Refining the Blood Collection Procedure for Macaques

By Viktor Reinhardt

Handling procedures for macaques assigned to re search often involve two circumstances that trigger anxiety and fear in the subjects: removal from the home environment and involuntary restraint. Common responses to this situation include a variety of distress reactions [1,2] that not only affect the well-being of the macaque but also the validity of research data [3-18].

Being a veterinarian and behavioral scientist, I recognized a need for refining the common blood collection procedure (Fig. 1). I thought that the intelligent nature of monkeys [19-23] should make it possible to condition them to cooperate during blood collection in their home environment. Anecdotal reports of laboratory monkeys and apes presenting a limb for blood collection [12,14,24-27] support the view that it is not necessary in all situations to restrain the animals during this common procedure.

Figure 1. Anxiety and fear associated with involuntary blood collection away from the familiar homecage may lead to substantial deviations of the subject's normal physiological functions, and hence introduce a dependent variable that is often overlooked by researchers [1].

METHODS

I worked with rhesus monkeys (Macaca mulatta) at the Wisconsin Regional Primate Research Center (WRPRC), and after several years felt confident enough to start training the animals to present a leg for venipuncture in their familiar homecage.

I conducted my first trial with the assistance of Russell Vertein, an experienced animal caretaker [28]. We worked with four pairs of 5-16 year-old female rhesus monkeys who were all familiar with us and showed no fear reactions when either of us approached their cages. Staff at the center previously habituated the monkeys to saphenous blood collection in a restraint apparatus away from their homecages. Each pair was housed in a 85 x 85 x 85 cm cage equipped with a squeeze-back.

During days 1-5 of the training, Vertein and I habituated the animals to accepting raisins offered through the mesh of the cage, while we reduced their cage space by about 75%. The monkeys had enough room to turn and move around. Some took and ate raisins on the first day, while others accepted them only on day 5. On days 6 and 7, we partially restrained the females as described above. Then, with my fingertips through the mesh of the cage front, I groomed the leg of each female for at least one minute and gave raisins as rewards thereafter. On days 8-15, I partly opened the cage door and cautiously reached for a leg of the female nearest to me. I gently lifted the animal's leg and stroked it for one minute, rewarded the animal with raisins, and repeated the procedure with the second female. On days 15-23, I lifted and gently pulled the leg of each female through the cage door. I kept each female in this sham venipuncture position for approximately 20 seconds before releasing it and offering some raisins in my hand. I pushed the squeeze-back into its normal position at the end of each training session, and the animals received a raisin reward again.

We completed the training on day 24 by collecting a blood sample from each animal via saphenous venipuncture. Three females promptly presented a leg on this occasion and showed no resistance during the procedure. The other five, although less cooperative, did not resist having their legs pulled through the cage door and having a blood sample collected. All animals accepted the food reward offered after venipuncture, thereby showing us that we had not lost their trust in the course of the training.

This pilot study with females was so encouraging that I decided to adapt the training program to males[29]. The second trial included 10 pair-housed, 4-12-year-old, male rhesus monkeys who we had also habituated to saphenous blood collection away from their homecages. Previously, I gave these males food treats during daily health checks, so they knew me. Each pair lived in a 85 x 170 x 85 cm high double cage provided with a privacy panel, two perches, and one restraint mechanism [30]. As a safeguard, I equipped all doors of the restraint compartments with a sliding Plexiglas panel. The panel had a smooth-edged (28 cm high and 8 cm wide) opening that allowed a male to extend a leg, but prevented it from protruding its head out of the cage (Fig. 2a,b,c). I trapped the target male by pulling the squeeze-back past the passage hole of the privacy panel [30]. The other male had free access to the rear portion of the cage and could keep visual contact with its companion.

I started each training session by enticing the target male (with raisins) to enter the restraint compartment of the double cage. By pulling the squeeze-back panel, I restricted the male to the front of the cage to the extent that he had to get into an upright position to turn around. Reaching a hand through the opening of the Plexiglas panel, I touched and stroked the male's back, grasped one of his legs and gently, yet firmly, pulled the leg out of the cage. I repeated this exercise until the male tolerated the procedure and spontaneously cooperated by presenting a leg in or through the opening of the Plexiglas panel. Once a male accepted venipuncture in this situation without showing any signs of resistance, I reduced the spatial restraint during the final session from approximately 80% to 75-50% floor area, giving the animal enough leeway to freely turn and move around.

I trained the ten males with strict consistency, and under no circumstances terminated sessions before pulling the subject's leg out of the cage for a one-minute period time, or before attaining a successful blood collection. Once achieving either of the two goals, I released the subject and offered raisins as a reward. Unlike the females, I considered the males successfully trained only if all of them actively presented a leg and accepted venipuncture without any signs of resistance. I made this modification to minimize the risk of getting bitten, because a voluntarily cooperating monkey should have no reason to attack in self-defense.

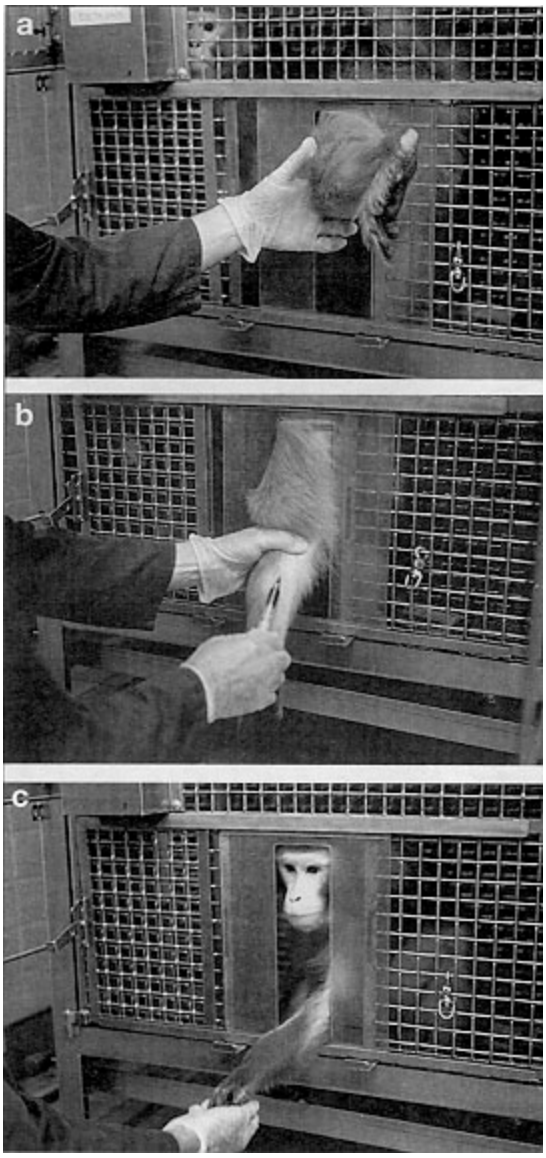

Figure 2a,b,c. Active cooperation of adult rhesus macaques (here, a ten-year-old male) during in-homecage blood collection can easily be achieved within less than a cumulative total of one hour of training. Trained animals promptly present a leg (Figure 2a) and show no distress response during venipuncture (Figure 2b) they accept food treats as positive reinforcement (Figure 2c).

I achieved my goal in 16 days. During this time, I invested, on average, a cumulative total of 20 minutes to overcome a male's resistance to having a leg pulled out of the cage while being restrained. I invested an additional 19 minutes to ensure that the animal actively presented a leg and displayed no resistance during blood collection, while having enough room to comfortably turn and move around (Fig. 2a,b). Total training time spent with a male ranged from 16-63 minutes, with a mean of 39 minutes distributed over 13 individual training sessions. Like the females, none of the males ever tried to bite, but they did not hesitate to take and eat raisins after completion of each training session (Fig. 2c). While training, I applied gentle, firm, and persistent patience, thereby making my actions predictable and non-threatening [29,31].

I applied this training protocol to six adult female stump-tailed macaques (Macaca arctoides) [31]. The animals' responsiveness was equally positive, and all subjects learned to actively cooperate during in-homecage venipuncture within a two-week period (Fig. 3). Total cumulative training time per individual ranged from 15 -45 minutes with a mean of 34 minutes.

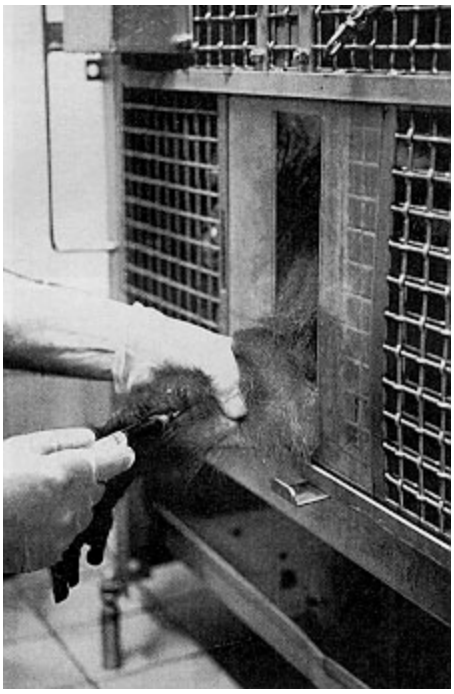

Figure 3. Adult stump-tailed macaques can be trained readily as adult rhesus macaques to actively cooperate during blood collection in their own cage.

Once trained, all rhesus and stump-tailed macaques cooperated during blood collection, not only with the trainer, but also with attending caretakers [28,29,31].

INFLUENCE ON CORTISOL LEVELS

I extended my studies to test the hypothesis that training the animals to cooperate during in-homecage venipuncture can help to avoid distress responses associated with the conventional involuntary blood collection procedures. Taking increased cortisol secretion as an indicator of distress [32-34], I analyzed the cortisol levels of two blood samples taken in 15-minute intervals from adult rhesus monkeys venipunctured while restrained on a table or in a specially designed restraint apparatus, and from monkeys trained to cooperate during venipuncture in their homecages [35-37]. Subjects were routinely bled in these fashions. The time required to get hold of an animal and draw a blood sample was 60-90 seconds in all three conditions.

Serum cortisol showed a statistically significant increase in animals venipunctured on the table or in the restraint apparatus (Table 1). The magnitude of cortisol levels was higher in the first than in the second mode of restraint. Neither females nor males showed a significant cortisol response to blood collection when trained to cooperate during the procedure while in their homecage (Table 1).

TABLE 1. Serum cortisol response of adult rhesus macaques to different blood collection procedures.

Condition of Venipuncture	15-minute Cortisol Increase
restrained on table	+63% (n=10 females)
restrained in apparatus	+50% (n=10 females) +52% (n= 6 males)
trained to cooperate in homecage	+14% (n=10 female) +13% (n=6 males)

Table 1. Serum cortisol response of adult rhesus macaques to different blood collection procedures.

These findings lead to the conclusion that training nonhuman primates to cooperate during venipuncture in their familiar home environment offers an important methodological refinement [3] for research protocols by eliminating significant cortisol responses. The training benefits not only the research subjects and research methodology but also animal care personnel by reducing the chances of being bitten, and by eliminating the health risk associated with carrying heavy animals from their cages to designated treatment areas [38]. Finally, the training offers an effective means of environmental enrichment [39] for the monkeys and for the animal caretaker by challenging them to establish and foster a relationship that is based on trust.

ACKNOWLEDGMENTS

I wish to express my sincere thanks to Doug Cowley, Steve Eisele, and Russell Vertein from the WRPRC for their guidance and assistance.
Received 11/20/95; accepted 12/12/195.

REFERENCES

1. Reinhardt, V. Impact of venipuncture on physiological research conducted in conscious macaques. J Exp. Anim. Sci.; 34: 212-217, 1991.

2. Reinhardt, V., Liss, C. and Stevens, C. Restraint methods of laboratory non-human primates: critical review. Animal Welfare; 4: 221-238, 1995.

3. Russell, W.M.S. and Burch, R.L. The Principles of Humane Experimental Technique. Charles C.Thomas Publishers, Springfield, MA, 1959.

4. National Institutes of Health (NIH). Guide for the Care and Use of Laboratory Animals. NIH, Bethesda, MD, 1985.

5. US Department of Health and Human Services. Principles for the utilization and vertebrate animals used in testing, research, and training. In: National Institutes of Health (NIH). Guide for the Care and Use of Laboratory Animals. NIH, Bethesda, MD, pp. 82-83, 1985.

6. European Economic Community Council Directive. On the approximation of laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes. Journal Officiel des Communautes Europeennes; L 358, December 18, 1986.

7. Canadian Council on Animal Care (CCAC). Guide to the Care and Use of Experimental Animals, Volume 1, 2nd Edition. CCAC, Ottawa, Canada, 1993.

8. Brown, M.J., Pearson, P.T., and Tomson, F.N. Guidelines for animal surgery in research and teaching. Am. J. Vet. Res; 54:1544-1559, 1993.

9. Prentice, E.D., Zucker, I.H. and Jameton, A. Ethics of animal welfare in research: The institution's attempt to achieve appropriate social balance. The Physiologist; 17: 17-20, 1986.

10. Zimmermann, M. Ethical principles for the maintenance and use of animals in research. Neurosc. Lett.; 73: 1, 1987.

11. Street, JW. and Jonas, A.M. Differential effects of chemical and physical restraint on carbohydrate tolerance testing in nonhuman primates. Lab. Anim. Sci.; 32: 263-266, 1982.

12. Walker M.L., Gordon T.P., and Wilson M.E. Reproductive performance in capture-acclimated female rhesus monkeys. J. Med. Primat.; 11: 291-302, 1982.

13. Anderson, J.H., and Houghton P. The pole and collar system. A technique for handling and training nonhuman primates. Lab Animal; 12(6): 47-49, 1983.

14. Herndon J.G., Turner J.J., Perachio A.A., Blank M.S., and Collins, D.C. Endocrine changes induced by venipuncture in rhesus monkeys. Physiol. Behav.; 32: 673-676, 1984.

15. Adams, MR., Kaplan, J.R., Manuck, S.B., Uberseder, B., Larkin, K.T. Persistent sympathetic nervous system arousal associated with tethering in cynomolgus macaques. Lab. Anim. Sci.; 38: 279-282, 1988.

16. Turkkan, J.S. New methodology for measuring blood pressure in awake baboons with use of behavioral training techniques. J. Med. Primat.; 19: 455-466, 1990.

17. Schwindaman, D. The 1985 animal welfare act amendments. In: Novak, M.A. and Petto, A.J., eds. Through the Looking Glass. Issues of Psychological Well-being in Captive Nonhuman Primates. American Psychological Association, Washington, DC, pp. 26-32, 1991.

18. Quimby, F.W. The role of the attending veterinarian in laboratory animal welfare. JA VMA; 206: 461-465, 1995.

19. Rumbaugh, D.M., Richardson, W.K., Washburn, D.A., SavageRumbaugh, E.S., and Hopkins, W.D. Rhesus monkeys (Macaca mulatta), video tasks, and implication for stimulus-response spatial contiguity. J. Comp. Psychol. Anim. Behav.; 103: 32-38, 1989.

20. Washburn, D.A., Hopkins, W.D. and Rumbaugh, D.M. Video-task assessment of leaming and memory in macaques: Effects of stimulus movement upon performance. J. Exp. Psychol. Anim. Behav.; 15: 393-400, 1989.

21. Reinhardt, V. Uncommon tool usage by captive primates. International Zoo News; 38(5): 13-14, 1991.

22. Washburn, D.A. and Rumbaugh, D.M. A comparative assessment of psychomotorperformance: Target prediction by humans and macaques. J. Exp. Psychol. Gen.; 121: 305-312, 1992.

23. Washburn, D.A., Harper, S., and Rumbaugh, D.M. Computer-task testing of rhesus monkeys (Macaca mulatta) in the social milieu. Primates; 35: 343-351, 1994.

24. Rosenblum, I.Y. and Coulston, F. Normal range of longitudinal blood chemistry and hematology values injuvenile and adult rhesus monkeys (Macaca mulatta). Ecotox. Environ. Safety; 5: 401-411, 198 1.

25. Billiard, R.B., Richardson, D., Anderson, E., Mahajan, D., and Little, B. The effect of chronic and acyclic elevation of circulating androstenedione or estrone concentrations on ovarian function in the rhesus monkey. Endocrinology; 116: 2209-2220, 1985.

26. Wall, H.S., Worthman, C., and Else, J.G. Effects of ketamine anaesthesia, stress and repeated bleeding on the haematology of vervet monkeys. Lab Animal; 19: 138-144, 1985.

27. Whitney, R.A. and Wickings E.J. Macaques and other old world simians. In: Poole T.B., ed. The UFAW Handbook on the Care and Management of Laboratory Animals, 6th edition. Churchill Livingstone, New York, NY, pp. 599-627,1987.

28. Vertein, R. and Reinhardt, V. Training female rhesus monkeys to cooperate during in-homecage venipuncture. Lab. Prim. Newsl.; 28(2): 1-3, 1989.

29. Reinhardt, V. Training adult male rhesus monkeys to actively cooperate during in-homecage venipuncture. Anim. Technol.; 42:11-17, 1991.

30. Reinhardt, V., Pape, R,. and Zweifel, D. Multifunctional cage for macaques housed in pairs or in small groups. AALAS Bull.; 30(5): 14-15, 1991.

31. Reinhardt V. and Cowley D. In-homecage blood collection from conscious stumptailed macaques. Animal Welfare; 1:249-255, 1992.

32. Selye, H. Hormones and Resistance. Springer, New York, NY, 1971.

33. Udelsman, R., and Chrousos, G.P. Hormonal responses to surgical stress. In: Chrousos G.P, Gold, D.L., and Gold, P.W., eds. Mechanisms of Physical and Emotional Stress. Plenum Press, New York, NY, pp. 265-267, 1988.

34. Torii, R., Kitagawa, N., Nigi, H. and Ohsawa, N. Effects of repeated restraint stress at 30-minute intervals during 24-hour on serum testosterone, LH and glucocorticoids levels in male Japanese monkeys (Macaca fuscata). Exp. Anim.; 42: 67-73, 1993.

35. Reinhardt, V., Cowley, D., Scheffler, J., Vertein, R., and Wegner, F. Cortisol response of female rhesus monkeys to venipuncture in homecage versus venipuncture in restraint apparatus. J Med. Primatol.; 19: 601-606, 1990.

36. Reinhardt, V., Cowley, D., Eisele, S., and Scheffler, J. Avoiding undue cortisol responses to venipuncture in adult male rhesus macaques. Anim. Technol.; 42: 83-86, 1991.

37. Reinhardt, V. Improved handling of experimental rhesus monkeys. In: Davis H. and Balfour A.D., eds. The Inevitable Bond: Examining Scientist-Animal Interactions. Cambridge University Press, Cambridge, UK, pp. 171-177, 1992.

38. Cowley, D., Vertein, R., Pape, H. and Reinhardt, V. Improving the work environment for animal care personnel with laboratory macaques. Anim. Technol.; 44: 129-135, 1993.

39. United States Department of Agriculture. Animal Welfare; Standards; Final Rule. Fed. Reg.; 56: 6426-6505, 1991.

Viktor Reinhardt has worked with primates for ten years. He currently consults for the Animal Welfare Institute. Please send reprint requests to the author at 4605 Crescent Road, Madison, WI 53711.

Reprinted with permission of the publisher.
This article originally appeared in *Lab Animal*, volume 25, number 1.