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Training Common Marmosets (*Callithrix jacchus*) to Cooperate During Routine Laboratory Procedures: Ease of Training and Time Investment

Jean McKinley, Hannah M. Buchanan-Smith, and Lois Bassett

Department of Psychology
University of Stirling

Keith Morris

MRC Human Reproductive Sciences Unit
Edinburgh, Scotland

The first author trained 12 laboratory-housed common marmosets (*Callithrix jacchus*) in pairs to assess the practicality of positive reinforcement training as a technique in the management of these nonhuman animals. Behaviors taught were (a) target training to allow in homecage weighing and (b) providing urine samples. Between 2 to 13, 10-minute training sessions established desired behaviors. Training aggressive animals only after they had been fed eliminated aggression during training. Trained animals proved extremely reliable, and data collection using trained animals was considerably faster than collection using current laboratory techniques. The results suggest that positive reinforcement training is a practical option in the management of laboratory-housed marmosets.

A review of early research into the care of common marmosets (*Callithrix jacchus*) reveals just how much progress has been made in housing and feeding of these nonhuman animals (Epple, 1970; Hiddleston, 1978; Lunn & Hearn, 1978; Poole,

Requests for reprints should be sent to Hannah M. Buchanan-Smith, Scottish Primate Research Group, Department of Psychology, University of Stirling, Stirling FK9 4LA, Scotland. E-mail: h.m.buchanan-smith@stir.ac.uk

Hubrecht, & Kirkwood, 1999). However, these articles also reveal few changes in how marmosets are managed during routine laboratory procedures.

The current Home Office (1986) code of practice for the housing and care of animals used in scientific procedures states that, for primates, "The least distressing method of handling is to train the animal to cooperate in routine procedures" (Sec 3, para 50). However, the literature that shows that training both facilitates data collection and reduces stress in primates in the laboratory largely concerns the training of macaques (Reinhardt, 1997). The *Universities Federation for Animal Welfare (UFAW) Handbook* (Poole et al., 1999) stated that marmosets can be trained for routine husbandry and experimental procedures but does not provide details on how to train, or the cost effectiveness of the training versus alternative practices. Anzenberger and Gossweiler (1993) used the tendency of marmosets to urinate immediately on leaving the nestbox each morning to develop a technique allowing in-homecage collection of urine samples. However, although useful in some settings, this technique may not be practical with cage-housed animals because of the need for specially designed apparatus and overnight confinement in the nestbox. In addition, this method only allows collection of first void and therefore is unsuitable for frequent sampling throughout the day.

Despite its growing popularity in the management of macaques, training has not been widely adopted in the management of laboratory-housed marmosets. One reason may be that the need for improved methods has not yet been perceived. Motivation to encourage cooperation is greatly increased when handling potentially dangerous animals (Kiley-Worthington, 1990). Due to their small size, marmosets are easily handled, and a stout pair of gloves is all that is required to protect the handler from bites and scratches.

Although handling these monkeys poses few problems in terms of human safety, it is widely noted that this can cause considerable distress, not only for the animal but for others housed in the same area (National Research Council, 1998). In addition, both removal from the homecage (Norcross & Newman, 1999) and methods used for capture can be problematic. The use of nets can result in injury; thus, the recommended method is to trap these animals in their nestbox (National Research Council, 1998). However, Poole (1998) reported that a secure place to hide or rest is one of the fundamental psychological needs of mammals. As marmosets both sleep in these boxes and retreat there when threatened, there is a potential welfare problem in using nestboxes as a means of capture.

Positive reinforcement training (PRT) rewards an animal for performing a desired behavior. No coercion is used; mistakes are ignored, not punished, leaving the animal to choose whether he/she will participate in the training program (Pryor, 1999). An example of this technique is target training. Here, the animal is rewarded for holding a specific object whenever it is presented. This simple behavior then can be used in a variety of ways; moving animals between locations, entering transport cages, and keeping animals at specific locations. This technique has been used to improve husbandry and veterinary care, reduce abnormal behavior and aggression, and promote the safety of personnel (Laule, Bloomsmith, & Schapiro, 2003/this issue).

However, when alternative methods of handling are sought, there are a number of practical reasons why PRT may not be considered. Many laboratory animals are destined for terminal studies; thus, their time in the laboratory may be limited, decreasing the return on initial time investment. Although appropriate in a zoo setting with relatively few animals, training is not widely regarded as practical in a laboratory housing hundreds of individuals. Although the need to invest time during the training process cannot be denied, the possibility that this investment may be recouped through faster data collection often is not considered. This is an important issue as changes intended to promote welfare stand little chance of being widely implemented unless they can be shown to be practical. There also is a widespread belief that animals will cooperate for rewards only as a consequence of food or water deprivation. Kiley-Worthington (1990) suggested that such beliefs are a legacy of the era of “Skinner box” experiments on reinforcement, and a glance through any behaviorist textbook does reveal numerous studies on the behavior of hungry and thirsty rats (e.g., Pearce, 1997).

The aim of this study was to assess the practicality of PRT with common marmosets in a laboratory setting. Recording weights and collecting urine samples through the use of metabolism cages are noninvasive procedures but usually require capture and removal from the homecage, potentially causing distress (Norcross & Newman, 1999). To avoid this, animals were trained to allow in homecage data collection. Research questions were as follows:

1. Can common marmosets be trained using solely positive reinforcement techniques and, if so, how much time investment is required?
2. Will trained animals cooperate reliably enough to allow regular data collection?
3. How does data collection using trained animals compare to those using current practices with regard to time required for each technique?

METHODS

Study Animals

The study animals were 6 male and 6 female common marmosets housed in pairs, with a mean age 1,188 days (± *SE* 232.37 days). Initial criteria for selection was that at least one member of each pair would take a food reward from the trainer’s hand. Pairs who showed aggression at this stage were not selected.

Although the standard practice at the unit was to refer to marmosets by identity number, all study animals were named prior to training. (See Table 1).

Housing and Husbandry

The marmosets were housed in male/female pairs in the same colony room at the Medical Research Council (MRC) Human Reproductive Sciences Unit, Edinburgh, Scotland. This room measured 2.7m × 3m × 5m and contained eight housing units, each subdivided into four sections measuring 55cm wide × 95cm high × 110cm deep, with one pair per section. Of the study animals, three pairs were housed in upper tier cages, and three pairs were housed in the lower tier. Cages had wood shavings as a floor substrate and were furnished with a nestbox, shelves, and two wooden logs. Some cages contained additional enrichment devices. Water was available *ad libitum* from a bottle mounted on the front mesh of the cage. Rooms were maintained at a temperature of 22 to 24°C and a relative humidity of 50%.

Food was provided once a day at about 1230h. The marmosets were fed with commercially manufactured primate pellets (Mazuri Primate Diet, E; Witham, Essex, England) and a variety of fresh fruit (banana, apple, pear, orange, tomato and grapes). This was supplemented by either a high protein porridge or a mixture of dried fruit and nuts. Of the food items available, the primate pellets were the least preferred and the most likely to be removed uneaten during morning cage cleaning. To encourage their consumption, the proportion of fresh fruit in the diet was reduced over weekends.

TABLE 1
Details of Study Animals

Pair	*Name*	*Sex*	*Age at Start of Study*	*Relationship*
1	Cecil	M	2 years 4 months	Siblings
	Coco	F	1 year 11 months	
2	Freddy	M	2 years 6 months	Siblings
	Foxy	F	2 years 6 months	
3	Iggy	M	2 years 6 months	None
	Iris	F	3 years 6 months	
4	Jambo	M	6 years 7 months	Father/daughter
	Jilly	F	1 year 6 months	
5	Kipper	M	1 year 6 months	Siblings
	Keltie	F	1 year 11 months	
6	Leo	M	2 years 8 months	None
	Lala	F	2 years 6 months	

Procedure

Of the study animals, Pairs 1, 2, and 3 were target trained first and then trained to urinate. The remaining three pairs (Pairs 4, 5, and 6) had the order of training reversed. When members of a pair learned the desired behavior at different rates, the trained animal was rewarded only when the behavior followed the verbal cue "go on" (i.e., spontaneous responses were no longer reinforced). These animals were asked to perform at intervals throughout each training session, thus maintaining their behavior while allowing the training of their partner to continue.

Three different food rewards were used. These, in order of preference, were small pieces of marshmallow, cornflakes, and chopped dates. Attempts were made to avoid the use of sweet items and use healthier rewards. Perhaps due to the varied diet the study animals received, these attempts proved unsuccessful. If any animal showed aggressive behavior toward a partner, training was terminated immediately, and a lower value food reward was used during the next session.

Reliability of Trained Animals

Reliability of the trained animals was assessed by recording weights and collecting urine for cortisol analysis (Bassett, Buchanan-Smith, McKinley, & Smith, 2003/this issue) and by calculating the percentage of required weights and urine samples obtained.

Target Training for in Homecage Weighing

Each training session lasted a maximum of 10 min, ending sooner if each animal had earned 12 rewards. When an animal held the target for 20 sec, the scales were introduced. Animals were considered trained when they remained on the scales long enough to allow their weight to be noted. A shaping procedure was used with training progressing in stages:

1. The target (a plastic spoon) was held at the front of the cage with the food reward held behind it. Males were offered a black target placed on the left-hand side and females a white target placed on the right. Initially the target was touched as the marmoset reached for the food. A reward was given when the correct target was touched. Incorrect responses were ignored.
2. The target was presented without the reward held behind it. Marmosets were rewarded when target touched.
3. The time the target had to be held before reward was given was gradually increased (see Figure 1).

FIGURE 1 Target trained female holds her target as male is given his.

FIGURE 2 Weighing: Female sits on scales as male waits until his target is presented.

4. Scales for weighing were placed in the cage and the target held in front of them. The marmoset was rewarded for climbing onto the scales and holding the target (see Figure 2).

Urine Training

Marmosets were rarely observed urinating but scent marked frequently, depositing a few drops of urine each time. Scent marking is a behavior that occurs fairly frequently in common marmosets (Epple, 1970; Stevenson & Poole, 1976) and, as observed by H. M. Buchanan-Smith, in this population in particular. It proved more practical to reinforce this behavior than to wait for urination. The criterion for success of urine training was that each animal scent marked on request 12 times per 10-min session.

As previously described, a shaping procedure was used with training progressing in stages. To allow immediate reinforcement of desired behaviors, a clicking sound was used as a bridging stimulus. Commercially available "clickers" proved too loud and startled the marmosets; therefore, the trainer (J. McKinley) created the sound by clicking her tongue. Five stages were employed during training:

1. The marmosets were taught to associate tongue clicking with a food reward (i.e., the trainer clicked her tongue and then rewarded both pair members). The association was considered formed when the marmosets moved rapidly to the front of the cages and reached for food as soon as the clicking sound was made.
2. Each pair in turn was observed by the trainer who waited for scent marking to occur spontaneously. Whenever a marmoset scent marked a branch, the trainer made a clicking noise and rewarded that animal.
3. When the rate of scent marking had increased, a verbal request was given as the animal moved toward the sites where scent marking occurred. An animal who then scent marked was rewarded.
4. Once the marmoset scent marked on verbal request, rewards were given only for marking one or two specific sites.
5. Holes were drilled at sites used by the marmosets to allow insertion of collecting vials.

Comparing Data Collection Using Trained Animals and Standard Laboratory Procedures

Weighing. The time taken to record the weights of the trained animals was compared to that taken using the current standard procedure. Data were collected when the study animals were weighed during another study (Bassett et al., 2003/this issue). Timing began when the cage door was opened. The monkeys were confined in their nestbox and then taken to the procedure room. Each, in turn, was removed and placed in a weighing cage, weighed, and then returned to the nestbox. Timing ended when cage door was closed after the monkeys were returned to their homecage. For the trained marmosets, timing began when the cage door was opened to allow insertion of the scales and ended when the door was closed after removal.

Urine versus blood sampling. Urine is not collected routinely in this laboratory. Therefore, time taken to collect urine samples was compared to that taken to collect blood samples, as many tests conducted on blood also can be carried out using urine. Data were collected during routine blood draws. The standard practice is to collect all animals due to have samples taken simultaneously. This is done by confining them in their nestboxes and transporting them to the procedures room. When all samples are collected, the animals are returned to their homecage. Timing began when the first cage door was opened and ended when the last animal was returned to the homecage. For urine sampling, timing began when first cage door was opened to allow insertion of the first collecting vial and ended when the last sample was removed and the door closed. In all cases, time recorded was divided by the number of samples obtained to give an estimate of time taken per sample.

RESULTS

Reliability

After training, during formal data collection, the trained animals proved extremely reliable with 100% of weights (n = 12) and 95% of required urine samples being successfully collected (n = 312).

Time Investment in Training

There was considerable variation in the speed with which each animal learned to perform the tasks. The time required to complete target training ranged from 2 to 12 sessions (20 min to 2 hr overall, M = 1 hr, 4 min, per pair), whereas urine training was accomplished in 3 to 13 sessions (30 min to 2 hr, 10min, M = 52 min, per pair). There was no difference by sex for either target training, $t(10) = 0.22$, $p = .83$, or urine training, $t(10) = 0.47$, $p = .65$. Figure 3 shows the number of sessions required for individual animals.

When the marmosets were grouped according to which behavior was learned first, animals who were urine trained first learned significantly faster than those who were target trained first, $F(1, 10) = 157$, $p < .001$. When urine training was conducted first, this behavior was established within a mean of 4.5 sessions (around 45 min, per pair). Target training was accomplished within two sessions (20 min, per pair). Figure 4 shows the mean number of sessions required per individual for both trained behaviors depending on training order.

Comparison Between Trained Animals and Routine Laboratory Procedures

When the time taken to record the weights of the trained animals was compared to weights recorded by the current standard procedure, data collection from

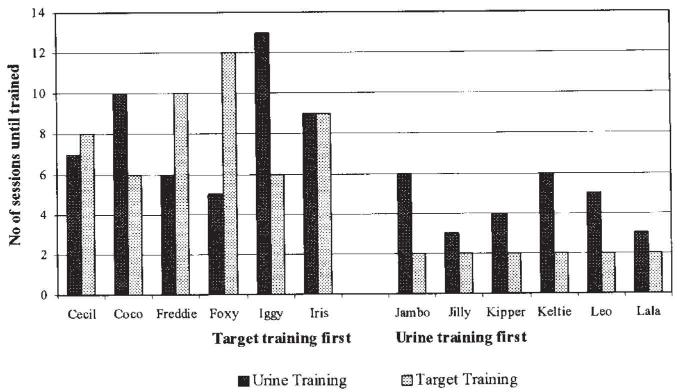

FIGURE 3 Number of training sessions required by each study animal to reach training criterion.

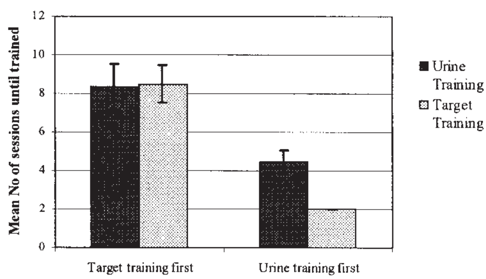

FIGURE 4 Mean number of sessions required for each behavior to reach training criterion by training order (bars show standard errors).

trained animals was considerably faster (see Table 2). Time taken per urine sample was less than that typically taken to collect blood samples.

DISCUSSION

This study demonstrated that training marmosets to cooperate with routine laboratory procedures could be accomplished using only PRT. One problem initially encountered while training pairs was four instances of aggression when the dominant animal tried to steal the reward. All of these incidences of aggression occurred when the monkeys were hungry (e.g., before they were fed), with three occurring on a Monday (after 2 days when the proportion of fresh fruit in the diet was reduced). Aggression was eliminated by training pairs with an aggressive member only after they had been fed and rewarding responses with cornflakes rather than marshmallow at the start of each session. Cornflakes are a less preferred food and were usually accepted three or four times and then discarded. Monday morning sessions were discontinued. These findings on aggres-

TABLE 2
Mean Time Required Per Sample Collected Using Trained Animals Compared With Standard Laboratory Procedures

Procedure	*Mean Time Per Sample*[a]
Weighing (standard procedure)	174.25
Weighing (trained animals)	14.75
Blood sample collection	542.8
Urine sample collection	184.6

[a]Given in seconds.

sion have far-reaching implications. They counter the widely held belief that food deprivation is necessary for successful training. Indeed, they even suggest that food deprivation may be counterproductive to the training process.

Weights were collected with 100% reliability. Around 5% of urine samples were lost, largely due to the same animal, not because he failed to provide a sample, but because he became adept at removing the collecting vial before the trainer. In a comparison of urine versus blood collection, it should be noted that it could be difficult to collect daily blood samples over a long period of time without damage to the femoral vein. In addition, there is a limit to how much blood can be taken from such a small animal before his or her health is compromised (Ferrell, 2003; Hearn, 1983). Training to provide urine samples could be particularly useful for studies of relatively long duration. However, as scent marking is associated with stress in marmosets (Bassett et al., 2003/this issue), there is the issue of rewarding a stress-related behavior (Sutcliffe & Poole, 1978; Watson, Ward, Davis, & Stavisky, 1999). However, the study animals did not continue to scent mark at high rates outside training sessions despite this behavior having been rewarded (Bassett et al., 2003/this issue). Many substances such as cortisol can now be measured in saliva (Lutz, Tiefenbacher, Jorgensen, Meyer, & Novak, 2000), and this may prove a more satisfactory replacement for blood and urine. Saliva can be collected at very regular intervals (i.e., 5 min), and training is minimal.

Although the marmosets learned both behaviors fairly quickly, there was considerable variation in the speed with which individual marmosets learned; it would seem that overcoming fear of humans was an important factor in their performance. Initially the marmosets were nervous and tended to take the food reward, then retreat to the back of the cage before eating. They only would remain holding their targets once this nervousness had been overcome during the training process. Some primates scent marked more frequently when nervous (Watson et al., 1999), and this did appear to be the case with these animals. Initial nervousness, although a hindrance during target training, was actually helpful during urine training, as frequent scent marking allowed frequent reinforcement of this behavior. By the time urine training was complete, the marmosets had become quite tame, and this made target training easier.

A positive consequence of PRT is that the caregiver–animal relationship is richer. Although this clearly has benefits for animal welfare, it has a potentially negative side effect. Personnel involved in terminal studies frequently maintain a degree of psychological detachment through avoidance of naming or anything else that might personify the study animals (Serpell, 1999). PRT could make detachment from the animal as a means of coping with euthanasia more difficult.

Once training was complete, data collection for trained animals was considerably faster than that collected using standard procedures, suggesting that time invested in training may be recouped later. In this study, time spent target training for weighing could be recovered within 8 to 20 sessions, depending on training order. However, as the marmosets were trained in pairs, with two sessions being conducted simultaneously, the number of sessions required per animal actually overestimates the actual time investment required. The basic behavior is extremely versatile and, once established, could be used for other procedures such as entering transport cages (Laule & Desmond, 1998). We conclude that training is a practical tool in the management of these animals and allows them to cope better with routine laboratory procedures (Bassett et al., 2003/this issue).

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