Conditioning laboratory cats to handling and transport

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As research subjects, cats have contributed substantially to our understanding of biological systems, from the development of mammalian visual pathways to the pathophysiology of feline immunodeficiency virus as a model for human immunodeficiency virus. Few studies have evaluated humane methods for managing cats in laboratory animal facilities, however, in order to reduce fear responses and improve their welfare. The authors describe a behavioral protocol used in their laboratory to condition cats to handling and transport. Such behavioral conditioning benefits the welfare of the cats, the safety of animal technicians and the quality of feline research data.

Fear responses in laboratory animals negatively impact their welfare, their ability to learn to new tasks and their value as research subjects[1]. Fear is a distressing emotion focused on particular objects or situations[2]. It can manifest in a range of behavioral responses, from escape behavior to defensive aggression[3], and a range of physiological 'stress' responses. In cats, stress responses may include tachycardia, increased blood pressure[4] and elevation of cortisol due to activation of the hypothalamic-pituitary axis. Stress can also cause behavioral changes such as increased hiding[5] or decreased food intake and social interactions[6].

Behavioral conditioning, the process by which an animal forms an association between a particular behavior and a particular reinforcing stimulus[3], can attenuate fear responses in laboratory animals. Conditioning has been used to reduce stress responses in numerous laboratory animal species, including rhesus macaques[7], dogs[8] and cats[9]. Reducing stress responses that can lead to changes in physiologic and behavioral measures[10,11] improves data quality and reliability. Conditioning animals to a research protocol prior to data collection can result in the animals becoming more cooperative subjects.

Positive reinforcement training, a type of behavioral conditioning, is an effective way to decrease stress responses in laboratory-housed chimpanzees related to handling[12]. In cats, positive reinforcement training may be more effective than forceful training techniques involving coercion[13]. Although cats are predators by nature, when restrained or confined, they exhibit many behaviors characteristic of prey species including flight reactions to noise and disturbance, avoidance responses to unfamiliar individuals and defensive reactions[14,15]. These types of responses can have substantial negative effects on the welfare of the cats, can decrease the quality of data collected in studies using cat and can inhibit the cats' ability to acquire new learned behaviors. Therefore, minimizing cats' fear responses and distress in research situations is important, especially for studies that target behavioral or physiological endpoints.

Many articles have been published about conditioning laboratory animal species, including dogs[16], primates[17–19], and rabbits[20,21]. Yet, although some recommendations have been made regarding housing and enrichment for laboratory cats[22], there is little information available about conditioning cats for handling and transport in a laboratory setting. To facilitate handling and transport of cats in our laboratory, our research group developed a protocol for conditioning singly

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housed laboratory cats to be handled by personnel and routinely transported in a cat carrier to another location for behavioral testing and sampling. In our experience, cats can be conditioned in the relatively short time span of ~1–3 weeks, depending on the cat. The time invested in conditioning benefits the welfare of the cats, the safety of animal technicians and the quality of feline research data.

TECHNIQUE

After transportation from a Class A commercial vendor to our laboratory animal facility, cats undergo an institutionally mandated 14-d quarantine prior to the start of any research studies in order to ensure their health status and to allow them time to acclimate to their new surroundings (R. Fish, personal communication). Following quarantine, the cats are individually housed without collars in large enclosures with a floor area of 1.37 m^2 and a height of 1.88 m (Kitty Condo: Ultralite Products, Inc., Rogue River, OR). Each enclosure is furnished with a litter pan, hiding boxes and elevated resting shelves, and enrichment toys are provided on a revolving schedule. The enclosures exceed the minimum space requirements recommended by the *Guide for the Care and Use of Laboratory Animals*[13]. While group housing is the standard recommendation for laboratory cats[13], our project, approved by our IACUC, requires individual housing to prevent disease transmission between infected and sham-infected individuals. Cats are in visual and vocal contact with conspecifics housed in the same room.

Upon arrival at the facility, cats often display a range of fearful behaviors, ranging from mild to profound. Many of the cats are reluctant to approach the front of the enclosure and, when people enter the room, will retreat to their hiding areas. They also may show other signs of timidity or even defensive aggression. Eventually, as they habituate to their new surroundings, the cats begin to display affiliative behavior toward familiar caretakers. However, they persist in fearful responses, and may exhibit escape or defensive behaviors, when handled for anything other than routine feeding, exercise and enclosure cleaning and when they encounter unfamiliar people.

Our research involves longitudinal behavioral studies which require that a research technician be able to approach a cat without it showing escape or retreat behaviors and easily transport the cat to the behavioral testing room using a cat carrier. In addition, research technicians must be able to weigh the cats and restrain them for physical examination and venipuncture on a predetermined schedule. Therefore, our overall goals were for each cat to voluntarily exit its enclosure, enter a cat carrier, calmly be transported within our facility and tolerate routine handling and restraint for physical examination. To meet these goals, we used a three-phase protocol to condition each cat to our behavioral research staff and handling procedures.

Conditioning protocol

We used the following protocol to condition a group of 13 young (6–18 months old), male, neutered, Class A, purpose-bred, specific-pathogen-free cats. The conditioning protocol began as soon as the quarantine period was completed. Each conditioning session lasted approximately 15–20 min per room (~6–7 cats per room), and sessions took place once daily on consecutive weekdays. The three phases were implemented consecutively; once each cat reached the goal or criterion for each phase, we moved onto the next phase of conditioning for that cat. Any other activities in the room, such as cleaning and husbandry, were suspended during each conditioning session. Within our research group, all research technicians who participate in behavioral testing were required to participate in the process of conditioning the cats as described. Multiple research technicians work with the cats; the desired response needed to be generalized to many individuals, not to just one familiar person. While conditioning the cats, we followed three general principles: i) adapt the conditioning protocol to meet individual behavioral requirements of each cat; ii) work slowly and in a positive manner; and iii) strive to maintain each individual cat below its threshold for anxiety and fear.

Phase one. The goal of the first phase of our protocol was to have the cat come to the front of its enclosure when a research technician approached and remain there while the door to the enclosure was opened. This behavior allows a research technician to enter the enclosure and to observe the cat without having to retrieve it from a hiding box. We used commercially available food treats (Whiskas Cat Treats, Mars, Inc., McLean, VA; Pounce Cat Treats, Del Monte Foods, San Francisco, CA) and highly palatable wet food (a/d, Hills Pet Nutrition, Inc., Topeka, KS) as primary reinforcers[2] during this phase. We chose these food reinforcers through unsystematic trials with each cat; individual cat preferences were noted on log sheets and used by the research staff. Whenever a research technician entered the room, he or she would gently place treats into each enclosure, regardless of the cat's response. Each cat's reaction was noted, and cats that hid in their boxes or crouched in the back of their enclosures were identified so that we could schedule increased time for conditioning those cats to the first phase. Over time, as the cats began to orient toward the research technicians (i.e., raising their heads or approaching the door), the treats were placed closer to the front of the enclosure. Once a cat reliably came forward to retrieve a treat while the research technician was in the room, the research technician increased his or her proximity to the enclosure.

FIGURE 1 | A cat willingly approaches the research technician as she comes close to the enclosure. The technician gives the cat a treat.

FIGURE 2 | A cat willingly enters a carrier for transport.

Next, the technician opened the door to the cat's enclosure and placed a small amount of canned cat food in a dish on the floor ~30 cm inside the enclosure door. Meanwhile, the research technician remained immobile outside the enclosure, trying to remain as neutral and unimposing as possible, and refraining from looking at, leaning toward or speaking to the cats. This last step was repeated, each time moving the food closer to the door of the enclosure, until the cat reliably came forward to receive its treat in the near presence of the research technician (**Fig. 1**). For our group of cats, an average of two sessions (ranging from one to three sessions) was required to reach this criterion.

Phase two. In the second phase of conditioning, the goal was to train the cat to allow gentle handling. As in the first phase, a research technician opened the enclosure door, placed a food treat on the floor and remained in place while the cat approached to eat its treat. While the cat was eating, the research technician first extended his or her hand toward the cat and spoke quietly to the cat. The research technician then gradually reached for, touched and, finally, petted the body and handled the neck (collar area) of each cat, withdrawing his or her hand if the cat showed any fear or crouching response. For our group of cats, an average of two sessions (ranging from one to four sessions) was required to reach this criterion.

Phase three. In the third and final conditioning phase, the goal was to train the cat to be transported in a cat carrier. First, the research technician brought into the room a cat carrier that is large enough to accommodate each cat comfortably. If the cat showed a reaction to the presence of the cat carrier, such as retreating, hiding or being unwilling to come to the front of the enclosure, the technician moved the carrier to the far side of the enclosure room. Then the technician gave the cat treats while the carrier remained stationary in the room. This procedure was continued until the cat resumed taking treats from the front of the enclosure while the carrier was in the room. Next, the technician gradually moved the carrier closer to the enclosure until it could be placed in front of the enclosure while the cat remained at the front of the enclosure. Then, the research technician opened the door of the carrier and placed treats inside, allowing the cat to explore the carrier (**Fig. 2**). Once the cat freely entered the carrier and consumed the treats, the technician closed the door to the carrier for 5–10 s with the cat inside (**Fig. 3**). He or she then opened the door and placed treats inside the cat's home enclosure to train the cat not to run when the door was opened and to efficiently exit the carrier after transport and return to its enclosure. This procedure was repeated, gradually increasing the amount of time the cat was inside the carrier with the door closed up to ~2 min. Finally, the research technician closed the carrier door with the cat inside, picked up the carrier

FIGURE 3 | A cat tolerates having the door of the carrier closed.

and carried it around the room. Then he or she opened the carrier door near the door to the cat's enclosure and placed treats inside the enclosure. The third phase was completed when each cat reliably approached the front of its enclosure, willingly entered a cat carrier and later exited the cat carrier after a variable amount of time and willingly entered its enclosure. In our group of cats, an average of seven sessions (ranging from six to nine sessions) was required to reach this criterion. All 13 cats were successfully trained to enter and exit the cat carrier for transport.

CONCLUSIONS

We trained cats in our laboratory to tolerate handling and transport using the conditioning approach described. We found that once a cat was trained, it continued to be easy to handle and willing to be transported in a carrier for a month or longer. We believe that this conditioning protocol could also be used to train cats for other husbandry tasks, such as being weighed on a scale, increasing both the efficiency and the safety of those procedures. The cats and the research staff both benefited from the time invested in this training; the cats showed decreased fear and distress responses and were easier to handle, and the biological relevance of behavioral and physiologic measures taken with these cats was increased.

The length of time required to condition each cat varied, but all cats were successfully conditioned within a few weeks. When working with more timid cats, our recommendation is always to end a conditioning session with a positive experience. In our laboratory, if a fearful response was noted at any time, the research technician would return to a previously successful task and reward that behavior again. By allowing more timid cats as much time as they needed to complete each phase, we were able to achieve our goal of making all the cats in our colony easy to handle and transport for our experiments.

It is important for cats to be familiar with the personnel doing the training. The effect of familiarity on animal behavior is well documented[11], and cats may be more willing to perform learned behaviors with familiar people. In fact, we observe that when unfamiliar staff members approach the cats, they consistently retreat to their hiding boxes. In contrast, our cats appear to recognize the research technicians that participate in the conditioning program and come to the front of their enclosures as soon as a technician appears. In our laboratory, all new staff members familiarize themselves to the cats by carrying out an accelerated version of the conditioning protocol described above. As has been documented in dogs[23], trained tasks will transfer to new handlers as long as the principles of handling remain the same. These practices ensure consistent handling and optimize the welfare of the cats.

In keeping with the 3-Rs principle of reduction[24], all of our cats were part of a larger study and needed to be willing participants for behavioral testing. Therefore, the whole population of cats participated in behavioral conditioning, and we did not include a control group of cats that did not undergo conditioning for comparison purposes. Although we observed a marked improvement in the cats' behavior and willingness to be handled by the staff members that participated in the conditioning protocol, these observations are subjective, and the lack of a control group makes an objective evaluation of this observed effect impossible. Despite this limitation, this short report should serve to increase awareness of conditioning in cats and detail the ease and efficiency with which a conditioning protocol may be completed. Future studies are needed to evaluate the effects of behavioral conditioning in cats in comparison with a control group that did not undergo a conditioning program. Standardized scoring of cats' behavior after conditioning, for example, using the Cat-Stress-Score[25], and the collection of physiological data, including heart rate and fecal[26] or urinary cortisol[27] measurements, would provide further objective evaluation of the effects of conditioning in cats.

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