1-10)
There was no significant difference in the mean number of stools analyzed between the groups (control = 3.6 ± 1.5/cat, treated = 4.6 ± 2.7/cat, \( P = 0.24 \)), nor was there an effect of day on S-IgA for either group (Controls: \( P = 0.88 \)) (Treated: \( P = 0.30 \)).

S-IgA values were greater for Treated than Control cats (6.73 ± 0.47 Vs. 6.04 ± 0.68 \( \log_e \mu g/g \), or 537 Vs. 110 \( \mu g/g \), \( P = 0.03 \)), and for treated cats that responded to treatment positively versus negatively (6.83 ± 0.41 Vs. 5.99 ± 0.14 \( \log_e \mu g/g \) or 925 Vs. 399 \( \mu g/g \)) (GEE \( P = 0.003 \)) (Table 5). There was a tendency for S-IgA to be greater for Treated cats rated as Content, compared with Control cats rated as Content (6.83 ± 0.41 Vs. 6.56 ± 0.23 \( \log_e \mu g/g \) or 925 Vs. 706 \( \mu g/g \), respectively) (GEE \( P = 0.06 \)). There was no significant difference in S-IgA as a result of differences in source, age or sex (\( P > 0.05 \)).

3.5 Effect of treatment on viral shedding and incidence of clinical URD

Only one Treatment cat (14%) developed URD, on day 23, compared with four (50%) of the Control cats (mean = d7 ± 2.9). The Cox-Proportional Hazards model determined that Control cats were more likely (HR: 2.37, CI: 1.35-4.15) to develop URD over time compared to Treatment cats (\( P < 0.0001 \)).

4.0 Discussion
We wanted to conduct preliminary investigations of whether enrichment, which included time out of the cage together with the learning of a complex behavior, may reduce frustration, increase secretory IgA resulting and reduce risk of contracting upper respiratory infections.

Over the 10 days of confinement, there was a trend for treated cats to be rated as Frustrated less frequently than non-treated cats (Figure 1). Once frustration subsided in both groups, control cats showed a high rate of apathy whilst treated cats became content suggesting that frustration left untreated may be a factor for onset of apathy. Further, apathetic behaviors were not observed in our previous studies of anxious (Gourkow et al 2014b) and content cats (Gourkow et al Phillips, 2015), also suggesting that frustration may be a specific risk factor for apathy in shelter cats. In farm animals, apathy, including loss of interest in the environment, inhibition of feeding, grooming and locomotion may be indicative of sickness (Broom, 2006). However apathy does occur in healthy farm and research animals following failure to avoid aversive conditions, or under conditions of social isolation and behavioral restriction (Broom, 1983; Dawkins, 1988; Mason, 2010; Irwin, 2001).

In this study, cats in the control group exhibited apathetic behaviors, such as abnormal sleeping (>80% of the time) together with inhibition of feeding, grooming and locomotion following on average 6 days of repeated failed attempts to exit the cage (pushing on the door, attempting to open the latch, hanging on the door and biting the cage bars, pawing at floor and walls) and failure to gain the attention of humans in the room (persistent meowing, pawing through door in response to humans in the room). Although sometimes used as an indicator of good welfare in cats (Kessler and Turner, 1997), excessive and deep sleep, particularly when correlated with
inhibition of feeding, grooming and locomotion has been associated with depression (Pedersen and Pratt, 1991) and respiratory infections (McCobb et al., 2005).

Our findings suggest that letting cats out of the cage and providing them with a stimulating activity was beneficial for their health. This was supported by greater levels of S-IgA in cats receiving treatment than in non-treated cats, indicating that the treatment had benefits over and above reducing frustration-related behaviors and preventing onset of apathy. However, other factors may have influenced the stimulation of IgA secretion. Rewarding activities cause dopaminergic neuron activation (DA) and thus increase the production of dopamine associated with a feeling of pleasure (Arias-Carrión and Pöppel, 2007). Rewards, which can be anything that the animal wants and attains, increase DA (Wise, 2006). As there are dopamine receptors in the gastrointestinal tract, where IgA is secreted (Kullmann et al., 1983), and that DA increases blood flow to the mucosa causing mucosal vasodilatation by up to 400% (Sjövall et al., 1984), increased dopamine may have contributed to the stimulation of IgA secretion. Thus, acquiring a desired resource, be it time out of the cage or access to a food reward and human attention during the training session, may have facilitated secretion of IgA, as all aspects of the treatment were potentially rewarding for the cats. Hence, even though it is not clear which aspect may have facilitated contentment, the benefits were apparent and it is even possible that there was a synergistic effect of the two factors. It has been proposed that giving cats a puzzle feeder may be similarly stimulating and rewarding (Rochlitz, 1997). In addition, simply increasing the level of activity can stimulate IgA production (Akimoto et al., 2003). The training session had several components, one of which was increased exercise, with most cats walking to the training room and moving about the room during the training session. The social aspect of the training session
(the presence of a familiar human) may also have contributed to increased IgA. In humans, looking at a picture of a favorite person can increase IgA (Matsunaga et al., 2008).

Control cats were more likely to develop upper respiratory disease over the course of the study than treated cats. Elevated S-IgA has consistently been reported to protect against URD in many species (Hewson-Bower and Drummond, 1996; Brandtzaeg, 2003; Stokes and Waly, 2006; Flies et al., 2012), and conversely IgA deficiency has been associated with persistent symptoms of URD (Brandtzaeg et al., 1987; Renegar and Small, 1991; Janzi et al., 2009). However, in a study of IgA knockout mice (IgA\/-\) generated by gene targeting, IgA was not required for prevention of influenza virus infection and disease, indicating that other Ig’s may serve to decrease susceptibility to URD (Mbawuike et al., 1999).

The small number of cats rated as frustrated upon admission in this study (15 cats out of 250, 6%) may suggest that the problem of frustration is not pervasive in admitted shelter cats, however, cats can become frustrated later during their stay even when rated as Content or Anxious upon admission (Gourkow et al., 2014a; Gourkow et al., 2014c). Moreover, sparse reports of frustration in shelter cat studies may be due to the nature of this problem. Frustration behaviors, with the exception of persistent meowing, occurred in short spurts and for less than 10% of waking hours, during which the cats were pacing, pushing items around the cage, biting the cage bars, pushing on the latch, hanging upside down on the bar, pawing at the floor or walls and destroying the contents of the cage. In between these bouts of frustration behavior, cats appeared calm and were resting or engaged in normal activities such as eating and grooming. In response to a Human Approach Test, Frustrated cats responded similarly to Contented cats.
(Gourkow and Phillips, 2015), in contrast to the cats rated as Anxious, which in 81% of cases responded with defensive retreat and 19% of cases responded with aggression (Gourkow et al., 2014b). Thus, depending on the sampling schedule, frustration behaviors can easily be missed. Similarly, captive tigers believed to be frustrated by confinement spend approximately 23% of their time pacing, 60% resting and the rest in various active behaviors (Mohapatra et al., 2014).

In this study, in addition to observing the behaviors, we also informally observed environmental cues such as a disrupted cage in which the contents within it were repositioned and spilt, as evidence of frustration in cats. A disrupted cage can also be an indicator of anxiety, however intentionally moving items around the cage to create a hiding area (McCune, 1992) mostly occurs at night and mostly in cats which are immobile during the rest of the day (McCune, 1992; Gourkow and Fraser, 2006).

Although some frustration behaviors such as pushing the paw through the cage door or meowing persistently to attract attention may have some potential benefits, as it is a common criterion for selection of an individual for adoption (Gourkow and Fraser, 2006), these behaviors are indicators of poor welfare due to their strong correlation to bar biting, pawing, hanging upside down on the cage railing and attempting to open the cage latch (Gourkow et al., 2014 a). Bar biting in particular, which has not been described in shelter cats previously, has been cited as indicator of poor welfare in sows (Edwards, 2010) and cattle (Wiepkema, 1984).

Aggression observed in this study was unpredictable and mostly occurred during otherwise friendly interaction with staff during cleaning of the cage. We assumed that the aggression may
have been due to irritation in response to the sight and smell of other cats and subsequently redirected towards any suitable target (e.g. staff during cleaning of the cage). However, this type of irritation is believed to last several hours (Beaver, 2004), and cats were not aggressive towards the handler when taken out of the cage for a training session, suggesting that it was not redirected aggression but rather irritable aggression in response to confinement (Beaver, 2004). Evidently it subsided when attaining the desired goal (exiting the cage). Either way, aggression is a risk factor for euthanasia in 95% of Canadian animal shelters (Caffrey et al., 2011) and is therefore an important behavior to address and treat in shelter cats.

The provision of cognitive enrichment by a person requires a time input of at least 10 minutes per cat per day; therefore future research could examine what level of reward is required to achieve the required benefit. Dopamine neurons fire a burst of spikes when the reward value is greater than expected, which is associated with the experience of pleasure (Bressan and Crippa, 2005); conversely they are inhibited when the reward is less than expected (Schultz, 1998). Rewards with less value than expected, delay or inability to acquire desired resources affects dopamine transmission associated with symptoms such as frustration, anhedonia (inability to experience pleasure) and apathy (Roesch et al., 2007; Schultz, 2010; Mitchell et al., 2011). Hence it is essential that sufficient reward is provided if our technique is utilized to control frustration. It is possible that this could be provided by cognitive enrichment of cats in their cages, which would be less time consuming, but this requires further research.

4.1 Limitations of study
While we recognize that this was not a definitive study of frustration in shelter cats, working from a base of 250 cats it provides preliminary indications of the benefits of providing cats with cognitive enrichment. Sample size in our study was small thus the results cannot be generalized to all shelter environments; however the results have important implications for hypothesis generation. In addition, our selection of cats frustrated upon admission may not be representative of the actual occurrence of these emotional states in shelter cats, as it is likely that frustration may occur in cats initially rated as Content but not receiving treatment to help them sustain this positive state. We examined just three potentially confounding factors, age, source and sex, in cats’ response to cognitive enrichment, and did not have sufficient numbers to test differences statistically. There may have been others, such as breed, but they would also have been difficult to detect with the small number of animals in each treatment. In addition our initial model of the emotions of cats in shelters focused on only anxiety, contentment and frustration, and these were validated in physiological tests. In this study we added another emotion, apathy, because it is particularly relevant to frustration studies, but it remains to be validated physiologically.

### 4.2 Conclusions

Enrichment that includes time out of the cage and a training session with a familiar human was shown to reduce frustration-related behavior and prevent the onset of apathy in shelter cats. There were also physiological and health benefits that appeared to derive from the production of intestinal immunoglobulin A. Further research with larger numbers of frustrated cats is warranted, to confirm these promising results with provision of cognitive enrichment.
Acknowledgements

We are grateful to the Morris Animal Foundation (Grant number D09FE-504) for their generous support of this research. We are grateful to Drs. Dean and Lavoy for their analysis of immunoglobulin A. We thank the staff of the British Columbia Society for the Prevention of Cruelty to Animals for access to facilities and support with cat care protocols. We are also grateful to Dr Sara C. Hamon from the Laboratory of Statistical Genetics, Rockefeller University, for assistance with statistical analysis, William Chen from Microtech Canada for his support with videotaping and to Alex Chiu for his diligent help with data collection.

References


Fig. 1: Proportion of cats rated as Content from days 1-10 in the Frustration Treatment (TF) and Control (CF) groups in a study on cognitive enrichment in an animal shelter in Canada. Day 1 n=15 cats: CF = 7, TF=8, Day 2: CF=7, TF= 7, Day 3: CF=7, TF=7, Day 4: CF=7, TF=6, Day 5: CF=7, TF=6, Day 6: CF=7, TF=6, Day 7: CF=7, TF=6, Day 8: CF=6, TF=6, Day 9: CF=4, TF=6, Day 10: CF=4, TF=6.
Table 1: Criteria used for the rating of mood during the intake assessment (one hour observation) and for daily assessment of mood (10 min / hour) in a study on human-cat interaction in an animal shelter in Canada (adapted from Gourkow et al 2014b).

<table>
<thead>
<tr>
<th>Mood / behavior</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frustrated</strong></td>
<td>Behavior observed &gt;10 % of awake time</td>
</tr>
<tr>
<td>Meow</td>
<td>Persistent and loud meowing, not related to anticipation of food Engaging in one or more behavior in a repetitive or persistent pattern: standing on hind limbs, pawing wall or floor, pushing paw through door, pushing on door latch, hanging on cage door with body inverted, biting or licking cage bars*.</td>
</tr>
<tr>
<td>Escape bouts</td>
<td>Persistent visual scanning of all areas of the cage</td>
</tr>
<tr>
<td>Scan</td>
<td>Hits or throws objects around the cage in a destructive manner using head, body or paws (not related to play) Spills food bowls, and litter. Pressing body or head on cage door.</td>
</tr>
<tr>
<td>Pace</td>
<td>Persistent, repetitive and rapid locomotion at cage door or circle pattern. Unpredictable, short burst of aggression such as biting or scratching during otherwise friendly interaction with a human (not accompanied by defensive behavior or vocalizations).</td>
</tr>
<tr>
<td>Aggression</td>
<td>All Anxiety behaviors and lie on side.</td>
</tr>
</tbody>
</table>

| **Absent**      | |
| **Content**     | Behavior observed > 80% per 24 hour |
| Sleep / rest    | Lying down, relaxed body posture with eyes closed or semi closed. |
| Lie on side     | Lying on flank, body and tail stretched, neck and ventral area exposed. |
| Front sit       | Sitting upright at the front of the cage, calmly observing activities. |
| Groom           | Licks body or paws, rubs head with paws (without chewing or pulling coat). |
| Eat / Drink     | Takes food or water into mouth. Does not spill food or water around the cage. |
| Walk            | High body posture, normal gait, functional locomotion to access areas of the cage (not on-going or repetitive). |
| Friendly        | Approaches and interacts with humans in an amicable manner. |
| Rub             | Rubs body or head on objects and cage door. |
| Absent          | All Anxiety and Frustration behaviors. |

<p>| <strong>Anxious</strong>     | Behavior observed &gt; 80% per 24hour |
| Flat            | Low body posture when lying down, sitting or standing for locomotion. |</p>
<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze</td>
<td>Remains completely immobile, body and head flattened, eyes wide open.</td>
</tr>
<tr>
<td>Hide</td>
<td>Body fully or partially hidden under bedding, behind or in litter box.</td>
</tr>
<tr>
<td>Crawl</td>
<td>Slow locomotion while keeping body flattened close to the ground.</td>
</tr>
<tr>
<td>Startle</td>
<td>Sudden and brief tensing of the body.</td>
</tr>
<tr>
<td>Retreat</td>
<td>Avoidance of human usually by retreating to the back of the cage (often accompanied by lip licking*).</td>
</tr>
<tr>
<td>Inhibition</td>
<td>Sleep, grooming, locomotion, drink/eat (particularly in the presence of humans).</td>
</tr>
</tbody>
</table>

**Absent** All other Contentment behaviours and all Frustration behaviours.

* Lip licking and bar biting were not included in the original study (Gourkow et al., 2014) but were added to this study as they were exclusively and frequently observed together with other anxiety and frustration behaviors respectively.

** Apathy was added to this study because the relevant behaviors were observed frequently.
Table 2: Proportion of cats rated as frustrated on admission in each rating category and days to first rating for Treated and Control groups in a study on cognitive enrichment in an animal shelter in Canada.

<table>
<thead>
<tr>
<th></th>
<th>Frustrated</th>
<th>Content</th>
<th>Apathetic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of cat days</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(and proportion)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, total 67</td>
<td>41 (61%)</td>
<td>18 (27%)</td>
<td>8 (12%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Treated, total 55</td>
<td>28 (51%)</td>
<td>27 (49%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Days to first rating</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(and number of cats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1± 0.0 (8)</td>
<td>4.4 ± 0.5 (5)</td>
<td>6 ± 0.0 (2)</td>
<td>0.09</td>
</tr>
<tr>
<td>Treated</td>
<td>1 ± 0.0 (7)</td>
<td>3.2 ±1.3 (4)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Positive and negative responses to treatment for cats given mood ratings of content or frustrated on the day of treatment in a study on cognitive enrichment in an animal shelter in Canada.

<table>
<thead>
<tr>
<th>Mood Rating (n= days)</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content (27 days)</td>
<td>2 0.07</td>
<td>25 0.93</td>
</tr>
<tr>
<td>Frustrated (28 days)</td>
<td>5 0.18</td>
<td>23 0.82</td>
</tr>
</tbody>
</table>
Table 4: Response to treatment according to characteristics, age [(senior and adults) versus juveniles], sex (male versus female) and source (owner-surrendered versus stray) in a study on cognitive enrichment in an animal shelter in Canada.

<table>
<thead>
<tr>
<th></th>
<th>Negative n (%)</th>
<th>Positive n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senior and adults</td>
<td>1 (14%)</td>
<td>24 (50%)</td>
</tr>
<tr>
<td>Juveniles</td>
<td>6 (86%)</td>
<td>24 (50%)</td>
</tr>
<tr>
<td>Females</td>
<td>6 (86%)</td>
<td>14 (29%)</td>
</tr>
<tr>
<td>Males</td>
<td>1 (14%)</td>
<td>34 (71%)</td>
</tr>
<tr>
<td>Owner-surrendered</td>
<td>6 (86%)</td>
<td>14 (29%)</td>
</tr>
<tr>
<td>Strays</td>
<td>1 (14%)</td>
<td>34 (71%)</td>
</tr>
</tbody>
</table>
Table 5: Effect of response to treatment on s-IgA (µg/g) in control and treatment groups and according to response to treatment in a study on cognitive enrichment in an animal shelter in Canada.

<table>
<thead>
<tr>
<th>Groups &amp; responses to treatment</th>
<th>Mean IgA</th>
<th>SEM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.04</td>
<td>0.240</td>
<td>0.027</td>
</tr>
<tr>
<td>Treated</td>
<td>6.73</td>
<td>0.178</td>
<td></td>
</tr>
<tr>
<td>Positive response</td>
<td>6.83</td>
<td>0.0361</td>
<td>0.003</td>
</tr>
<tr>
<td>Negative response</td>
<td>5.99</td>
<td>0.103</td>
<td></td>
</tr>
</tbody>
</table>