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Training Rhesus Macaques for Venipuncture Using Positive Reinforcement Techniques: A Comparison with Chimpanzees

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As more emphasis is placed on enhancing the psychological well-being of nonhuman primates, many research facilities have started using positive reinforcement training (PRT) techniques to train primates to voluntarily participate in husbandry and research procedures. PRT increases the animal's control over its environment and desensitizes the animal to stressful stimuli. Blood draw is a common husbandry and research procedure that can be particularly stressful for nonhuman primate subjects. Although studies have demonstrated that chimpanzees can be trained for in-cage venipuncture using PRT only, fewer studies have demonstrated success using similar techniques to train macaques. It is often assumed that macaques cannot be trained in the same manner as apes. In this study, we compare PRT data from singly housed adult rhesus macaques (*Macaca mulatta*; n = 8) with data from group-housed adult chimpanzees (*Pan troglodytes;* n = 4). All subjects were trained to place an arm in a 'blood sleeve' and remain stationary for venipuncture. Both facilities used similar PRT techniques. We were able to obtain repeated blood samples from 75% of the macaques and all of the chimpanzees. The training time did not differ significantly between the 2 species. These data demonstrate that macaques can be trained for venipuncture in a manner similar to that used for chimpanzees.

Abbreviation: PRT, positive reinforcement training

With increased emphasis placed on enhancing the psychological well-being of nonhuman primates, many research facilities are using positive reinforcement training (PRT) techniques to train primates to voluntarily participate in husbandry and research procedures such as blood sampling and administration of injections. PRT is becoming accepted as an important component of behavioral management programs for nonhuman primates.[1,29] Positive reinforcement techniques are a form of operant conditioning[30] in which the trainer reinforces desired behaviors (such as coming to the front of the cage) by rewarding the animal when it performs the target behavior.[19,27] By allowing the subject to exercise choice in how they perform these behaviors,[10,13] PRT increases the animal's control over its environment (for example, the subject can choose not to cooperate). PRT also desensitizes the animal to stressful stimuli (such as injections), thereby increasing overall well-being and creating a better experimental subject. PRT techniques generally are preferred over negative reinforcement training, in which the subject performs a desired behavior to avoid or escape from a negative stimulus.[6,10]

The first step in positive reinforcement training is to pair a primary reinforcer (for example, a desired food item) with an initially neutral stimulus (such as a whistle or click) to establish this stimulus as a secondary reinforcer.[10,12] Subjects then are trained to perform various tasks by reinforcing successive movement (that is, shaping) toward the desired behavior.[10,19] Each time the subject performs a behavior successively closer to the target behavior, the trainer reinforces it by 'clicking' (or using another secondary reinforcer) and then rewarding with a food treat.

Positive reinforcement techniques have been used to train nonhuman primates to perform various veterinary and research procedures,[3,21,27] including moving a body part (a thigh) close to the front of the cage for exams or injections,[18,29,32] taking oral medications,[7] and remaining still for blood pressure measurement[31] or blood samples.[11,18,32] PRT also has been used to decrease aggression and increase affiliative behavior in group-housed primates.[28]

One common veterinary procedure that can be particularly stressful for primates is venipuncture. In many facilities, animals either are sedated or put into special cages and restrained for blood collection, both of which can be stressful, and can alter the levels of stress hormones such as cortisol.[24] Although chimpanzees can be trained for in-cage venipuncture using PRT,[9] few studies have reported success in using PRT only to train macaques.[17] In the present investigation, we demonstrate that macaques can be trained by using positive reinforcement training only and that the training takes a reasonable amount of time.

Materials and Methods

Subjects. The subjects for this study were 8 adult (age, 7 to 10 y) male rhesus macaques (*Macaca mulatta*). Monkeys were housed singly (for experimental purposes) at the Oregon National Primate Research Center, in double cages (48 × 27 × 32 in.) located in a single room. All animals were rated as exploratory in a simple behavioral assessment.[5] The monkeys were assigned to a study in which they were fed twice daily with a high-fat diet (Test Diet, Richmond, IN) and provided produce and other treats daily. Water was available ad libitum. The lights were

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Figure 1. Adult male rhesus macaque with arm in blood sleeve. The monkey was trained to put his arm in the blood sleeve and hold onto the peg at the distal end until released by the trainer. While 1 trainer worked with the monkey, another provided the secondary reinforcement (clicking) and the juice bottle, which was removed if the monkey did not perform the appropriate behavior. The blood sleeve for the chimpanzees was similar.

on 12 h per day, from 0700 to 1900, and the temperature was maintained at 24 ± 2 °C.

In addition, 4 adult (16 to 43 y) chimpanzees (*Pan troglodytes*, 3 males and 1 female) were used for this study. Chimpanzees were socially housed in either indoor–outdoor runs or corrals[25] at the Department of Veterinary Sciences of The University of Texas MD Anderson Cancer Center (Bastrop, TX). Social groups ranged in size from 2 to 15 animals. Animals were fed a commercially available pelleted diet and fresh produce for a total of 6 meals daily. Water was available ad libitum.

Subjects at both facilities participated in behavioral management programs to ensure their psychological health and well-being. Animal care programs at both facilities are compliant with federal and local regulations regarding the care and use of research animals and accredited by AAALAC International. Studies at both institutions were approved by the respective institutional animal care and use committee.

Training. The ultimate training goal for all subjects was to place an arm in a 'blood sleeve'[18] and remain stationary for blood draw (Figure 1). Both facilities used a similar training plan (Table 1). Because the chimpanzees were housed in a social group, they were trained to approach a designated place in their enclosure, where sleeve training occurred. They had also been previously trained to present a body part for injection prior to blood sleeve training. The monkeys had no previous training experience.

Subjects were trained to place an arm in the blood sleeve and remain stationary during venipuncture through a process of reinforcing successive approximations of the target behaviors. The first step was to desensitize[10] the subjects to the presence of the blood sleeve by rewarding them when they made movements to inspect or touch it. Subjects then were trained to put their arm in the sleeve and hold a peg located at the end of the sleeve (Figure 1). To encourage subjects to continuously hold the peg, we provided constant access to a juice bottle with a favorite drink (fruit-flavored beverage for monkeys and fruit juice for chimpanzees). If the subject broke position (for example, pulled arm out of the sleeve) prior to being released by the trainer, the trainer would immediately both remove the continuous reinforcement of the juice bottle and cue the desired behavior. Once the subject was in the desired position, the trainer immediately reinforced with the secondary reinforcer (for example, clicking), and the juice bottle was reinstated. This procedure by which we selected to reinforce the higher quality of behavior (for example, keeping arm in sleeve) while not reinforcing the lower quality behavior (for example, pulling arm out) is referred to as selective reinforcement.[20] All subjects used the bottle, except for 1 rhesus monkey and 1 chimpanzee. These animals were provided with constant access to small treats during training. The shaping plan is detailed in Table 1.

Training sessions for the rhesus monkeys were approximately 5 to 10 min (average, 5.1 min) in length, 2 to 3 times per week. Training sessions for chimpanzees were also approximately 5 to 10 min (average, 7.3 min) in length, but subjects were trained 1 to 4 times per week. The length of the training sessions was dictated by the attitude of the subject. Sessions were stopped if the subject lost focus on the trainer or became agitated. Chimpanzees were considered to be reliably trained to give blood in the blood sleeve when blood was obtained in 4 of 5 sessions. Rhesus macaques have relatively small brachial veins, and the phlebotomist was not always able to draw blood even if the monkey remained stationary during the whole procedure. Therefore, the rhesus were considered to be reliably trained when they remained stationary and allowed venipuncture (including redirections of the needle) in 4 of 5 sessions.

Although the trainers working with the macaques had less experience with PRT (0.5 to 3 y) than did those working with chimpanzees (2 to 5 y experience), the macaque trainers themselves were trained in PRT techniques by the personnel who worked with the chimpanzees. The more experienced personnel also traveled to the macaque facility to assess training efforts and were available for consultations, thus ensuring similar philosophies and methodologies of nonhuman primate training between the facilities.

Blood sleeve. The blood sleeve[18] used in this study was originally designed at the chimpanzee facility as a way to safely obtain blood samples from the apes. The original design was modified for use with the rhesus monkeys in the current study (Figure 2). The back plate of the rhesus sleeve (made of 0.5-in. thick acrylic) was designed to cover the opening of the cage, where it was held in place on the top and bottom with hooks. The length of the circular arm sleeve (made of 0.128-in. thick acrylic) was 9.5 in. and the diameter was 2.75 in. The top of the arm sleeve was cut off, to allow access to the brachial vein for venipuncture and blood draw.

Statistics. For each subject, we calculated the amount of time (in minutes) and the number of sessions that it took to reliably perform the task (that is, put arm in blood sleeve and accept blood draw according to the described criteria). The assumptions of normality and homoscedacity were tested for all variables. Because transformations did not normalize the data, we used nonparametric analyses to examine differences between the 2 species. Data are presented as mean ± SEM. Alpha values were set at 0.05. SYSTAT 11 (Systat Software, San Jose, CA) was used for all analyses.

Results

Using predominantly positive reinforcement with some selective reinforcement techniques, we were able to train all

Table 1. Shaping plan for voluntary blood collection using the blood sleeve

Step	Description
1	Desensitize (that is, expose subject to stimulus to make stimulus neutral)[20] the subject to the presence of the blood sleeve by hanging sleeve on cage and rewarding any exploration of or movement toward sleeve.
2	Establish cue (a signal that will elicit a behavior) for putting arm in sleeve and holding peg.
3	Reinforce (that is, reward) progressive movement toward touching sleeve, including sitting in front of the blood sleeve, putting arm in sleeve, and touching peg.
4	Reinforce subject for holding onto peg. Work on increasing the duration of time monkey will spend holding peg (up to 30 s).
5	Desensitize the subject to the implements needed during the blood collection procedure (for example, alcohol swabs, gauze pads, vacuum phlebotomy tubes, needles).
6	Desensitize subject to touching sensation (with neutral object such as capped pen) on the ventral forearm. Slowly increase duration of time (up to 1 min).
7	Desensitize to the sensation of a capped needle on forearm. Work on increasing duration (up to 2 min).
8	Desensitize to the sensation of pressure on the vein without actually breaking skin by utilizing a blunt needle resting on top of the skin. Do not pierce. Work on increasing duration (up to 2 min).
9	Desensitize to real needle just beneath the skin. Work on duration (up to 2 min). Give bonus (a large, special treat; for example, big piece of banana or can of cola for chimpanzees, large piece of banana for monkeys) to subject for allowing blood to be drawn.
10	Once the subject reliably allows blood draw, transfer the behavior to appropriate personnel (for example, veterinary or research staff).

8 monkeys to put their arms in the sleeve, hold the peg until released by the trainer, and allow insertion of a needle. Six of these 8 animals were reliably trained for our target behavior and allowed blood draws. As expected, training time varied considerably across subjects: some learned the task quite easily, whereas others took much longer. An average of 257.5 ± 30.9 min (50.3 ± 5.1 training sessions; 6.6 ± 0.7 mo) was needed to train each monkey for this task. In contrast, we reliably obtained blood from all 4 chimpanzees in this study (average time, 219.0 ± 24.2 min; 31.0 ± 3.1 sessions; 7.0 ± 1.9 mo). The average amount of time to reliably train the 6 rhesus macaques and 4 chimpanzees to put their arm in a blood sleeve and remain stationary for a blood draw did not differ (Mann–Whitney $U = 9$, $P = 0.52$; Figure 3) between species. However, significantly more sessions (Mann–Whitney $U = 1.5$, $P = 0.03$) were needed to train the rhesus than the chimpanzees.

Discussion

Although an increasing number of facilities are including PRT techniques as part of their behavioral management plans, few studies have shown successful training of blood sampling from macaques by using primarily PRT.[17] In this study, we demonstrate that adult male rhesus macaques can be trained for blood draw by using PRT in a manner similar to that used to train chimpanzees. Most of the published accounts on venipuncture in macaques have used some sort of mild restraint, such as squeezing the back of the cage.[23] Positive reinforcement techniques have several advantages over this type of negative reinforcement training. PRT can desensitize animals to potentially stressful stimuli, such as injections, thereby reducing fear and anxiety toward procedures.[10] In addition, by allowing subjects to cooperate with the procedures, positive reinforcement training gives animals a sense of control over their environment,[10] which contributes to well-being.[14,16] PRT increases mental stimulation for subjects[8] and therefore can be an effective form of psychological enrichment, which may result in a reduction of stress-related behaviors. For example, threat behaviors toward caretakers decreased after initiation of a PRT program for several species of New World monkeys.[26] By reducing stress and variability associated with maintenance procedures, training can increase the value of the nonhuman primate model in research.

In addition to the utility of reducing stress associated with maintenance and research procedures, other benefits warrant training monkeys to cooperate voluntarily. Training can reduce the need for sedation, which in turn can reduce variability that might be introduced by pharmacological agents. Even in situations that require sedation, training subjects to accept injections can alter stress-related variables. In a recent study, chimpanzees that voluntarily presented a limb for injection of anesthesia had significantly lower hematological indicators of stress, including neutrophils, glucose levels and white blood cell counts than did animals that were not trained.[8] In addition, although PRT involves a substantial initial investment of time (for example, in this study, 4 to 8 mo to train the rhesus for blood draw), procedures ultimately take less time with trained animals than with untrained animals, saving time in the long run. In one study, weighing laboratory marmosets (a relatively common husbandry procedure) took significantly less time with trained versus untrained monkeys.[15] In addition, working with a cooperative animal is safer for caretakers and handlers, compared with working with a highly stressed animal.[22,26]

Although we were able to train all the monkeys to put their arm in the blood sleeve and accept venipuncture, we were able to obtain repeated blood samples from only 75% of them. Of the 2 animals that did not acquire the target behavior, 1 monkey allowed venipuncture but had a clotting abnormality that required extra attention during blood collection. Therefore, training was discontinued for this animal. The other monkey would allow the needle to be inserted but would pull his arm away from the trainer during the actual blood draw. Because not every subject may accept blood draws, identifying traits or other variables that might help predict a cooperative nature would be helpful. For example, in a previous study,[5] we found that temperament, as measured by response to novelty, correlated with successful training of female rhesus macaques. Monkeys that immediately inspected a novel object presented to them as part of a temperament assessment were easier to train than were monkeys that withdrew from and did not inspect the novelty.

The amount of time (measured in minutes) involved in train-

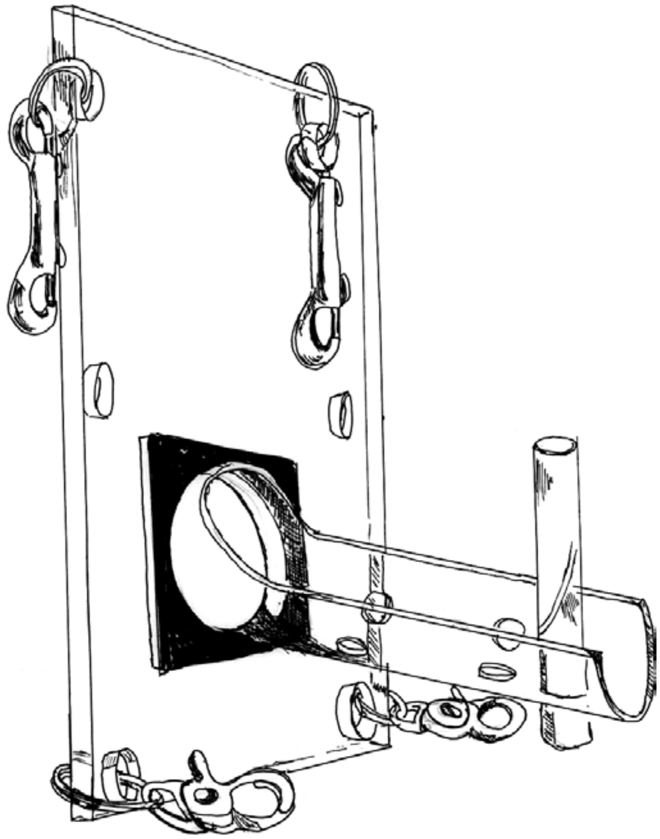

Figure 2. Blood sleeve used for training adult male rhesus macaques. The sleeve was designed for the cages used at the macaque facility. The back plate, which was cut so that it covered the cage door, was made of 0.5-in. acrylic, and the arm sleeve was made of 0.125-in. acrylic. The arm sleeve was 9.5 in. long, with a diameter of 2.75 in. Illustration credit: J. Gregory Johnson.

ing did not differ between the rhesus and chimpanzees in this study. Whereas the rhesus were naïve to training at the start of the study, the chimpanzees had been trained to present a body part for injection before blood sleeve training began. They also were trained to approach a specific location in their enclosure and remain stationary during blood sleeve training. The time involved in training the monkeys might have been reduced if they had been exposed to other types of training prior to the blood sleeve.

Although the total number of minutes required to train was similar between the 2 species, significantly more sessions were needed to successfully train the monkeys than the chimpanzees, indicating that the sessions were shorter for the monkeys than for the chimpanzees. Training times were dictated, in large part, by the subject's attitude. Sessions were terminated if the subject stopped paying attention to the trainer or became agitated. Remaining flexible and adapting to the behavior of the subject are important aspects of training. An animal that is easily distracted may require shorter, more frequent training sessions than does a more focused animal.

Husbandry practices may also have affected the time needed to train the animals. Factors such as housing situation, husbandry practices, and diet can affect training results.[4] For example, in a previous study,[4] training rhesus macaques to touch a target placed on the outside of their cage required more time for pair-housed versus singly housed animals. The chimpanzees and rhesus in this study were reared in different housing situations. However, the chimpanzees were trained to separate from their group prior to the start of the study. Despite the differences in the housing situations, we were still able to train the rhesus in a comparable amount of time.

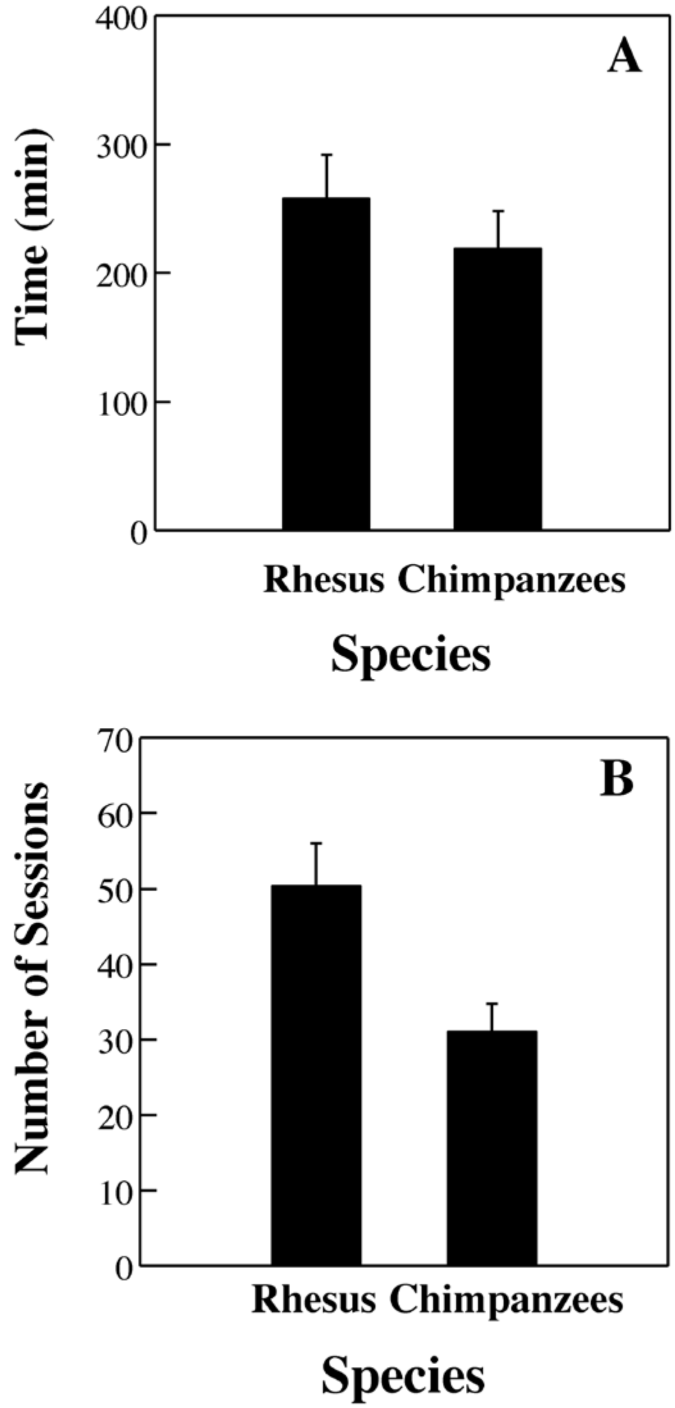

Figure 3. Amount of time (min; A) and number of sessions (B) needed to train 6 rhesus macaques and 4 adult chimpanzees to put their arm in a blood sleeve and remain stationary for blood draw. Overall, training took 4 to 7 mo for the rhesus and 4 to 12 mo for the chimpanzees. Error bars represent SE.

Training macaques for venipuncture using a blood sleeve may not be feasible in all situations. Macaques have relatively small brachial veins, making it difficult to collect blood from the arm. Clearly, similar PRT techniques could be used to train animals to present veins that are easier to work with, such as the femoral or saphenous. Regardless, the data in this report suggest that the use of PRT techniques to train caged rhesus macaques to present for venipuncture not only is possible, but also is feasible.

Because a recent review suggests that captive primates may require as long as 3 mo to acclimate to the changes in housing conditions that are typical of preparations for research,[2] this acclimation period may provide an ideal opportunity to use PRT to train the animals for voluntary venipuncture and other similarly useful behaviors.

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