Training Nonhuman Primates to Cooperate With Scientific Procedures in Applied Biomedical Research

Leah Scott, Peter Pearce, Sarah Fairhall, Neil Muggleton, and Jeremy Smith

Biomedical Sciences
Dstl Porton Down
Salisbury, Wiltshire, England

This report provides a brief overview of aspects of training nonhuman primates who have been, and continue to be, used in this laboratory. The research context involves applied behavioral studies in which animals are trained to perform complex operant behavioral sequences, often in their homecage environment. In such studies, animals have freedom to choose whether to engage in appetitively reinforced behavioral tests that employ neither food deprivation nor fluid management. This background of operant conditioning has provided an insight to, and a context for, animal training both as an adjunct to general laboratory management and as a way to expedite scientific procedures. Thus, training has potential implications for both well-being and scientific quality, although it must be considered an adjunct to the provision of socialization with conspecifics in high quality diverse housing systems and not as an alternative to such provision. The importance of discussion and consideration of alternative procedures cannot be overemphasized.

This article describes practical experience gained over more than 20 years in the training of nonhuman primates to cooperate with scientific procedures in an applied biomedical research setting. A number of techniques and approaches will be described that exemplify the culture in which the work is conducted.

The work of this laboratory has involved applied research in behavioral pharmacology and toxicology. The provision of high quality, healthy animals from in house colonies of marmosets (Callithrix jacchus) and rhesus monkeys (Macaca

Request for reprints should be sent to Leah Scott, Biomedical Sciences, Dstl Porton Down, Salisbury, Wiltshire, SP4 OJQ, England. E-mail: lascott@dstl.gov.uk
mulatta) has underpinned all the work. The opportunity to observe animals in breeding and peer groups has influenced staff attitudes and approaches positively to the care and use of the animals.

The nature of the applied research objectives has necessitated the development and application of measures of both spontaneous and conditioned behavior. This has provided opportunities to develop and validate a diverse range of novel experimental techniques for characterizing the common marmoset as a model in biomedical research and refining the use of rhesus monkeys in behavioral and pharmacological research. A cornerstone of the approach adopted has been the interrelationship between good welfare and scientific quality; the work is considered as a joint venture involving scientists, animal care staff, and the animals themselves.

**METHODS**

**Rationale for Homecage Testing**

Presentation of behavioral tests to nonhuman primates traditionally involves removing animals from their own cages and testing them in a remote location such as an operant chamber. In this laboratory, the emphasis on homecage testing exemplifies the culture that has been engendered, and this has provided a robust basis for training animals to cooperate with scientific procedures. The major feature of such testing is that although at times short-term separation from cage mates is necessary, such separation is undertaken within the confines of an extremely familiar environment, which would be expected to lead to significantly less stress in all concerned. Preliminary unpublished results in this laboratory suggest that short-term separation from a cage mate in an unfamiliar environment raises marmoset heart rate (as measured by radiotelemetry techniques) by about 30% compared to separation with visual contact within their cage environs.

Being unrestrained, animals are free to choose whether to engage in any task presented. Successful completion of conditioned tasks generally is associated with an opportunity to access preferred food treats, which normally are not incorporated in their regular diet. In such circumstances, no food or water deprivation is employed; negative reinforcement is never used. If animals choose not to engage in the task, they are free to move elsewhere in their enclosure.

Furthermore, homecage testing provides substantial levels of stimulation for all the animals in the room. This stimulation applies not only for the animal under test, as demonstrated by their willingness to participate, but also to others in the room who attend to the activities going on. They are able to do so because of the cage extensions, which allow observation of the whole room. It recently has been demonstrated, by means of 24-hr activity monitoring, that husbandry practices and the room activities associated with homecage testing have an impact on daily time...
budgets for marmosets—although it would be unwise to speculate about the desirability of such changes.

**Development of the Approach**

Traditional approaches to operant conditioning involve isolation of the subject in a sound-attenuated, temperature, and humidity-controlled environment such as that shown in Figure 1. When such approaches were used in this laboratory more than 20 years ago, adequate levels of performance were achieved, but isolation-induced vocalization was common and marmosets took many months to attain satisfactory baselines of performance on relatively simple schedules.

In parallel studies at that time, there was a requirement to quantify visually guided reaching in marmosets, and a simple apparatus that readily could be attached to—and detached from—the homecage was developed. The success of this approach (D’Mello, Duffy, & Miles, 1985) established confidence in the utility of homecage testing approaches and formed the basis for the subsequent development of methods for measuring more sophisticated behavioral indexes, such as attentional set shifting in marmosets and rhesus monkeys in the homecage (Crofts, Muggleton, Pearce, Nutt, & Scott, 1999). Homecage presentation of tasks, wherever practicable, now is the preferred approach.

![FIGURE 1 An example of a marmoset pressing a lever to obtain reward in an operant box of traditional design, which is remote from the homecage.](image)
Training Marmosets

Our operant training approaches are exemplified by recent and continuing studies in marmosets (Pearce, Crofts, Muggleton, & Scott, 1998). Animals are trained to perform complex cognitive tasks by responding to icons presented on a touch sensitive screen, which is positioned such that the animals can access the test equipment from a rigid cage extension on their homecage. In keeping with all operant training, it is essential to ensure that animals are (a) strongly motivated by the reward to be offered and (b) that they are familiar with the auditory and visual cues associated with availability of that reward.

Marmosets first are trained to lick up to 0.1 ml of banana milkshake (which has been demonstrated to be a powerful reinforcer in this and other laboratories) from a licker spout within a 5-sec period in response to a 1-sec tone. This is presented every 8 sec and, following establishment of reliable contingent licking, animals are trained to touch a colored square icon that initially fills the whole screen. Touching the screen is followed by the tone to indicate that the reward is available. They are encouraged to touch the screen by placing pieces of mastic or colored stickers on the screen. Once individuals touch the screen reliably, the mastic or stickers are removed and the animals encouraged to touch the screen unprompted to obtain the reward (see Figure 2). The dimensions of the icon then are gradually reduced until it approximates the size of the stimuli to be used in discrimination studies (see Figure 3). In this laboratory, the training protocol is successful without human participation.

Training sessions generally last for 30 min. When animals have achieved a predetermined level of performance, subsequent testing sessions of varying task com-

FIGURE 2 Marmoset responding to icons presented on a touch sensitive screen—making the correct response is rewarded with access to banana milkshake. The animal is free to return to the main body of the homecage at any time.
complexity last for 15 min or terminate when 60 trials have been completed. It is important to note that the test, as presented, is self-paced. When the task increases in complexity, such as when the animal has to shift its attention from one icon type to another, fewer responses are made initially per test session because animals make more errors and choose to spend less time in close proximity to the touch screen.

The homecage testing approach is flexible in that a number of tasks can be presented sequentially, which maximizes the amount of information that can be collected from individual subjects. In an ongoing marmoset study, aspects of cognitive behavior and muscle function are investigated using different devices positioned on or near the cage front. Training for all testing procedures follows broadly similar patterns of successive approximation.

Leaving (and Returning to) the Homecage

It is not practicable to conduct all scientific procedures in the animal’s homecage. Although it is possible to train some species to present limbs for blood sampling and drug administration, it sometimes is necessary to remove animals from their familiar environments for more complex procedures. When this is necessary, options for macaque species include the use of crushback cages or netting (in larger enclosures) with subsequent “manhandling.” Neither option is ideal. Both can induce confrontation and high levels of stress for all concerned. Moreover, in many circumstances health and safety considerations dictate a “hands-off” approach. The preferred option for some studies in this laboratory has been to train animals to cooperate with pole and collar handling systems. Collars of an appropriate size and type do not ap-
pear to interfere with social behavior. Animals are readily trained to cooperate with attachment/detachment of a pole, and trained scientists/care staff direct the movement of animals toward the required destination.

In this laboratory, all rhesus monkeys (with or without collars) are trained to respond to voice commands. They are housed in large, interlinked cages with access to well-equipped outdoor pens. When transfer to another location is required, knowledgeable, sympathetic staff use a consistent approach involving the animals’ individual names and established methods of animal training (e.g., positive reinforcement, successive approximation) to encourage them to leave their home enclosure and enter another enclosure or transport box. To train animals to enter a transport box, which is attached to a door in the lower portion of the cage, they initially are separated into individual cages and the linkers closed. Early stages may involve positioning of staff above the level of the top of the cage; voice tones, which vary from one-word commands to softer reinforcement tones, are employed. Reducing the available space in the cage also may be used in the early stages. Once trained, animals will enter the transport box with little encouragement and generally following a single command. Grapes, which do not form part of the animals’ regular diet, are used as a reward to reinforce this activity. As with other aspects of homecage testing, appropriately designed cages greatly facilitate the development and implementation of innovative techniques that are beneficial to animals, researchers, and animal care staff.

Eye Tracking

Studies of visual tracking are generally conducted in human and nonhuman primates. This is one of a number of areas of research that necessitate accurate positioning of the subject’s head. When the subject is human, this does not present difficulties but traditional methods for nonhuman primates frequently involve highly invasive techniques and substantial restraint to restrict movement.

In some areas of work, when gaze orientation is under study and sophisticated neurophysiological recordings are not necessary, it is practicable to develop systems that do not necessitate substantial levels of restraint. Procedures involving voluntary cooperation can be devised, and this approach has been used successfully to track direction of gaze and pupil size in rhesus monkeys. To collect such information in humans, a commercially available system involving a pair of small cameras mounted on a headband (see Figure 4) has been employed. This arrangement would not be practicable for use with freely moving rhesus monkeys.

“Traditional” options would have involved the use of restraint chairs and the surgical implantation of scleral coils and head posts to limit head movement and facilitate measurement of gaze direction. Such techniques were not considered necessary in view of the nature of the study and the commitment of this laboratory.
to using noninvasive or minimally invasive procedures wherever practicable. It was therefore decided to investigate options for a system based on cooperation.

Rhesus monkeys are trained to enter a specially modified transport box on voice command and then to place their heads through a hole in the roof to access a fruit-flavored drink, delivered via a specially designed ensemble that also defined the position of the subjects’ eyes (Figure 5). This design is pivotal to the success of the approach. Subsequently, the animals are trained to respond by first fixing and then shifting their gaze in response to icons presented on a monitor screen. After a short test session of approximately 15 min (daily for 5 days per week), animals are returned to their cage mates. Throughout training and testing, animals are free to move within the constraints of the transport box and are not forced to engage with any the test procedures. For some areas of applied vision research, the technique may offer an alternative to more invasive methods.

CONCLUDING REMARKS AND RECOMMENDATIONS

This report gives a brief overview of aspects of training nonhuman primates that have been, and continue to be, used in this laboratory. The background of applied behavioral studies, in which animals are trained to perform complex behavioral sequences—often in their home cage environment—has provided an insight to, and a context for, animal training as an adjunct to general laboratory management and as a way to expedite scientific procedures.

A number of lessons have been learned over the years, and the concept of teaching the right lesson from the outset is a key element of all animal training—regardless of whether the training takes place in the home cage. The importance of this
concept was evident from homecage studies with rhesus monkeys in this laboratory 15 years ago.

There was a requirement to collect a precise measurement of reaction time, and the paradigm required a response key to be pressed until a signal light was illuminated. The animal then was required to release the response key as quickly as possible. All animals in the group, except one, learned the task very quickly, and their performances were consistent and predictable. When the animal who had not learned the task was filmed, it was clear that this animal was not performing the test properly. He was unable to detect illumination of the stimulus light because he was pressing the response key with his nose rather than his finger. The introduction of a small Perspex surround ensured that responses could be made only in the manner intended. This is an example of teaching the right lesson and of the value of observation, video recording, and appropriate evaluation.

Undoubtedly, there are benefits to be gained in terms of animal welfare and scientific quality, although these benefits will be optimized only if the prevailing culture of the laboratory is appropriate. At a very basic level, this involves striking an “appropriate” balance between human and nonhuman primate interactions. Furthermore, training and interactions with humans should be considered as adjuncts to the provision of socialization with conspecifics in high quality diverse housing systems and not as alternatives to such provision.

Communication on training issues is extremely important, whether by establishing personal contact, visiting facilities, sharing experiences in multidisciplinary forums, or publishing methodological details in mainstream physiological, behavioral, and applied scientific reports in specialist
primatological and welfare journals. Publication of reports on unsuccessful techniques also is extremely useful as valuable lessons can be learned. Critical evaluation and widespread dissemination and discussion of relevant findings are extremely important issues.

There is a continuing requirement for creativity, lateral thinking, and open-mindedness to consider how aspects of techniques and approaches developed and implemented by other laboratories might be adapted and adopted to refine procedures. Spending time with individuals who have relevant practical experience can de-risk such ventures substantially and can pay real dividends in terms of animal welfare and scientific quality.

ACKNOWLEDGMENTS

We acknowledge the contributions of many who have been influential in their thinking and approaches—especially Viktor Reinhardt, Hal Markowitz, Joachim Jaekel, and Trevor Poole. We acknowledge the commitment over the years of many teams of scientists, animal care staff, and technical support staff at Dstl Porton Down, which has contributed to the culture in which animal training has been undertaken and continues to be fostered.

REFERENCES