Published in final edited form as:

Am J Primatol. 2012 August ; 74(8): 712–720. doi:10.1002/ajp.22015.

Positive Reinforcement Training in Squirrel Monkeys Using Clicker Training

Timothy E. Gillis, Amy C. Janes, and Marc J. Kaufman

McLean Imaging Center, McLean Hospital, Harvard Medical School, Belmont, MA 02478, USA

Abstract

Nonhuman primates in research environments experience regular stressors that have the potential to alter physiology and brain function, which in turn can confound some types of research studies. Operant conditioning techniques such as positive reinforcement training (PRT), which teaches animals to voluntarily perform desired behaviors, can be applied to improve behavior and reactivity. PRT has been used to train rhesus macaques, marmosets, and several other nonhuman primate species. To our knowledge, the method has yet to be used to train squirrel monkeys to perform complex tasks. Accordingly, we sought to establish whether PRT, utilizing a hand-box clicker (which emits a click sound that acts as the conditioned reinforcer), could be used to train adult male squirrel monkeys (*Saimiri boliviensis*, N=14). We developed and implemented a training regimen to elicit voluntary participation in routine husbandry, animal transport, and injection procedures. Our secondary goal was to quantify the training time needed to achieve positive results. Squirrel monkeys readily learned the connection between the conditioned reinforcer (the clicker) and the positive reinforcer (food). They rapidly developed proficiency on 4 tasks of increasing difficulty: target touching, hand sitting, restraint training, and injection training. All subjects mastered target touching behavior within 2 weeks. Ten of 14 subjects (71%) mastered all tasks in 59.2 ± 2.6 days (range: 50–70 days). In trained subjects, it now takes about 1.25 minutes per monkey to weigh and administer an intramuscular injection, one-third of the time it took before training. From these data, we conclude that clicker box PRT can be successfully learned by a majority of squirrel monkeys within two months and that trained subjects can be managed more efficiently. These findings warrant future studies to determine whether PRT may be useful for reducing stress-induced experimental confounds in studies involving squirrel monkeys.

Keywords

Positive Reinforcement Training; Clicker Training; Operant Conditioning; Conditioned Reinforcer; Squirrel Monkey; *Saimiri*

Introduction

Nonhuman primates living in research laboratory environments experience regular stressors including physical/chemical restraint, phlebotomy procedures, and they experience stress during husbandry practices such as homecage cleanings/transfers and weighing [Prescott & Buchanan-Smith, 2003]. As chronic stress can alter nonhuman primate physiology, brain structure, and brain function [Coe et al., 1982; Lyons et al., 2007; Patel et al., 2008] and can

confound some types of research studies [Lyons et al., 2000], it is advantageous to carry out routine procedures in ways that minimize stress [Laule et al., 2003].

It has been proposed that operant conditioning methods can be used to improve laboratory nonhuman primate behavior and cooperation. One behavioral training method, positive reinforcement training (PRT), utilizes operant conditioning techniques to teach animals to voluntarily perform simple behaviors, for which they earn incentives [Schapiro et al., 2003]. Training that engenders voluntary behavior can modify an animal's perception from learned helplessness to cooperative participation, and can mitigate stresses associated with the conduct of routine laboratory procedures [Prescott & Buchanan-Smith, 2003; Desmond & Laule, 1994]. In this way, PRT may help reduce animal anxiety, possibly yielding studies with greater data reliability. Furthermore, PRT may facilitate reduced use of anesthesia or sedation in some procedures as well as reduced use of negative reinforcement strategies [Veeder et al., 2009] such as a squeeze cage apparatus. PRT also may help alleviate social problems, ease introductions between animals and their caretakers or to novel stimuli, diminish stereotypic behaviors [Coleman & Maier, 2010], augment enrichment measures, and increase safety for research staff [Laule et al., 2003].

A number of examples have been published showing that PRT is effective in nonhuman primates. Reinhardt [2003] utilized a PRT method that obviated the need to use a squeeze cage (which itself can increase stress and anxiety levels by confining subjects to a small space), to enhance macaque cooperation during blood collection procedures. Scott et al. [2003] utilized PRT to train rhesus macaques to depart from and return to their home cages without the use of the pole and collar system. Coleman et al. [2008] used this method to train macaques to insert their arms into a sleeve mechanism used for venipuncture. Macaques first were rewarded for approaching and then touching the sleeve mechanism, and then gradually were trained to insert their arms into the apparatus and remain stationary for up to two minutes to allow personnel to conduct blood draws.

A typical PRT strategy temporally links presentation of a positive reinforcer (e.g., food) with a bridge [Veeder et al. 2009] or conditioned reinforcer. Much research has explored one type of PRT referred to as clicker training. Clicker training (described below) is commonly used in companion animals and in other animal species and has become a common training methodology for use with laboratory primates. In a study by Schapiro et al. [2003], clicker training was used in macaques initially to train basic targeting behaviors and then used to facilitate training on more complex behaviors. Fernström et al. [2009] used the clicker method to train several behaviors including targeting, collaborating, box-training, and initial injection training.

Clicker training in nonhuman primates also has been used in common marmosets to train subjects to voluntarily undergo home cage weighing and to provide urine samples [McKinley et al. 2003]. As clicker PRT has been successfully used in multiple laboratory nonhuman primate species, including chimpanzees [Coleman et al. 2008; Videan et al. 2005], rhesus macaques [Shapiro et al. 2003; Fenstrom et al. 2009], and sooty mangabeys [Veeder et al. 2009] among others, we explored whether a PRT approach could be used to train squirrel monkeys (*Saimiri boliviensis*), another NHP species commonly used in laboratory research. In a preliminary study presented by Rogge et al. [2011], PRT was used to train both owl monkeys and squirrel monkeys to touch a target and present a hand. Our primary goal was to determine whether it is possible to develop and implement a PRT regimen involving several more complex behaviors key to laboratory management of squirrel monkeys, to see if it was possible to elicit monkeys' voluntary participation in basic husbandry, animal transport, and injection procedures. We conducted our study in a colony of 14 adult male squirrel monkeys, by building on initial training of simple targeting

behavior similar to that used by Rogge et al. [2011]. Our secondary goal was to quantify the training time investment needed to achieve positive results, to determine whether PRT can be implemented economically.

2. Methods

2.1 Subjects

Subjects consisted of fourteen healthy male Black-Capped Squirrel Monkeys (*Saimiri boliviensis*) obtained from the University of South Alabama Primate Breeding and Research Laboratory, aged 2–3 years, with average weights between 680g – 980g. Initially, subjects were individually housed in standard squirrel monkey 2-tiered cage racks, (24"×17"×27", stainless steel, grid floor, cylindrical 6" steel perch, squeeze-back mechanism). Subjects were received in 3 separate cohorts over a year and a half. After six weeks in quarantine for tuberculosis testing, and an additional 4–6 week acclimation period, subjects were pair housed in standard macaque cages (26"×24"×30", stainless steel, grid floor, cylindrical 24" steel perch, squeeze-back mechanism, Figure 1, Top).

Subjects were fed a diet of commercial monkey pellets (LabDiet 4045). Feedings occurred twice daily with rations of 3–4 pellets in the AM and 4–5 pellets in the PM, with both feedings coming after training sessions had been completed. Subjects also were provided with an assortment of fresh fruit, peanuts, baby carrots and mealworms. The housing room ran on a 12-hr light/dark cycle of 7 AM to 7 PM. Each cage contained a plastic swing made from a small curved section of PVC pipe and white plastic chain supplied from a local hardware store. Hanging mirrors supplied by Primate Products Inc. also were provided in each cage. A variety of colorful toys were placed on the cage floor. A small 13" color television was used to provide visual enrichment. A radio also played music from various local radio stations during daylight hours. All subjects were fitted with specially made polypropylene collars with 2" long, 1.8mm trade twisted stainless steel chains with ½" diameter split rings. The chains were attached to the collars with colorful zip-ties to assist in subject identification. This method provided a safe and effective means to handle animals for weighing and transport, and also allowed for the pole and collar method to be used for veterinary care and injection administration.

Each subject underwent weekly weight checks. The majority of animals slowly increased from their baseline body weight by a range of 50–100g over the course of sixteen months. It would be difficult to posit with any certainty the cause of weight gains, which could be attributable to any of the following: fattening cycles of males as they reach sexual maturity, the addition of the training food reinforcers to the diet, or natural growth in captivity [DuMond & Hutchinson, 1967; Ausman et al., 1985.]

All procedures were conducted in compliance with established guidelines as described in the National Institutes of Health Guide for Care and Use of Laboratory Animals. In addition, all procedures were reviewed and approved by the McLean Hospital Institutional Animal Care and Use Committee (IACUC). This research also adhered to the legal requirements of the USA and to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non Human Primates. The studies described in this manuscript took place at McLean Hospital during the period from fall 2006 to spring 2008.

2.2 Training Tools and Criteria Establishment

Training Tools—The primary step in the clicker training process is establishing the significance of the “click” sound. A hand-held box clicker, available at www.clickertraining.com or at a local pet store) served as the conditioned reinforcer. Squirrel monkeys were presented with the conditioned reinforcer (the click sound), which

was immediately followed by a positive reinforcer, a palatable food item such as Cheerios. Initially, conditioned and positive reinforcers were paired every 30 seconds for 10-minute trials. A more significant reinforcer, such as a mini marshmallow, grape, peanut, or banana slice (alternated daily to vary rewards), was provided after the final reinforcer pairing of each trial. This method was used in all ensuing training to signal to the subject that the current trial was completed and that they were being rewarded for successful completion of ten desired responses. Once it became evident that subjects understood that a click sound preceded a treat (two trials each), the conditioned reinforcer was used to signal to a monkey that he was engaging in a desired behavior. Using the click instead of a primary reinforcer allowed for tighter temporal coupling between a desired behavior and a reward. Thus, monkeys were better able to identify behaviors they were encouraged to perform.

Behavioral Training—Each subject was trained on a daily basis (Monday – Friday). Trials were conducted 1–6 times per day depending upon the difficulty of the task being trained. For example, more target training trials per animal can be completed per unit time than injection training trials, which take longer to complete. Each trial lasted until 10 correct responses were made. The conditioned reinforcer was presented immediately after the desired behavior and signaled the availability of a positive reinforcer. Food rewards (positive reinforcers) were pre-arranged to be easily accessible, yet hidden from subject view. Cheerios cereal pieces (subjects preferred yogurt flavored Cheerios) were used and broken into small enough portions to be eaten quickly. These were used for the first 9 correct behavioral responses. A more significant reinforcer (described above) was used for the tenth and final response of each trial. A stopwatch was utilized to time each trial and a notebook/journal was used to log each subject's performance on each trial.

Performance Criteria—A behavior was considered learned when a subject could consistently and confidently repeat it successfully 10 times within 2 minutes. In some instances, subjects were able to repeat a behavior consistently, however they hesitated or exhibited fear responses (vocalizations such as alarm peeps, cackling and yapping [Winter et al. 1966], nervous grabbing/pulling, excessive hand rubbing, self-scratching [Bassett et al. 2003], evading to the back of the cage, acute diarrhea or biting at the trainer). In these cases, a task was repeated until a subject could perform it without exhibiting fearful behavior. The sequence of training tasks is shown in Table 1.

2.3 Basic Training – Targeting

Stationary Targeting—Trials began by presenting a green Flexible Original Nylabone (target) at the front of the home cage. The clicker was hidden to ensure that subjects focused on the target. Subjects initially were shaped [Laule, et al. 2003] to approach the target. Each time a subject moved closer to the target, the conditioned reinforcer was presented, followed by the positive reinforcer. Once the target was touched by a finger or hand, touching became the new criterion to receive reinforcement. When the target was touched, the conditioned reinforcer immediately was presented followed immediately by the positive reinforcer. Once a subject consumed the positive reinforcer, the process was repeated. As subjects mastered the behavior (by performing this task 10 times within 120 seconds), the criterion was altered to require touching of a moving target.

Moving Target and Duration Touching—The target was presented at different locations at the front of the cage, and subjects were reinforced by touching the target at different locations. When the task was mastered (10 touches within 120 seconds), the criterion was altered to require subjects to hold the target. Subjects initially were trained to hold the target for 2s and upon mastery were required to hold it for up to the 5s. Any incorrect holds (of durations differing from the required time) were noted and not rewarded.

After this behavior was mastered, targeting behavior was applied to more practical laboratory tasks.

2.4 Handling Training

Foot Targeting—Training began by changing the target from the green nylabone to a yellow one (by wrapping it with yellow vet wrap) to avoid confusing the subject, since this task required a foot (but not a hand touch) response. The target was presented through the cage bars. Initially, subjects would incidentally touch the target with one foot while climbing around the cage, a behavior that was rewarded. Once one-foot touching was deliberate and could be repeated, the criterion was changed to require touching by both feet, followed by the requirement to sit on the target for up to 5 seconds or until the conditioned reinforcer was presented. Foot target training was applied further to acclimate subjects to being caught without the use of the wire technique (use of a wire with a small hook on the end to “fish” for the subject’s collar chain, in order to pull it through the cage bars to attach to a metal leash) or strenuous glove catching.

Glove Desensitization—The foot target was held in the palm of a protective gloved (Inoculator 1550 by Hex Armour) hand inside the cage opening. Subjects were taught to sit on the target. Subjects were required to repeat this task until they could sit on the target without hesitation. Once this criterion was achieved, the target was removed and subjects were taught to sit on the gloved hand itself. The shaping process began with subjects just touching the glove palm with one foot and jumping off. This was followed by touches with both feet and then by sitting for up to 5 seconds in the glove palm. This progression desensitized subjects to the presence of the trainer’s gloved hand in the cage.

Collar Chain Holding—Next, subjects were trained to accept being caught/held by the short chains hanging from their collars. The shaping process involved presentation of a thumb and index finger so as to pinch and hold the subject’s chain. Initial trials rewarded subjects for moving close to the trainer’s fingers and touching them. Once subjects performed this step repeatedly in the allotted time, subjects were trained to accept the trainer touching their chains. The trainer positioning a gloved hand inside the subject’s home cage in the same location each time while extending the thumb and index finger. The subject was rewarded for approaching the hand and allowing the trainer to touch the chain with at least one finger. As subjects met the criterion, subjects next were required to accept trainer holding of the chain. The conditioned reinforcer and positive reinforcer were given whenever a subject’s chain was held for up to 3 seconds without struggling or resistance. Next, while holding a subject’s chain, the trainer would lift the subject into the air as it held onto or perched on the trainer’s hand.

Chain (Leash) Attachment—Training continued by attaching a longer chain/leash, approximately 24”, to the subject’s chain with a 1.5” swivel eye bolt snap (chain material and swivel eye bolt snaps provided by McMaster-Carr). Subjects were presented with the conditioned reinforcer and positive reinforcer each time the chain was successfully clipped and unclipped from their collars for a total of 10 repetitions. Criterion was considered reached when this could be done consistently within 120 seconds.

Pole Training—The next phase involved introducing a standard squirrel monkey handling pole. Subjects then were required to perform the same target training/sitting behavior as before on the pole. Subjects initially were rewarded for touching the pole with one foot. Next, subjects were rewarded for jumping on and off the pole with both feet. Finally, subjects were rewarded for staying on the pole for up to 5 seconds.

Chain and Pole Method—Leash attaching and pole sitting training then were paired together. Once the leash was attached, it was threaded through the eyelet at the end of pole. Next, repetitions first of pulling subjects taut to the pole and then loosening began. Once subjects comfortably accepted being pulled taut to the pole without resistance (pulling away) or fear (alarm calls, nervous hand rubbing or scratching, etc.) and repeatedly performed this behavior 10 times within 2 minutes, it was considered mastered. The final step of this training segment required subjects to be moved outside of their home cages while holding comfortably and calmly onto the pole. Once subjects mastered this task, injection training began.

2.5 Injection Training

Tail, Leg and Syringe Training—A chain was attached to the subject's collar and threaded through the pole eyelet. Chains then were pulled tight, allowing each subject to grasp onto the pole for removal from his home cage. The proximal part of each subject's tail gently was grasped and held against the pole, allowing the monkey to support itself with hands and feet. The conditioned reinforcer and the positive reinforcer were presented each time a subject calmly cooperated with this task. When subjects could repeatedly perform this behavior 10 times within 2 minutes, the behavior was considered mastered. After subjects were comfortable with tail holding, their tails were gently pulled toward the end of the pole, lengthening the subject's body along the length of the pole. Each time the subject performed this behavior without resistance, he was rewarded. This training continued until subjects could repeat this behavior 10 times within 2 minutes.

Next, each subject was extended gently along the length of the pole and the trainer held his leg. As subjects became comfortable with this procedure, their legs (alternating between left and right) then were gently straightened along the length of the pole. When leg stretching could be performed consistently with both legs without resistance or struggling, 10 times in 2 minutes, it was considered learned. Subjects then were presented with a capped needle and syringe while extended along the pole. The needle and syringe were pressed to the subject's thigh muscle with some pressure to simulate an injection. This was repeated until subjects allowed 10 repetitions within 2 minutes without moving, trying to grab the syringe, or exhibiting behaviors emblematic of fear, such as distress calls or trying to pull their leg away to avoid syringe contact.

3. Results

3.1 Targeting

All 14 subjects successfully learned basic targeting behavior (Figure 2). Training to criterion took a mean (SE) of 10.9 ± 0.4 days (range: 10–13 days, Figure 3). It took 23.8 ± 1.1 trials for subjects to learn targeting behavior, including stationary, moving target and duration touches (Figure 4). The fastest subject mastered basic targeting behavior in only 13 trials while the slowest subject took 28 trials to meet the same criterion.

3.2 Yellow Target/Hand-Sitting

Thirteen out of 14 subjects learned the yellow target/hand-sitting task (Figure 1 Middle; Figure 2). Mastery of this task took on average (SE) 16 ± 1.0 days (range: 11–22 days, Figure 3) and 31.6 ± 2.0 trials (range: 24 – 43 trials, Figure 4). One subject did not meet criterion in the required amount of time. Although that subject did perform required behaviors, he did not do so without exhibiting stressful and fearful behaviors (vocalizations, hesitation and avoidance).

3.3 Handling Training: Chain Holding through Pole and Leash

Ten of 14 subjects mastered handling training (Figure 2). These subjects accomplished this task on average in 17 ± 3.4 days (range: 13 – 25 days, Figure 3) over 40 ± 2.8 trials (range: 30–58 trials, Figure 4). Four subjects did not achieve task criteria. Three of these subjects would not consistently allow the trainer to hold their chain and clip on a leash without extended periods of avoidance, presentation of alarm calls, or fearful biting of the trainer's gloved hand. The fourth subject did not meet the chain holding criteria at all. He would allow the trainer to touch the chain briefly but would avoid or pull away from the trainer if he felt any sense of being held in place.

3.4 Injection Training -Tail, Leg and Syringe Training

Injection training was completed successfully by 12 of 13 subjects. The 3 shaping stages took on average (SE) 14.8 ± 1.2 days (range: 11–25 days, Figure 3) and 23.6 ± 1.7 trials (range: 16 – 36 trials, Figure 4). One subject did not meet criteria in the training time. He was very fearful out of his home cage on the pole and never acclimated to having the trainer hold his legs.

3.5 Overall Training Summary

Ten of 14 monkeys (71%) met training criteria on all tasks, taking on average (SE) 118.4 ± 4.1 trials (range: 98 – 138 trials) and 59.2 ± 2.6 days (range: 50 – 70 days) to meet all criteria.

4. Discussion

The goal of this study was to determine whether PRT could be implemented as an effective laboratory behavioral training strategy for use with squirrel monkeys. Our results indicate that clicker training qualifies as a method capable of eliciting a number of desired responses from squirrel monkeys. The time investments needed to establish the positive reinforcer and clicker relationship and apply it to train several routine laboratory behaviors were minimal (just over 2 months on average). Squirrel monkeys readily recognized the connection between the clicker and the reward and rapidly developed a willingness to work on shaping processes in order to receive food reinforcers for their performance of more complex tasks.

While there was a substantial amount of inter-individual variation in performance of the different tasks, our data show that the fundamental targeting behavior can be successfully learned within 2 weeks. Rogge et al. [2011] showed that squirrel monkeys could learn a simple targeting behavior in 1.6 ± 0.8 training sessions. Our data expands upon this initial finding to show that with an additional 20 or so trials, animals can learn to target a moving object as well as to touch and hold onto that object for up to 5 seconds. Our data also show that using targeting behavior as a foundation, the majority of monkeys (71%) could be trained to master a set of more complex behaviors within an 8-week training period. Thus, it appears that PRT methods such as clicker training can be used in squirrel monkeys as they have been used in other nonhuman primate species including macaques [Coleman et al. 2008, 2010; Fernstrom et al. 2009], chimpanzees [Lambeth et al. 2006; Videan et al. 2005; Pomerantz and Terkel 2009], marmosets [Bassett et al. 2003, McKinley et al. 2003], and sooty mangabeys [Veeder et al. 2009].

Clicker training PRT served a dual purpose. It taught subjects to voluntarily cooperate during routine laboratory procedures while at the same time, reduced the need to use practices that normally would induce stressful responses. Presentation of novel stimuli (target, foot target, gloves and pole) initially evoked behaviors commonly displayed only when the animals were under stress. These behaviors were rapidly extinguished after a few

PRT trials in the majority of animals. Not surprisingly, tasks requiring the most amount of time to train were those that took the longest to alleviate fear/stress responses, including hand sitting and chain holding. Lambeth et al [2006] showed through collection of physiological data (hematology and serum chemistry profiles) PRT was effective at reducing stress levels in captive chimpanzees. Pomerantz and Terkel's [2009] work also tested the affects of PRT on the wellbeing of zoo-housed chimpanzees. Their data suggests that PRT serves as an effective method to reduce abnormal and stress-related behaviors, promote prosocial affiliative behaviors and reduce aggression toward caretakers. We hypothesize that our training methods would produce similar physiological and behavioral improvements in trained versus untrained squirrel monkeys.

During the hand-sitting task, squirrel monkeys displayed fearful vocalizations and behaviors such as alarm calls, avoidance and the occasional defensive response of biting at the trainer's gloved hand. Subjects taking the longest to learn this task, upwards of 20 days, would cycle through 5–6 successful trials followed by periods of regression, characterized by more frequent fearful behaviors, refusal to train, and non-acceptance of food rewards [Videan et al. 2005].

It is interesting to note that prior to initiating this research, our technique used to transfer subjects from dirty to clean cages or for weighing procedures was to catch them with large Kevlar gloves. Thus, our subjects may have developed a fear response and sensitization to the appearance of gloves that may have delayed their desensitization, prolonging the time it took to reach criteria on hand sitting behavior. We hypothesize that had our clicker method glove training been initiated in glove-naïve animals, more monkeys may have mastered this task more quickly than we observed.

A similar pattern was observed for chain holding behavior. While some subjects easily learned to perch on the glove, some became fearful when the glove was moved directly toward them. We found that it was possible to gradually shape acceptance of this process by slow conditioning. However, once restraint was applied by holding onto chains, subjects would regress to avoiding the glove altogether. This regressive pattern was prolonged in 3 of 14 monkeys. Ultimately, these 3 subjects learned the behavior, however some hesitation and fearful behavior persisted.

Similarly, in the handling task, when some monkeys were pulled taut to the pole, this restraint caused them to exhibit behaviors indicative of stress similar to those described in the work of Down et al [2005]. Thus, the restraint step required more trials than required for simpler tasks. In the syringe presentation task, many subjects initially attempted to grab and pull the syringe out of the trainer's hand. Over time, subjects became familiar with the syringe stimulus and ignored it. However, since part of the real stimulus involves a needle stick, training needs to continue with trials including sticks, in order desensitize the most uncomfortable part of this task. In our studies, 2 of 4 animals that receive regular saline injections (because they serve as control subjects in a longitudinal drug administration study) hesitate leaving their home cage when they anticipate receiving an intramuscular injection.

The PRT method we applied to train squirrel monkeys on routine laboratory behaviors proved successful and the time investment required to achieve success was economical (approximately 2 months). Clicker PRT helped subjects overcome fear through a structured training and conditioning regimen and engendered voluntary participation on tasks previously met with varying degrees of resistance. Trained subjects now are more easily managed and most subjects readily volunteer for cage changing and weighing. Training has obviated the need to use the pole and collar method for these husbandry procedures and

monkeys readily sit on the trainer's gloved hand to be either transferred to a clean cage or the weighing box (Figure 1 Middle and Bottom). We no longer need to use glove-catching procedures, which eliminates the stress induced by this form of capture and restraint [Bowers et al. 1998] and eliminates the risk of an animal being injured or getting loose in our housing room.

Our training regimen not only benefits our subjects but also saves substantial amounts of time during the conduct of routine procedures. For example, it now takes about 45 seconds to administer an intramuscular injection and 60 seconds to weigh a subject; by contrast, before we began our training regimen, the completion time for the same procedures took about 3 times longer (~135 seconds to administer an IM injection and 3 minutes to weigh each subject). Training thereby saves approximately two-thirds the amount of time it takes to complete our injection and husbandry weighing procedures compared to pre-training times. Because it took on average 60 days (2 months) to train each animal, at month 3 post-training, we began saving 2/3 the amount of time per month to perform our monthly activities. Similarly, in months 4 and 5, we also saved 2/3 of the initial time. Therefore, by the end of month 5, we estimate that we had saved six-thirds (or 2 months) time. Thus, our training program essentially "paid back" the time invested within 5 months time. Moreover, since many of the monkeys reached criteria before the 60-day mark, time savings already were accruing before full competency had been reached by all subjects.

Further, our subjects seem to enjoy participating in new clicker training activities, as evidenced by their excitement behaviors such as vocalizing peeps and chirps when the trainer enters the room and trilling and twittering when food rewards are visible. Shaking behaviors such as banging swings against the cage wall also are commonly displayed whenever the trainer sets up [Winter, et al. 1966]. Because of this, we believe our training regimen provides environmental enrichment for the animals.

It is possible that our results would have been improved had we started the study using an already-optimized training regimen. In this regard, at training onset, we experimented with different food reinforcers to characterize reward preference and consumption rates to determine the most effective and rapidly consumed reward. We found that the smaller and most readily chewed and swallowed food worked the best. In addition, some early trials were used to identify an appropriate target stimulus, an item that could be easily manipulated by the trainer but robust enough to withstand the repeated grabbing force imposed by squirrel monkeys. We also wish to point out that the criteria we selected to establish mastery of particular behaviors was arbitrary. However, criteria were selected because they served as stepping-stones to condition more complex desired behaviors, and we believe the criteria set provided useful indices of progress and mastery. Thus, it is possible that different results could be obtained if different tasks are used or different criteria are established. Further, the reward-based learning paradigm we used may itself introduce confounds into certain research designs (e.g., those that utilize other types of reward based reinforcement). Notwithstanding these limitations, we believe that our initial results warrant additional studies to optimize the use of PRT with clicker training in squirrel monkeys, and to assess whether the training method reduces stress hormone levels.

In summary, we found that the clicker training PRT method can be economically applied and can be beneficial both for managing and for providing environmental enrichment to laboratory squirrel monkeys. PRT reduced overall fear and perceived stress, and thus has the potential to eliminate or reduce stress-induced experimental confounds in laboratory squirrel monkeys.

Acknowledgments

Supported in part by NIH grants R01DA09448 and K01DA017324.

References

- Ausman, LM.; Gallina, DL.; Nicolosi, RJ. Nutrition and metabolism of the squirrel monkey. In: Coe, CL.; Rosenblum, LA., editors. *Handbook of Squirrel Monkey Research*. New York: Plenum Press; 1985. p. 349-378.
- Bassett L, Buchanan-Smith H, McKinley J. Effects of Training on Stress-Related Behavior of the Common Marmoset (*Callithrix jacchus*) in Relation to Coping With Routine Husbandry Procedures. *Journal of Applied Animal Welfare Science*. 2003; 6:221–233. [PubMed: 14612270]
- Bowers C, Crockett C, Bowden D. Differences in stress reactivity of laboratory macaques measured by heart period and respiratory sinus arrhythmia. *American Journal of Primatology*. 1998; 45:245–261. [PubMed: 9651648]
- Coe CL, Franklin D, Smith ER, Levine S. Hormonal responses accompanying fear and agitation in the squirrel monkey. *Physiology and Behavior*. 1982; 29:1051–1057. [PubMed: 6298843]
- Coleman K, Maier A. The use of positive reinforcement training to reduce stereotypic behavior in rhesus macaques. *Applied Animal Behavior Science*. 2010; 124:142–148.
- Coleman K, Pranger L, Maier A, Lambeth SP, Perlman JE, Thiele E, Schapiro SJ. Training rhesus macaques for venipuncture using positive reinforcement techniques: a comparison with chimpanzees. *Journal of the American Association for Laboratory Animal Science*. 2008; 47:37–41. [PubMed: 18210997]
- Desmond T, Laule G. Use of positive reinforcement training in the management of species for reproduction. *Zoo Biology*. 1994; 13:471–477.
- Down N, Skoumbourdis E, Walsh M, Francis R, Buckmaster C, Reinhardt V. Pole-and-collar training of macaques: a discussion by the Laboratory Animal Refinement and Enrichment Forum. *Animal Technology and Welfare*. 2005; 4:157–161.
- Dumond FV, Hutchinson TC. Squirrel monkey reproduction: The “fatted” male phenomenon and seasonal spermatogenesis. *Science*. 1967; 158:1067–1070. [PubMed: 4964560]
- Fernström AL, Fredlund H, Spångberg M, Westlund K. Positive reinforcement training in rhesus macaques-training progress as a result of training frequency. *American Journal of Primatology*. 2009; 71:373–9. [PubMed: 19195008]
- Lambeth S, Hau J, Perlman J, Martino M, Schapiro S. Positive reinforcement training affects hematologic and serum chemistry values in captive chimpanzees (*Pan troglodytes*). *American Journal of Primatology*. 2006; 68:245–256. [PubMed: 16477594]
- Laule GE, Bloomsmith MA, Schapiro SJ. The use of positive reinforcement training techniques to enhance the care, management, and welfare of primates in the laboratory. *Journal of Applied Animal Welfare Science*. 2003; 6:163–173. [PubMed: 14612265]
- Lyons DM, Parker KJ, Zeitzer JM, Buckmaster CL, Schatzberg AF. Preliminary evidence that hippocampal volumes in monkeys predict stress levels of adrenocorticotrophic hormone. *Biological Psychiatry*. 2007; 62:1171–1174. [PubMed: 17573043]
- Lyons DM, Lopez JM, Yang C, Schatzberg AF. Stress-level cortisol treatment impairs inhibitory control of behavior in monkeys. *Journal of Neuroscience*. 2000; 20:7816–7821. [PubMed: 11027246]
- McKinley J, Buchanan-Smith HM, Bassett L, Morris K. Training common marmosets (*Callithrix jacchus*) to cooperate during routine laboratory procedures: ease of training and time investment. *Journal of Applied Animal Welfare Science*. 2003; 6:209–220. [PubMed: 14612269]
- Patel PD, Katz M, Karssen AM, Lyons DM. Stress-induced changes in corticosteroid receptor expression in primate hippocampus and prefrontal cortex. *Psychoneuroendocrinology*. 2008; 33:360–367. [PubMed: 18222612]
- Pomerantz O, Terkel J. Effects of positive reinforcement training techniques on the psychological welfare of zoo-housed chimpanzees (*Pan troglodytes*). *American Journal of Primatology*. 2009; 71:687–695. [PubMed: 19434627]

- Prescott MJ, Buchanan-Smith HM. Training nonhuman primates using positive reinforcement techniques. *Journal of Applied Animal Welfare Science*. 2003; 6:157–161. [PubMed: 14612264]
- Reinhardt V. Working with rather than against macaques during blood collection. *Journal of Applied Animal Welfare Science*. 2003; 6:189–197. [PubMed: 14612267]
- Rogge JR, Sherenco KD, Malling R, Thiele E, Schapiro SJ, Lambeth SP, Williams LE. Establishing a positive reinforcement training program in neotropical primates: a comparison of squirrel and owl monkeys [Abstract]. *American Journal of Primatology*. 2011; 73 (Suppl):84. [PubMed: 20665715]
- Schapiro SJ, Bloomsmith MA, Laule GE. Positive reinforcement training as a technique to alter nonhuman primate behavior: quantitative assessments of effectiveness. *Journal of Applied Animal Welfare Science*. 2003; 6:175–187. [PubMed: 14612266]
- Scott L, Pearce P, Fairhall S, Muggleton N, Smith J. Training nonhuman primates to cooperate with scientific procedures in applied biomedical research. *Journal of Applied Animal Welfare Science*. 2003; 6:199–207. [PubMed: 14612268]
- Veeder C, Bloomsmith M, McMillan J, Perlman J, Martin A. Positive Reinforcement Training to Enhance the Voluntary Movement of Group-Housed Sooty Mangabeys (*Cercocebus atys atys*). *Journal of the American Association for Laboratory Animal Science*. 2009; 48:192–195. [PubMed: 19383217]
- Videan EN, Fritz J, Murphy J, Borman R, Smith HF, Howell S. Training captive chimpanzees to cooperate for anesthetic injection. *Lab Animal*. 2005; 34:43–48. [PubMed: 15861158]
- Winter P, Ploog D, Latta J. Vocal Repertoire of the Squirrel Monkey (*Saimiri sciureus*), its Analysis and Significance. *Experimental Brain Research*. 1966; 1:359–384. [PubMed: 4958770]

Figure 1.

Top: Pair-housed squirrel monkeys in their home cage; Middle: Subject being transported on gloved hand; Bottom: Subject voluntarily entering a weighing box.

Figure 2.
Numbers of subjects meeting performance criteria on each task (total N=14).

Figure 3.

Days to meet criteria on each task and on all tasks for monkeys mastering all tasks (N=10)

Figure 4.

Trials to meet criteria on each task and on all tasks for monkeys mastering all tasks (N=10).

Table 1**PRT Training Protocol**

PRT Protocol
1. Establish secondary-primary reinforcer relationship
2. Target Training
Stationary target
Moving target
Duration touch
3. Foot Targeting
One foot touch
Two feet touch
Duration touch
4. Glove Desensitization
Glove and foot target
Glove alone touch
Glove duration touch
5. Collar Chain Holding
Fingers touch
Allowing chain touch
Allowing chain hold
Lifting subject
6. Chain Attachment
Clipping/unclipping chain
7. Pole Training
Pole touching
Pole sitting
8. Chain and Pole
Pulling taut to pole
Removal from home cage
9. Injection Training
Tail hold
Pole extension
Leg hold
Leg extension
Capped needle presentation