Journal of the American Association for Laboratory Animal Science
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Vol 53, No 1
January 2014
Pages 61–68

Refining the Pole-and-Collar Method of Restraint: Emphasizing the Use of Positive Training Techniques with Rhesus Macaques (*Macaca mulatta*)

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The pole-and-collar method is one of several techniques that enable the safe transfer of a nonhuman primate from its home environment into a restraint chair without the need for sedation. It has been used within the scientific community for decades. Traditional methods to train animals for pole-and-collar use rely primarily on aspects of negative reinforcement, with very little incorporation of positive-reinforcement techniques. With increasing emphasis on animal training and welfare, research facilities are incorporating positive-reinforcement training into husbandry and experimental procedures. Here we demonstrate the feasibility of training rhesus macaques (*Macaca mulatta*; *n* = 8) to cooperate for pole-and-collar transfer to a primate restraint chair. By using predominantly positive-reinforcement techniques, with supplemental elements of negative reinforcement, macaques were trained in a mean of 85 training sessions (a mean of 1085 min of training time). We also provide tools for investigators using the pole-and-collar method to help them successfully incorporate positive-reinforcement training into their procedures. This refinement has the potential to improve animal welfare and enhance the value of nonhuman primate models in research.

Abbreviations: NRT, negative reinforcement training; PRT, positive reinforcement training; PTS, Primate Training Specialist.

Physical restraint of a laboratory animal is defined as "the use of manual or mechanical means to limit some or all of an animal's normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation" (p 29).[20] Some common restraint methods used with laboratory primates include 'squeeze' cages, manual restraint, and restraint boxes or chutes. The use of specifically designed primate restraint chairs is the preferred method of restraint for various research studies that require nonhuman primates to sit in place for sustained periods of time. Restraint chairs have been used with nonhuman primates since 1917.[17] Over the years, researchers have used chair restraint with a variety of nonhuman primate species, including squirrel monkeys (*Saimiri sciureus*), baboons (*Papio papio*), pig-tailed macaques (*Macaca nemestrina*), stump-tailed macaques (*M. speciosa*), cynomologus macaques (*M. fascicularis*), and rhesus macaques (*M. mulatta*).

Methods for transferring nonhuman primates from their home enclosures into restraint chairs vary among institutions, among different laboratories within the same institution, and among different types of restraint chairs. Here we focus on a frequently used method of transfer—the 'pole-and-collar' technique. In this method, nonhuman primates are fitted with a neck collar to which a pole can be attached and used to guide the animals from their home cage to the restraint chair.[9,28] The pole-and-collar technique is reported to have advantages over other restraint techniques, such as the use of immobilizing agents, in that the "animals can be trained and restrained effectively, safely, rapidly and humanely" (p 47).[1] However, the effect of this technique on animal welfare is debated. For example, in Europe, the use of the pole-and-collar system is not a recommended method for restraint and is not considered good practice.[10] To date, there have been no published scientific assessments of the animal welfare implications of using the pole-and-collar transfer technique. In a study that assessed behavioral and physiologic responses to chair restraint (without evaluation of the pole-and-collar process used to transfer to the chair), even though the monkeys showed behavioral habituation (that is, they appeared calm), a physiologic measure (cortisol) continued to respond to the chairing procedure as a stressor, with repeated restraint in the chair.[28] Clearly more research is needed to understand the welfare implications of restraint for nonhuman primates and the training used to prepare animals for these procedures.

The pole-and-collar method was first described as a restraint method 30 y ago,[1] and this method is still used today by many in the scientific community. In this method, the cage wall is moved to confine the animal to a small portion of the cage; when the pole is attached to the animal's collar; the squeeze mechanism is released. When the animal resists, it is held firmly by the pole until resistance stops, and then the force is reduced. The animal is then transferred to the restraint chair. Once the animal is secured into the chair, food treats are provided.[1] These traditional methods to train nonhuman primates for pole-and-collar restraint primarily use a combination of 2 training techniques including 'flooding,' a form of habituation in which the animal is

Received: 31 Jan 2013. Revision requested: 08 May 2013. Accepted: 19 Jul 2013.
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exposed to an aversive stimulus full-on until it becomes habituated to it, and force training,[1] a negative-reinforcement training (NRT) technique in which the removal of an aversive stimulus immediately after a desired behavior increases the frequency of the behavior occurring over time. When aversive techniques are applied in the framework of NRT, they are very effective in achieving the desired behavior, but it may be argued that there is an inherent cost to the animal's welfare by being forced to cooperate through the threat of an aversive event (that is, the squeeze mechanism or force from the pole) that may elicit fear or anxiety.[26] In the current study, we provide a refinement to these traditional methods.

With the recent release of the revised *Guide for the Care and Use of Laboratory Animals*,[20] there has been an increase in attention toward training techniques used in laboratories. The *Guide* includes multiple recommendations to incorporate training methods into husbandry and experimental procedures to promote voluntary cooperation from subjects and specifically addresses the addition of positive reinforcement with animals placed in restraint devices to help them adapt to equipment and personnel (p 29).[20] Prior to initiating training and attempting to refine the restraint process, it is important to have a good understanding of training terminology and technique relative to operant and classic conditioning as well as those terms pertaining to nonassociative learning (that is, habituation techniques [Figure 1]).

The methods used in the refinement of the pole-and-collar procedure in this manuscript were a combination of operant conditioning and classic conditioning techniques as well as systematic desensitization. Positive-reinforcement training (PRT) is a form of operant conditioning in which the animal receives rewards for desired behavioral responses. Through shaping, subjects are reinforced for cooperating with individual steps, ultimately leading toward acquisition of the goal behavior of allowing the pole to attach to the collar and exiting the cage to sit in the restraint chair. By using classic conditioning, the macaque learns to understand the relationship between a secondary reinforcer (that is, mechanical bridge) and a primary reinforcer (that is, food). Habituation, by using systematic desensitization in the refined procedure (instead of flooding as in the traditional procedure), and counter-conditioning were key elements in our refinement of pole-and-collar training. Positive reinforcement was provided throughout the training process for appropriate behavior. Given the need for animals to be fully trained and exhibiting calm behavior throughout the procedure within a particular timeframe, when a macaque did not progress after several attempts at a particular step by using only PRT methods, the squeeze mechanism (the element of NRT used in the context of the current study) was incorporated into the training, by using both systematic desensitization and counter-conditioning techniques.

In the last 2 decades, there has been an increase in laboratory facilities applying PRT techniques to the daily management of nonhuman primates to teach them to cooperate voluntarily with various husbandry, veterinary, and research procedures.[4-7,12,13,15,23,24,32] PRT has many benefits, including reduced distress,[6,24] enhanced flexibility in captive management,[4,5,32] reduced use of anesthesia,[7,29] and increased cognitive stimulation.[14,30] The cooperative restraint training previously described[3] offers a refinement in preparing rhesus macaques for restraint by using an enclosed primate chair that does not require the use of the pole-and-collar method. Animals are transferred to the 'box chair' and trained to stick their head through an opening on top of the chair, by using a combination of PRT, desensitization, and NRT techniques.[3] However, to date, there are no published studies describing a detailed, stepwise approach to using PRT methods for teaching nonhuman primates to cooperate with the pole-and-collar transfer method of restraint. The aims of the current study are to determine the feasibility of using mostly PRT techniques to apply the pole-and-collar method to transfer rhesus macaques into restraint chairs and to provide descriptive information on the time and the techniques needed for this refinement. We also assessed whether male or female macaques were trained more quickly by using these refined techniques.

Materials and Methods

Subjects and housing. Subjects were 8, mother-reared juvenile (4 male, 4 female) rhesus macaques (*Macaca mulatta*) ranging in age from 2 to 4 y. All animals were assigned to the same research protocol. All subjects were pair-housed indoors, in adjoining standard primate caging (71 cm × 61 cm × 76 cm), at the Yerkes National Primate Research Center. All subjects were fed chow twice daily, supplemented by fruits and vegetables and other enrichment (for example, seed mixture on a foraging board). Water was available ad libitum. None of the subjects had been involved in previous research studies, and none had been exposed to a formalized animal training program. Animal use procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals*[20] and were approved by the Institutional Animal Care and Use Committee of Emory University.

Training information. This training was a joint effort by the personnel in one research laboratory interested in incorporating more PRT into their own pole-and-collar methods, and the Primate Training Specialist (PTS) at the Yerkes center. To establish consistency among all trainers, training guidelines (that is, a shaping plan and training documentation)[22] were created which included the steps needed to reach the goal behavior: attaching the pole to the collar, guiding the animal's movement toward the primate chair, and restraining the animal in the chair. Through classroom-style learning and one-on-one training with the animals, all 6 trainers, who had roughly the same level of training experience, met regularly with the Primate Training Specialist to 1) establish consistency in training; 2) assure an understanding of correct training terminology (Figure 1) and technique, 3) assure that all personnel were using appropriate body language and attitude when interacting with the animals, 4) assure that all personnel had an understanding of basic rhesus macaque behavior (for example, identifying signs of fear, aggression, or abnormal behaviors), and of judging the monkeys' responses to training steps to determine when a monkey was ready to move through the next step of the shaping plan.[22] Once trainers began training independently, additional consultation with the Primate Training Specialist took place on an as needed basis.

The laboratory group strived to assign one trainer per animal, especially early in the training process, as this is recommended to maximize consistency.[18,22,33] However, due to staffing constraints, 2 of the monkeys had 2 trainers. In these cases regular communication, reviewing of each other's training records, and observing each other's training sessions were done to improve consistency. All trainers used the same type of mechanical bridge during training sessions as another way to increase consistency. Training sessions were conducted 5 times per week for 10 to 20 min each. This training schedule was selected based on research suggesting that once-daily training is more conducive to training success than other training frequencies.[8] Animals were separated from their cage-mates during training

Classic conditioning	A type of learning in which a neutral stimulus is paired with an unconditioned stimulus that elicits a reflex response. After the conditioned stimulus is followed repeatedly by the unconditioned stimulus, the association between the 2 stimuli is learned. The conditioned stimulus alone will then elicit the same reflex response.
Counter-conditioning	Processes of actively pairing something positive (that is, a secondary and primary reinforcer) with an aversive stimulus until the stimulus loses its ability to adversely influence the behavior and becomes more neutral over time. Counter-conditioning expedites the desensitization of a novel stimulus by associating it with a reinforcer.
Flooding	A method that involves habituation. Full-on exposure to a situation that elicits relatively high levels of anxiety, usually for prolonged periods, until there is adaptation to the situation, as reflected in reduction in anxiety. The animal has no way to avoid the stimulus.
Habituation	The loss of response to a stimulus after the animal's repeated exposure to it. Habituation can be accomplished using 2 techniques: systematic desensitization and flooding.
Negative-reinforcement training	The removal of an unpleasant action or aversive stimulus immediately after the desired behavior occurs, whose purpose is to increase the frequency of the behavior over time.
Operant conditioning	A type of learning in which the behaviors are altered primarily by regulating the consequences that follow them.
Positive-reinforcement training	A pleasant action or event whose purpose is to increase the frequency that the behavior will occur again. Frequency of the behavior increases by the addition—immediately after the behavior—of something the animal desires.
Primary reinforcer	Something that the animal finds inherently rewarding. A primary reinforcer usually satisfies a biologic need (that is, food, water, and so forth).
Regression	The (usually temporary) return of a behavior to an earlier stage of the learning process.
Secondary reinforcer (mechanical bridge)	Something that initially had no significance to an animal that then became desired by the animal because of the secondary reinforcer's association with other desired, primary reinforcers (often food). Once established, the association can be very strong; provision of the secondary reinforcer indicates the precise moment at which the desired behavior occurred and bridges the gap in time between the behavior and when the animal receives a food reward.
Shaping	Developing a new behavior by dividing it into small increments or steps and then reinforcing one step at a time until the desired behavior is achieved. Shaping is a technique that is based on principles of operant conditioning.
Systematic desensitization	A method that involves habituation. In systematic desensitization, an animal's perception of an event is changed gradually, as evidenced by the animal's lack of response to the event when compared with a previous baseline response to that event (that is, an aversive event). In this method, the animal can approach or back away; they are in control.

Figure 1. Training terminology modeled after that in references 11 and 25.

sessions, but remained in visual access to one another; subjects were trained in varying order across days.

The pole-and-collar system designed by Primate Products (Woodside, CA) was used. Prior to the start of beginning training, animals were sedated (ketamine, 5 to 10 mg/kg) and a plastic collar was loosely fitted around the monkey's neck, to which a metal capture pole could be attached during future training. After collar placement, the monkey was returned to its cage and no further procedures were done for at least 48 h to allow habituation to the collar. The chair used in this assessment was custom built, with an open-air design that allowed access to all parts of the monkey's body, similar to the commercial chair available through Primate Products

Animal training process. Trainers followed the designed shaping plan for pole-and-collar and chair training (Figure 2). All trainers used a handheld 'clicker' (mechanical bridge; available at most pet stores) as a secondary reinforcer. The use of a mechanical secondary reinforcer is encouraged in training complex or potentially stressful behaviors, because it is a way of communicating to the animal the exact moment at which the desired behavior occurred and that a reward will be delivered.[16] The monkeys were taught to associate the click sound with the subsequent presentation of a food reward (primary reinforcer) by clicking and immediately providing a small piece of food (for example, a raisin, small apple or banana pieces, cereal) repeatedly. Once this association was made, and animals began responding to the click sound in anticipation of receiving a food reward, formal training began.

Equipment (for example, the chair and poles) was left in the animal room to begin habituation. Shaping included systematic desensitization and counter conditioning techniques. Positive reinforcement training was used as much as possible. Trainers first cued subjects to the back corner of their cage by using a small hand gesture and asked them to remain in this 'training position' by using the verbal command 'stay' as the pole approached. The trainer 'clicked' when the subject responded appropriately by staying calmly in position as the pole moved closer to its body in small increments and provided the animal with positive reinforcement.

The restraint chair and other tools needed in the restraint training process (e.g., chair, poles) are left in the room during each training procedure to help habituate the macaques to these objects.

If the animals are paired, separate for training sessions. Use a panel that allows visual access, if possible.

Begin training with 'click-and-treat' **(**Step 1**).** Stand directly in front of the macaque with a nonthreatening body posture, avoiding any eye contact. Holding the clicker by your side, click, and immediately provide a treat to the macaque. If the animal does not take the treat, place it in the cage (or on the ledge of the cage). Repeat this process until the macaque takes the treat from your hand and the association between the click and food is made. The animal will begin responding to the click sound in anticipation of receiving a food reward (e.g., raising hand, looking in the direction of treats, and so forth).

Once the macaque is comfortable taking treats and the association between the clicker and food has been established, reinforcements are provided only when the macaque is in the 'training position', that is, the location in the cage that the animals should be in for all training sessions (for this training, this position is the back corner of the cage). Cue the macaque to position by using a small, nonthreatening, hand gesture. Once the animal moves to the location needed, click and immediately provide reinforcement **(**Step 2). Note: This is the position in which the macaque will be for pole attachment.

Gradually increase the amount of time the macaque stays in position, establishing the verbal 'Stay' command. Click and reinforce the macaque for staying in the position for extended periods of time.

Begin using counter-conditioning and systematic desensitization techniques. Holding the pole in a neutral position (that is., by your side), click and reinforce the monkey for remaining calm when the pole is in neutral position.

While the animal is in the training position, gradually move the pole slowly (about 1-in. increments) toward the front of the cage. Click and reinforce the monkey for staying in the training position for each 1-in. increment as the pole slowly approaches (Step 3).

Using counter-conditioning and systematic desensitization, slowly move the pole toward the macaque and begin touching a neutral part of its body (for example, leg; Step 4). Reinforce the macaque for remaining calm and staying in the training position.

Continue with counter-conditioning and systematic desensitization techniques until the macaque tolerates the pole touching its neck or collar and hooking of the pole onto the collar with little to no struggling (Step 5).

When the pole is attached to the collar for the first few times, immediately release and reinforce with highly preferred food items. Slowly increase the duration for which the pole is attached to the animal's collar (Step 6). Reinforce the macaque for staying in the training position.

If a second pole is deemed necessary for safe transfers, slowly bring the pole toward the macaque until the animal allows it to be attached to its collar. Click and reinforce for cooperation with these steps. If a second trainer is needed, slowly incorporate this trainer into the training sessions to allow the animal to habituate to the additional person.

With the pole attached to the collar and by using counter-conditioning and systematic desensitization, click and reinforce the macaque for staying in the training position as the chair is brought closer and closer to the front of cage.

Slowly open the cage door all the way (this step may require additional time and training for the subject to stay in position as the door opens) and gradually bring the macaque out of its home cage and toward the chair (Step 7).

Guide the collar into the neck yoke. Secure into place before removing poles; reinforce the macaque for calm behavior. The first time the animal is put into the chair, do not restrain the arms or legs; leave the animal in position for a few seconds before returning it to its home cage. Again, reinforce with highly preferred food items.

Slowly increase the duration for which the macaque is in the chair, before moving on to additional stabilization of arms and legs (if necessary). Provide reinforcement to the macaque for calm behavior during the ride to the procedure area.

Figure 2. Shaping plan for pole-and-collar and chair training. Note that the specific steps recorded during training steps are indicated throughout the plan.

Monkeys were gradually made familiar with the pole and with the pole approaching and touching a neutral area of their body. Trainers began by holding the pole in a neutral position, generally by their side, sounding the clicker, and providing reinforcement to the monkey for remaining calm. Gradually the pole was moved closer toward the monkey's cage, and again, the trainer sounded the clicker and provided reinforcement when the macaque remained calm in the appropriate position. These steps continued until the trainer was able to insert the pole gently into the cage and touch the pole on a neutral area of macaque's body with little reaction. If animals showed signs of fear or aggression as the pole approached, the trainer slowly backed the pole away until the macaque appeared more comfortable, and then the steps began again. Similar gradual processes were taken with the next steps of the pole approaching the collar, the pole touching the collar, the pole attaching to the collar, and the macaque being transferred and secured into the primate chair. Positive reinforcement, in the form of small, highly preferred food items, was provided to macaques for cooperating (that is, sitting calmly with the pole touching collar) throughout the training process. The amount of training time spent with each macaque was dependent on the animal's response to each step in the training. Sessions varied in length, because trainers attempted to end each session with a positive outcome before macaques became agitated or lost interest in the training.

When the macaques were not progressing within the time allotted for the shaping procedures to meet experimental study deadlines (which varied for each subject depending on when the animal was assigned to the study protocol but were within a 1- to 4-mo time frame), the squeeze mechanism was applied to accelerate training progress. The squeeze mechanism (an element of NRT) was used to encourage the macaque to move to the front of the cage; when the macaque reached the front, the squeeze mechanism was released, and positive reinforcement was delivered immediately. The use of NRT was limited, and the goal was to eliminate its use over time. To keep the decision of when to incorporate the squeeze mechanism consistent among all trainers, a timeframe was included in the training guidelines. Therefore, 4 wk before the start of experimental procedures that required the monkey to be restrained in the chair for a period of time, the training progress was evaluated jointly by the trainer and the Primate Training Specialist. On the basis of the current state of training, if it was deemed unlikely the animal would be fully trained in the remaining time, the Primate Training Specialist supplied an alternative training plan to move the monkey more quickly through the training steps. This progress was accomplished generally by habituating the macaque to the use of the squeeze mechanism and then using it during training sessions as necessary. Systematic desensitization and counter-conditioning to the squeeze mechanism was a gradual process: the trainer released the latches to the squeeze, sounded the clicker, and provided reinforcement to the macaque for remaining calm. The squeeze mechanism then was moved forward in small increments (that is, 1 in. at a time), with the trainer using the clicker and reinforcing the macaque for continuing to remain calm during each step, until the animal was in the position that the trainer needed to attach the pole, and the squeeze mechanism was released. In some cases, to promote animal and trainer safety, a second pole and trainer were used; in these cases, the macaque was habituated to these changes. In contrast to the more traditional method, the entire training process catered to each individual macaque's training progress and the overall time lines dictated by the study protocol.

Documentation of training sessions. To monitor training progress, trainers recorded information about each training session including the session duration (in minutes), the highest training step reached for each session (step 1, establishing the bridge; step 2, stays in training position; step 3, tolerates the pole approaching; step 4, tolerates the pole touching a neutral area of the body; step 5, tolerates the pole touching the collar; step 6, tolerates the pole attaching to the collar; and step 7, exits cage on pole and transfers to chair), and whether negative reinforcement (that is, the squeeze mechanism) was used. Macaques were considered reliably trained for each of these steps when they cooperated 100% of the time during a single training session (for example, when the macaque stayed in the training position during the entire session as the pole approached, the trainer then began working toward step 4) or 75% of the time during each of 4 consecutive training sessions.

Statistical analysis. Because the data were not normally distributed, a nonparametric Mann–Whitney test was conducted (SPSS Statistics v17, IBM, Armonk, NY) in an effort to determine any sex-associated differences in the total number of training sessions needed to complete the goal behavior, the total number of training sessions that included the use of the squeeze mechanism, and the percentage of training sessions for each step of the training process.

Results

All 8 macaques were trained to move from the home cage into a restraint chair in a mean of 85 training sessions over a mean of 17 wk (mean of 1085 min total; Table 1). Due to restrictions in the total duration of training, all macaques required some use of the squeeze mechanism, during at least one step in their training (Figure 3). One of the macaques (animal J) reached the final behavior by using PRT almost exclusively (the squeeze mechanism was used only 9 times or during 7% of total number of training sessions). The other macaques were trained by using a preponderance of PRT, which was supplemented with some use of the squeeze mechanism (used at least once during 9% to 45% of the total training sessions per macaque). When the squeeze mechanism was used during training, the macaque moved to the front of the cage and received a reward, the pole was attached to the collar, a food reward was provided, the squeeze mechanism was released, and the animal immediately received another reward.

All macaques were trained by using only PRT techniques during steps 1 through 4 (Table 1). The most challenging steps for the macaques to cooperate with were allowing the trainer to touch the collar with the pole (required a mean of 32 sessions, 7% of which included the use of the squeeze mechanism), attaching the pole to the collar while the macaques stayed in the training position (required a mean of 25 sessions, 45% of which included the squeeze mechanism), and exiting the cage (required a mean of 34 sessions, 66% of which included the squeeze mechanism; Table 1). There was interindividual variability among the subjects in the number of sessions needed. For example, NRT was incorporated into step 5 for macaque L after a substantial amount of effort was put forth with PRT (for example, the squeeze mechanism was only incorporated into 12 of the 77 sessions needed to train the macaque to tolerate attachment of the pole to the collar), but the other macaques needed only 8 to 48 training sessions of PRT methods only to obtain this behavior. Time constraints were more pronounced during the training for specific macaques; these constraints also influenced the pace of training and the percentage of sessions into which NRT was incorporated. For example, macaque F was trained in 33 sessions, 45% of which incorporated the use of the squeeze mechanism (Table 1), to ready it for experimental testing when needed.

A Mann–Whitney test indicated no significant differences between male and female macaques when the total number of sessions required to achieve the goal behavior, the total number of training sessions that included the use of the squeeze mechanism, and the percentage of training sessions needed for each step were compared. Because our training subjects were very close (that is, within 2 y) in age, we could not analyze for age-associated effects.

Discussion

This assessment demonstrates the feasibility of applying a combination of classic and operant conditioning with habituation techniques for training a complex behavior involving the restraint of laboratory nonhuman primates. All subjects in the current study were trained by using predominantly PRT techniques (55% to 93% of the total training sessions) supplemented by elements of negative reinforcement (that is, the use of a squeeze mechanism) for pole-and-collar transfer to a primate chair, where they then were restrained. All subjects were trained to move from the home cage into a restraint chair in a mean of 85 training sessions over a mean of 17 wk of training. Male and female monkeys had similar responses to the training. Having multiple trainers may have slowed the monkeys' progress, but this practice reflects what is done in most research laboratories, and we believe these findings are more likely to generalize to other training situations. This PRT-based method required more time than do traditional techniques[1] and can be very challenging to implement given the time constraints of research. A balance

Table 1. Number of training sessions required for each subject to reach the criterion performance during each step of the training process

	Training subject (sex)								Mean no.	Mean %
Training steps	E (female)	F (female)	G (male)	I (male)	J (female)	K (female)	L (male)	M (male)	of sessions per step	of sessions with NRT
Step 1: Associates between click and treat	1	7	11	2	21	9	NA	3	8	0
Step 2: Stays in training position	2	9	3	3	4	5	2	3	4	0
Step 3: Tolerates pole approach	4	NA	18	3	68	12	7	11	18	0
Step 4: Tolerates pole touching neutral body area	NA	NA	13	4	6	6	NA	3	6	0
Step 5: Tolerates pole touching collar	8	NA	NA	9	NA	48	77[a] (12)	19	32	7
Step 6; Tolerates attachment of pole to collar	9	8[a] (6)	37	53[a] (18)	14[a] (3)	20[a] (18)	7[a] (7)	48[a] (36)	25	45
Step 7: Exits cage on pole and transfers to chair	8[a] (3)	9[a] (9)	22[a] (22)	15[a] (9)	11[a] (6)	3[a] (3)	4	7	10	66
Total no. of sessions	32	33	104	89	124	103	97	94	85	
Total time (min)	640	660	1560	890	1860	1545	679	846	1085	

NA, the training step was not used (that is, the macaque did not need to acquire that behavior to progress in training; the behavior was train in conjunction with the following step; or the behavior was incorporated during an earlier step).
All macaques were trained to the goal behavior of attaching the pole to the collar, exiting the cage, and transferring to the chair.
[a]NRT (that is, the squeeze mechanism) was incorporated during training; the number of sessions in which NRT was incorporated is given in parentheses.

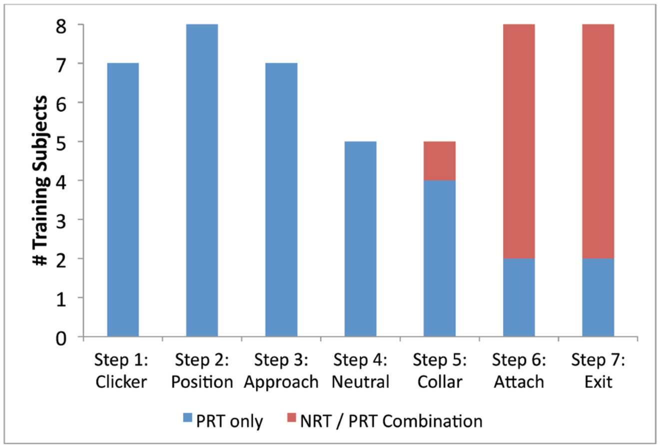

Figure 3. The number of macaques trained with PRT only or with a combination of PRT and NRT during each training step.

must be struck between PRT and the use of aversive stimuli that may be needed to advance the training in a timely manner.

The learning process varies among animals and, due to regression, does not follow a consistent, increasing trajectory. Therefore, once the macaques began experiencing that the pole-and-collar restrains their movements, the steps were more difficult and required more training time. Regression in performance was common after the initial restraint (that is, 6 monkeys showed regression after the time when the pole was first attached to the collar), and, although temporary, required additional time to work through. Regression should be considered a normal part of the training process, and it will likely vary across individuals, by steps in the training, and in its duration and magnitude. This is important to keep in mind when preparing animals for chair restraint. Using counter-conditioning procedures reduces the perceived aversiveness of a stimulus and may reduce the magnitude of regression. The squeeze mechanism was used to move more quickly through certain steps in the training process, but its use was limited, and its magnitude was based on each macaque's response.

Given time constraints, it was impractical to use only PRT techniques to prepare macaques for pole-and-collar transfer and chair restraint. Overall, subjects experienced the squeeze mechanism on average at least once during 26% of their training sessions, whereas the majority of the training sessions were conducted with PRT only, carefully shaping the desired behavior. When the squeeze mechanism was used, it was always released after the appropriate behavior, that is, moving to the cage front, away from the squeeze mechanism, to allow the pole to attach to the collar. When the squeeze mechanism was released, the macaque was provided with a reward. Applying a combination of PRT and limited NRT resulted in all macaques being trained to fully cooperate for research procedures within the set time frame, making this training regimen an effective one.

Physical restraint is likely a stressful procedure for laboratory primates.[18,19,21,27,28,31] Incorporating more PRT techniques is one way of reducing the potential adverse effect of procedures such as pole-and-collar transfer and chair restraint. The *Guide*[20] states that "habituating animals to routine husbandry or experimental procedures should be encouraged whenever possible as it may assist the animal to better cope with a captive environment by reducing stress associated with novel procedures or people. The type and duration of habituation needed will be determined by the complexity of the procedure. In most cases, principles of operant conditioning may be employed during training sessions, using progressive behavioral shaping, to induce voluntary cooperation with procedures." (p 65 to 66).[20] The training we describe here required daily sessions and a mean of 17 wk to complete, which is considerably longer than the few weeks required by most traditional training methods.[1] The value of the additional time is difficult to assess and was not formally evaluated. However, studies of laboratory primates in other training contexts show that they experience less stress when PRT is applied than when other training methods are used. For example, chimpanzees (*Pan troglodytes*) trained by using PRT techniques to voluntarily present for injections had significantly lower physiologic measures correlated with stress than when they were anesthetized and sampled by more traditional means.[12] Common marmosets (*Callithrix jacchus*) were trained with PRT to urinate on request. Those that were trained showed less behavioral evidence of stress when exposed to mildly stressful, routine husbandry procedures, as compared with nontrained animals.[2] There have been no assessments looking at the welfare implications of the pole-and-collar method. It would be valuable to conduct additional assessments of training methods used to prepare animals for restraint procedures that include behavioral and physiologic dependent measures as indicators of stress to determine whether PRT reduces those measures, as has been documented in other research situations.[2,12]

The training method we describe here is a modern refinement of more traditional methods.[1] This refinement has developed from many other recent advances in the use of PRT in laboratory animals.[4-7,12,13,15,23,24,32] Anderson and Houghton[1] state that "conditioning and training animals to allow restraint is an effective technique to prevent stress." However, in that traditional approach,[1] which has been widely applied by others, positive reinforcement and systematic desensitization are not used to shape the animal's behavior or to help the animal progress through the steps of training, and no counter-conditioning is used to help the animal overcome fear during the process. Fruit and reassuring verbal tones are provided once the animal is secured in the chair,[1] but providing a reward after a fearful event may not be as effective in decreasing fear during that event if timing is not right and there is a delay between the aversive stimulus and the reward.[25] It is a common misconception that providing food to an animal after the behavior is complete means the behavior was trained by using PRT. Instead, we used systematic desensitization and classic counter-conditioning methods. By actively pairing a positive reinforcer with an initially aversive event, that event gradually is perceived as more neutral, and animals learn to anticipate the reinforcement they receive for cooperating with that event.[25]

A pure PRT approach involves no use of force or coercion; the animal progresses through the steps of training at its own pace, participation in the training sessions is the animal's decision, and positive reinforcement is provided contingent on correct behavior and throughout the entire process. If the animal chooses not to participate in a training session, or not to engage in a certain behavior that is requested by the trainer, there is no consequence to the animal. This approach may not always be feasible in a research environment, but we suggest that it is possible to achieve a balance between by using PRT and completing training in a timely manner, as we did in the current study. The traditional methods used for the pole-and-collar technique are successful in accomplishing the end result, but our incorporation of additional PRT in the pole-and-collar method are offered as a refinement to these traditional methods. Bliss-Moreau and colleagues[3] offer an alternative refinement of restraint chair training, using a combination of PRT and NRT methods to train adult rhesus macaques to cooperate with restraint involving a different restraint chair design.

Training methods to achieve compliance in animals through the use of less-aversive techniques should be applied whenever possible. Understanding shaping, systematic desensitization, and counter-conditioning; using PRT; and limiting aversive stimuli, particularly for behaviors that are stressful and restrictive of the animal's movement, will advance animal welfare and improve the quality of science by reducing the potential confounding influence of stress on animal subjects.

Acknowledgments

This project was supported by NIH/NCRR Grant P51OD011132 to the Yerkes National Primate Research Center, and the following individual NIH/NINDS grants (TW): RO1NS049474, R01NS054976, R01NS042937, R01NS062876, and P50NS071669. The Yerkes Center is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. We would like to thank Xing Hu, Yuxian Ma, Damien Pittard, Annaelle Devergnas, and Karen Rommelfanger who helped with the training, and five anonymous reviewers whose edits and suggestions helped improve the paper.

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