Working With Rather Than Against Macaques During Blood Collection

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Training macaques to cooperate during blood collection is a practicable and safe alternative to the traditional procedure implying forced restraint. It takes a cumulative total of about 1 hr to train an adult female or adult male rhesus macaque successfully to present a leg voluntarily and accept venipuncture in the homecage. Cooperative animals do not show the significant cortisol response and defensive reactions that typically occur in animals who are forcibly restrained during this common procedure.

Blood collection is probably the most common handling procedure nonhuman primates are subjected to in research institutions. Traditionally, it is accomplished by forciblyrestraining the subject because it is believed that "all monkeys are dangerous" (Ackerley & Stones, 1969, p. 207), that "nonhuman primates can be very difficult and even dangerous to handle," and that "restraint is therefore necessary and desirable to protect both the investigator and the animal" (Robbins, Zwick, Leedy, & Stearns, 1986, p. 68). Indeed, a subdued monkey will try to show self-defensive aggression. Therefore, "despite rigorous observance of all precautions, bites and scratches are frequent" (Valerio et al., 1969, p. 45; Zakaria, Lerche, Chomel, & Kass, 1996).

Figure 1. Traditional blood collection procedure.

THE VARIABLE

Because of "adverse conditioning or fear" (Robbins, Zwick, Leedy, & Stearns, 1986, p. 68) enforced restraint during blood collection is an extremely alarming situation (see Figure 1) that affects physiological equilibrium, thereby increasing data variability and the number of research subjects needed to achieve statistically significant results (Brockway, Hassler, & Hicks, 1993). It has been shown that compulsory restraint changes normative:

1. Cortisol secretion in rhesus (Elvidge, Challis, Robinson, Roper, & Thorburn, 1976; Fuller, Hobson, Reyes, Winter, & Faiman, 1984; Hayashi & Moberg, 1987; Line, Markowitz, Morgan, & Strong, 1991; Puri, Puri, & Anand-Kumar, 1981)

and Japanese macaques (Torii, Kitagawa, Nigi, & Ohsawa, 1993) as well as in capuchin monkeys (Dettmer, Phillips, Rager, Bernstein, & Fragaszy, 1996);

2. Progesterone secretion in baboons (Albrecht, Nightingale, & Townsley, 1978; Goncharov et al., 1979);
3. Testosterone secretion in rhesus macaques (Hayashi &Moberg, 1987; Puri, Puri, & Anand-Kumar, 1981) and baboons (Goncharov et al., 1979);
4. Adrenal androgen secretion in rhesus macaques (Fuller, Hobson, Reyes, Winter, & Faiman, 1984);
5. Prolactin secretion in rhesus macaques (Quadri, Pierson, & Spies, 1978);
6. Growth hormone secretion in rhesus macaques (Mason et al., 1968);
7. Follicle stimulating hormone secretion in rhesus macaques (Todd et al., 1999);
8. Glucagon secretion in squirrel monkeys (Myers, Mendoza, & Cornelius, 1988);
9. Glucose regulation in rhesus, stump-tailed (Streett & Jonas, 1982) and Celebes macaques (Yasuda, Wolff, & Howard, 1988);
10. Serum glutamic-oxalacetic transaminase activity in rhesus macaques (Cope & Polis, 1959);
11. Aspartate aminotransferase and alanine aminotransferase activity in long-tailed macaques (Landi, Kissinger, Campbell, Kenney, & Jenkins, 1990);
12. White blood cell count in rhesus macaques (Ives & Dack, 1956; Loomis, Henrickson, & Anderson, 1980) and baboons (Goosen, Davies, Maree, & Dormehl, 1984);
13. Blood concentration in rhesus macaques (Loomis, Henrickson, & Anderson, 1980);
14. Blood pressure and heart rate in rhesus macaques (Golub & Anderson, 1986) and marmosets (Schnell & Wood, 1993);
15. Acid-base balance in squirrel monkeys (Manning, Lehner, Feldner, &Bullock, 1969) and Barbary and lion-tailed macaques (Bush, Custer, Smeller, & Bush, 1977); and
16. Respiration rate in rhesus and long-tailed macaques (Berendt & Williams, 1971).

    Surprisingly, traditional blood sampling is officially "expected to produce little or no discomfort" (Scientists Center for Animal Welfare, 1987, p. 12). In line with this, many investigators tacitly ignore their subjects' stress responses during blood collection (Reinhardt & Reinhardt, 2000).

THE REFINEMENT

In an attempt to reduce the stress reaction during blood collection, six individually caged adult (8 to 12 years old) female rhesus macaques (*Macaca mulatta*) were trained to cooperate during femoral venipuncture in the homecage (see Figure 2). The subjects were used to being immobilized on a table for this procedure (see Figure 1).

Figure 2. Refined blood collection procedure.

The effect of the training was assessed by drawing two blood samplesthe first at 13:15 ±1 min and the second at 13:30 ±1 minfrom each animal during the conventional procedure involving forced restraint and, on another day, during the refined procedure involving voluntary cooperation. The animals lived in 70 cm × 75 cm × 77 cm large upper row cages. They were fed commercial dry food at 7:30 and fruit at 15:00. The macaques were subjected to no external disturbanceincluding personnel walking in the hallwaysfor 1.5 hr before the first blood drawing at 13:15. Both during the conventional and during the refined procedure, venipuncture occurred 60 to 90 sec after the caretaker had entered the animal room. The time lapse did not differ between the two conditions (conventional 76 ± 12 sec vs. refined 73 ± 14 sec; $t = 0.399$, $df = 5$, $p > .1$).

The blood samples were analyzed for serum cortisol as an indicator of stress. The first samples were used to assess basal levels; the second samples served to evaluate the magnitude of the cortisol response 15 min after venipuncture. Mean cortisol concentrations of the first samples did not differ under both conditions, $t = 0.226$, $df = 5$, $p > .1$ (Table 1). Cortisol concentrations of the second samples, however, were significantly higher under the restraint condition than under the cooperation condition, $t = 3.910$, $df = 5$, $p < .005$ (Table 1). The magnitude of the endocrine response to venipuncture was significant (+68%), $t = 4.834$, $df = 5$, $p < .001$, when the subjects were restrained, but it was insignificant (+14%), $t = 1.135$, $df = 5$, $p > .1$, when they cooperated (see Table 1).

THE TRAINING PROTOCOL

The following protocol was used to train the subjects of this study as well as 12 adult pair-housed female, 5 adult single-housed male, 10 adult pair-housed male rhesus macaques, and 6 adult pair-housed female stump-tailed macaques (*M. arctoides*). The animals were used to being immobilized mechanically in their homecages during routine procedures such as ketamine injection.

Step 1

Establish an affectionate relationship with the trainee. She or he should come to the front of the cagerather than retreat to the backwhen you enter the room. The subject must trust you; only then will it be safe to proceed with the training.

Step 2

With the help of the squeeze-back, the subject is confined in the front quarter of the cage. In this position, freedom of movement is considerably restricted, but the subject has enough leeway to turn around. The animal is reassuringly talked to, gently scratched through the mesh, and offered some raisins. After a minute or two, the squeeze-back is pushed back and raisins again are offered. This exercise is repeated on different days until the animal is relaxed and accepts the food reward.

Step 3

The subject again is restricted and enticed with raisins and/or gently prodded to face the left or right side of the cage. The subject's leg is touched and groomed through the opening of the door. After a minute or two, the squeeze-back is pushed back and raisins offered. This sequence of events is repeated on different days until the animal stops retracting the leg and accepts the food reward.

Step 4

The restricted subject's leg is gently and firmly pulled through the opening of the door and held firmly for about 1 minute. The squeeze-back is pushed back and the subject rewarded with raisins. The goal of Step 4 is achieved when the animal shows no signs of resistance such a trying to retract the leg or to turn around.

TABLE 1
Cortisol Responses of Six Rhesus Macaques to Traditional and Refined Blood Collection

BLOOD SAMPLING PROCEDURE	MEAN CORTISOL CONCENTRATIONS		DIFFERENCE (SIGNIFICANCE)
	First Sample	Second Sample	
Traditional (restraint)	20.1 ± 4.5 µg/dl	33.8 ± 5.3 µg/dl	$p < .001$
Refined (cooperation)	19.6 ± 3.0 µg/dl	22.3 ± 5.0 µg/dl	$p < .1$

Step 5

The squeeze-back is pulled only so far as to prompt the trainee to come forward. The animal is in full control of the situation and has enough room to turn around freely and avoid being touched. The trainer encouragingly asks the subject to present a leg behind or through the opening of the door. An animal who refuses to cooperate is not punished in any manner but simply does not receive a food reward. This exercise is repeated on different days until the animal actively presents a leg and shows no resistance during blood collection from the femoral vein (see Figure 2) or saphenous vein. Once this goal is achieved, the animal is praised and rewarded with raisins.

The training protocol outlined here was applied successfully not only by the author but also by two animal caretakers, Vertein (Vertein & Reinhardt, 1989) and Cowley (Reinhardt & Cowley, 1992).

THE TIME INVESTMENT

The total number of training sessions per animal ranged from 2 to 27. Individual training sessions lasted from a few seconds to 5 minutes, depending on the trainee's responsiveness. Cumulative time to reach the training goal (Step 5) ranged from 16 to 69 min with a mean of 38.5 min (see Table 2). There was a tendency, statistically insignificant, for pair-housed subjects requiring less training time than single-housed subjects; female rhesus: $t = 0.621$, $df = 16$, $p > .1$; male rhesus: $t = 0.469$, $df = 13$, $p > .1$ (Table 2). Females and males did not differ in the time needed to train them; rhesus pair-housed: $t = 0.025$, $df = 20$, $p > .1$; rhesus single-housed: $t = 0.065$, $df = 9$, $p > .1$ (see Table 2).

Although traditional blood sampling procedures usually require at least two peopleone to help restrain the subject, one to puncture a vein and draw blood (see Figure 1)only one person is required to do this procedure with a trained subject (see Figure 2). Once trained, all animals cooperated not only with the trainer but also with the attending care personnel as well as with experienced personnel from other facilities.

TABLE 2
Time Investment to Achieve Active Cooperation of Macaques During Blood Collection in the Familiar Homecage

Subject, Housing	n	Time Investment (Minutes)
Female rhesus, single-housed	6	44.3 ± 16.6
Female rhesus, pair-housed	12	39.0 ±18.0
Male rhesus, single-housed	5	43.6 ± 18.7[a]
Male rhesus, pair-housed	10	38.8 ± 18.6[a]
Female stump-tailed, pair-housed	6	33.5 ± 10.0[b]

[a] Data originally published in Reinhardt (1991). [b] Data originally published in Reinhardt and Cowley (1992).

CONCLUSIONS

Training macaques to cooperate voluntarily during blood collection is a practical alternative to the traditional procedure implying forced restraint and stress. Working with a cooperative rather than against a resisting monkey (a) eliminates the handler's risk of becoming the target of defensive biting and scratching; (b) refines research methodology by controlling the extraneous variable of stress; and (c) provides high quality mental stimulation both to the animal and to the handling person. The initial time investment in the training quickly pays off in a safe handling procedure that no longer requires a second person to control the resisting subject. It should be noted that the idea of training macaques to cooperate during blood collection is not new. There are reports from 10 different research facilities where macaques have been trained voluntarily to present a leg for blood collection (Reinhardt, 1997). Surprisingly, however, this simple, yet effective, refinement technique is applied only sporadically while the traditional technique relying on force still is prevailing.

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