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Conditioning Laboratory Cats to Handling and Transport

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Fear responses in laboratory animals negatively impact their welfare, their ability to learn to new tasks, and their value as research subjects[1]. Fear may be defined as a distressing emotion focused on particular objects or situations[2]. It may be manifest in a range of behavioral responses, from escape behavior to defensive aggression[3], and a range of physiological “stress” responses.

One method that may be used to attenuate fear responses in laboratory animals is behavioral conditioning, the process in which an animal forms an association between a particular response and a particular reinforcement situation[3]. Conditioning has been used to reduce stress responses in numerous laboratory animal species, including rhesus macaques[4], dogs[5], and even cats[6]. By conditioning animals to a research protocol prior to data collection, animals may become cooperative subjects.

With conditioning, stress responses that can lead to changes in physiologic and behavioral measures can be avoided[7,8], thereby improving data quality and reliability. In cats, stress responses may include tachycardia, increased blood pressure[9], elevation of cortisol due to activation of the hypothalamic-pituitary axis, and behavioral changes such as increased hiding[10] or sickness behaviors such as decreased food intake and social interactions[11]. Conditioning, using positive reinforcement training, has been shown to be an effective way to decrease stress responses in laboratory housed chimpanzees when handled[12]. In cats, positive reinforcement training, versus forceful training techniques involving coercion, may be especially important[13]. Although cats are predators by nature, when restrained or confined, they may 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 significant negative effects on the welfare of the cats, can decrease the quality of data collected, and inhibit the acquisition of new learned behaviors. Ensuring that fear responses and distress are minimized is a critical step, especially when behavioral or physiological endpoints are targeted.

Many articles have been published about conditioning other laboratory animal species, including dogs[16], primates[17–19], and rabbits[20,21]. Although some recommendations have been made regarding housing and enrichment for laboratory cats[22], there is a paucity of information in the literature 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 housed laboratory cats to handling by personnel and to routine transport in a cat carrier to another location for behavioral testing and sampling. In our experience, conditioning of cats can be accomplished in a relatively short time, approximately one to three weeks depending on the cat. The time invested benefits the welfare of the cats, the safety of animal technicians, and the quality of feline research data.

Upon arrival at our facility after transportation from a Class A commercial vendor, cats often display a range of behaviors, from mildly to profoundly fearful. Many of the cats are reluctant to approach the front of the enclosure and will retreat to their hiding areas when people enter the room. They may show other signs of timidity or even defensive aggression. At our laboratory animal facility, the cats complete an institution-mandated 14 day quarantine period prior to the start of any research studies in order to ensure their health status and provide a minimum period for stabilization/acclimation (Dr. Richard Fish, DACLAM, personal communication). Eventually, as they habituate to their new surroundings, the cats begin to display affiliative behavior toward familiar caretakers. However, they persist in fearful responses when handled for anything other than routine feeding, exercise, and enclosure cleaning and when encountering unfamiliar people. At these times, they may exhibit escape or defensive behaviors.

Following quarantine, the cats are individually housed in large enclosures[a] (floor area of 1.37 m^2, height of 1.88 m), without collars. Each enclosure is furnished with a litter pan, hiding boxes, elevated resting shelves, and enrichment toys 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 housed cats[13], our project, approved by our institutional animal care and use committee, 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. The cats participate in longitudinal behavioral studies that require them to be approached by research technician without showing escape or retreat behaviors, and be easily transported to the behavioral testing room via a cat carrier. In addition, the cats are restrained for physical examination, weight evaluation, and venipucture on a predetermined schedule.

Our overall goals are for each cat to be able to voluntarily exit its enclosure, enter a cat carrier, be calmly transported within our facility, and to tolerate routine handling and restraint for physical examination. To meet these goals, we apply the following general principles: 1) adapt the conditioning protocol to meet individual behavioral requirements of

[a]Kitty Condo: Ultralite Products, Inc., Rogue River OR

each cat, 2) work slowly and in a positive manner, and 3) strive to work below each individual cat's threshold of anxiety and fear.

Our laboratory employs a three phase protocol for conditioning cats to behavioral research staff and handling procedures. The conditioning protocol begins as soon as cats complete their quarantine period. Each conditioning session lasts approximately 15 – 20 minutes per room (approximately 6–7 cats/room), and sessions are conducted once daily on consecutive weekdays until phase criterion is reached. Any other activities in the room, such as cleaning and husbandry, are suspended during a conditioning session. This allows the research staff and the cats to obtain the best and most efficient results. The three phases of the protocol and data from a group of 13 young (6 months-1.5 year old), male neutered, Class A, purpose-bred specific pathogen free cats are detailed below.

Phase One

The first phase of our protocol is designed to develop a positive association between each cat and the research technicians. The goals of this phase are to have each cat come to the front of its enclosure when research technicians approach, and to remain in the front when the door to the enclosure is opened. This allows research technicians to monitor the cat's appearance and to enter the enclosure without having to retrieve the cat from a hiding box. For this to be accomplished, we use commercially available food treats[b],[c] and highly palatable wet food[d] as primary reinforcers[2] to establish a positive association with the appearance of these people in front of their enclosure. These food reinforcers were chosen by unsystematic trials with the cats; individual cat preferences were noted on log sheets and used by the research staff.

To begin the first phase, initially treats are gently tossed into enclosures whenever a research technician enters the room, regardless of the cat's response. Multiple research technicians work with the cats; the desired response needs to be generalized to many individuals, not to just one familiar person. Each cat's reaction is noted, and cats that hide in their boxes or crouch in the back of their enclosures are identified so that they can be scheduled for increased time to condition to the first phase. Over time, as cats begin to orient toward research technicians, including raising their heads or approaching the door, the treats are given closer to the front of the enclosure. Once a cat reliably comes forward to retrieve a treat while the research technician is in the room, the research technician increases her proximity. The door to each cat's enclosure is opened and a small amount of canned cat food is placed in a dish on the floor approximately 30 cm inside the enclosure door, while the research technician remains immobile outside the enclosure. Each research technician remains as neutral and least imposing as possible, not looking at, leaning toward, or speaking to the cats. This step is repeated, incrementally moving the food closer to the door of the enclosure until the cat is reliably coming forward to receive its food treat in the near presence of the research technician (FIGURE 1). For our population of cats to reach this

[b]Whiskas® Cat Treats: Mars, Inc., McLean, VA
[c]Pounce® Cat Treats: Del Monte Foods, San Francisco, CA
[d]a/d®: Hills Pet Nutrition, Inc., Topeka, KS

criterion, this phase lasted an average of 2 once a day sessions over two days with a range of 1–3 once a day sessions over 1–3 days.

Phase Two

Once cats reliably move to the front of the enclosure when research technicians approach, they begin the second phase: training the cats to allow gentle handling. The goal of this phase is for cats to allow research technicians to open the enclosure, speak quietly, reach for, and handle the cat's neck (collar area) without evoking a fear or crouching response. This phase builds on our initial phase, the process by which the cat was conditioned to approach when a research technician open the enclosure door, placed a food treat on the floor, and remained in place. Phase two begins with an extension of the hand while the cat is eating. Over the following sessions, the research technician gradually reaches for, touches, and finally pets the body and handles the neck of each cat. For our group of cats, this phase took an average of 2 once a day sessions over 2 days, with a range of 1–4 once a day sessions over 1–4 days.

Phase Three

The final conditioning phase, training the cat to a cat carrier and transport, is begun once the cats will allow the research technician to allow gentle petting of the torso and collar area without retreating or showing a fear response. The goal for this phase is for cats to approach the front of their enclosure, willingly enter a cat carrier, and then exit the cat carrier after a variable amount of time and willingly enter their enclosure. For this final phase, a cat carrier that is large enough to comfortably accommodate each cat is brought into the room. If the cats show a reaction to the presence of the cat carrier, such as retreating, hiding, or unwillingness to come to the front of the enclosure, the carrier is moved to the far side of the enclosure room and the cat is given treats while the carrier remains stationary until the cat is comfortable with the carrier in the room and resumes taking treats from the front of the enclosure. The carrier is gradually moved closer to the enclosure until it is able to be placed in front of the enclosure with the cat remaining at the front of the enclosure. Then the research technician opens the door of the carrier and tosses treats inside, and allows the cat to explore the carrier (FIGURE 2). Once the cat will freely enter the carrier and consume the treats, the door to the carrier is closed for 5–10 seconds with the cat inside (FIGURE 3). The cat is then released, and treats are placed inside his enclosure. Tossing treats inside the enclosure is an important step, as it trains the cat not to bolt when the door is opened, and to efficiently exit the carrier following transport and return to its enclosure. This is repeated while gradually lengthening the amount of time the cat is inside the carrier with the door closed, up to approximately 2 minutes. Finally, the carrier door is closed with the cat inside, and the carrier is picked up and carried around the room. The carrier door is then opened near the door to the cat's enclosure, and treats are tossed inside the enclosure. In our cats, this phase took an average of 7 once a day sessions over 7 days, with a range of 6–9 once a day sessions over 6–9 days, and all cats were able to be trained to both enter and exit the cat carrier for transport.

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. The effect of familiarity on animal behavior is well documented[8]. In fact, we observe that when unfamiliar staff members approach the cats, they consistently retreat to their hiding boxes. New staff members become "familiar" by working with the cats in an accelerated version of the described protocol. Similarly, in dogs[23], trained tasks will transfer to new handlers as research staff availability changes as long as the principles of handling remain the same. We have found that once trained, even after a month or more the cats persist in their ease of handling by familiar staff and willingness to be transported by carrier.

As described here, cats in our laboratory were able to be trained to tolerate handling and transport easily and in a relatively short period of time. The number of days for conditioning varied with the cats' individual temperaments, but all cats were successfully conditioned. By allowing more timid individuals to have as much time as needed to pass each phase, we were able to achieve our goal of having cats that were willing participants in handling and transport. Our general guideline, when working with more timid cats, is to always end a conditioning session with a positive experience. If a fearful response is noted at any time, the research staff would return to a previously successful task, even if simply approaching the enclosure, and reward that behavior again. In our laboratory, both cats and research staff benefit from the time invested in this training as the cats are easier to handle and show decreased fear and distress responses during handling. This training can also be transferred to other husbandry tasks, such as being weighed on a scale using food treats as a lure, and will increase both the efficiency and safety of these procedures. The effect of familiarity with personnel doing the training is important, and cats may be more willing to perform these tasks with persons with whom they are familiar. Our population of cats appears to recognize the research technicians that participate in the conditioning program, and come to the front of their enclosures as soon as they appear. This not only increases the ease and ability of research staff to work with the cats and their rapport with the cats, but increases the accuracy of behavioral and physiologic measures, and the welfare of the laboratory cat.

In an effort to be cognizant of the "3-R" principle of reduction[24], all of our cats needed to be willing participants in our behavioral testing, as they were part of a larger study. We feel that our conditioning protocol increases our ability to work with the cats, therefore, this whole population of cats participated in behavioral conditioning, and this represents a limitation of the paper. While we feel that there is striking improvement in the cats' behavior and willingness to be handled by the staff members that participate in the conditioning protocol, this is very subjective, and the lack of a control group makes an objective evaluation of this impossible. Our intention with this short report is to increase awareness of conditioning in cats, and detail the ease and efficiency with which a conditioning protocol may be completed. Future studies that look at the effects of behavioral conditioning in cats using a control group that did not receive a program of conditioning, as well as standardized scoring such as the Cat-Stress-Score[25], would be a valuable addition to the literature. Physiologic data, including heart rate and fecal[26] or urinary cortisol[27] measurement would provide further objective evaluation of the effects of conditioning in cats.

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FIGURE 1.
Cats will willingly approach the research technicians as they come close to the enclosure.

FIGURE 2.
Cats willingly enter their carriers for small food treats.

FIGURE 3.
Cats begin to tolerate having the door of the cat carrier closed and being transported in a carrier from room to room.