Cortisol Response of Female Rhesus Monkeys to Venipuncture in Homecage Versus Venipuncture in Restraint Apparatus

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Cortisol response of ten single-caged adult female rhesus monkeys during venipuncture in a restraint apparatus was compared with cortisol response of ten paired and five single-caged adult female rhesus monkeys during venipuncture in the homecage. Results demonstrated that in-homecage venipuncture offers a methodological improvement for research protocols that require blood collection of undisturbed animals.

Key words: Housing. Handling. *Macaca mulatta*

INTRODUCTION

Blood sampling via venipuncture is probably the most common experimental procedure that laboratory rhesus monkeys are subjected to. The procedure is generally unsatisfactory because of the animals' resistance to it. Such resistance may jeopardize unbiased research and should therefore be avoided. It has recently been shown that female rhesus monkeys can easily and quickly be trained to cooperate during in-homecage venipuncture (unpublished results). This training method was developed in an attempt to minimize distress reactions of research animals to blood collection.

The present investigation compares peripheral cortisol concentrations, taken as indicators of stress, in female rhesus monkeys during the new in-homecage venipuncture procedure with those of female rhesus monkeys during a conventional venipuncture procedure in which an animal is temporarily transferred from its homecage into a restraint apparatus. It was hypothesized that removal from the familiar homecage would be more distressing than actual venipuncture and that an animal's cortisol response would therefore be negligible during in-homecage venipuncture.

The study also addresses the question of whether subordinate partners of compatible pairs show higher cortisol concentrations, as a symptom of social distress, than their dominant companions. This issue has been raised repeatedly as a potential problem of pairing rhesus monkeys for the purpose of providing them means to express their social disposition.

MATERIALS AND METHODS

Blood samples (1 cc) were collected from 25 adult (average age 11. 8 ± 3.5 yr), healthy, non- pregnant, and non-lactating female rhesus monkeys (*Macaca mulatta*) according to two protocols. In the conventional procedure, a monkey is removed from its homecage to a transport box and transferred into a restraint apparatus. A blood sample is drawn by puncturing a saphenous vein, after which the animal is returned to its cage and rewarded with favored food. In the improved procedure,

a monkey is temporarily restrained in its homecage by means of the movable back wall of the cage, and a blood sample is taken by puncturing a saphenous vein (Fig. 1). The restraint mechanism is then released, and the animal is rewarded with favored food.

Ten single animals were assigned to the conventional procedure. They were accustomed to it for several years, and there was no need to force them to enter the transport box and the restraint apparatus. It was also not necessary to immobilize them in the restrainer since they readily presented a leg for venipuncture.

Fifteen animals were assigned to the improved procedure with which they were acquainted for 2-4 weeks. Five animals were singly housed, ten animals lived in five compatible pairs. Reducing their cage space by 75% with the movable back wall was sufficient to ensure their readiness to have a leg gently pulled through the open cage door and a blood sample drawn (Fig. 1). There was no need to immobilize them during this procedure.

Taking unidirectional fear-grinning and yielding as signs indicative of subordination, rank relationships of paired companions were determined one day before the first blood sample was collected.

Blood samples were collected by one caretaker with whom the animals were familiar. Each monkey was bled on a different day at 13:15 ± 1 min. and again at 13:30 ± 1 min. The first samples at 13:15 were used to assess basal cortisol concentrations. The second samples at 13:30 served to evaluate the magnitude of cortisol response 15 min. after venipuncture.

The animals were subjected to no external disturbance for 1.5 hr before the first blood drawing. The actual venipuncture occurred 60-90 sec after the caretaker had entered the animal room. Paired animals were not separated while one of them was venipunctured (Fig. 1).

Blood samples were centrifuged at 2,000 rpm for 10 min. and the serum stored at - 20T within I hr of collection. Subsequent analysis for cortisol was done with a Clinical Assays Gamma Coat Cortisol Kit (Dade, Baxter Travenol Diagnostics, Cambridge, MA, USA). All samples were done in duplicate.

Paired monkeys lived in 85cm x 85cm x 85cm cages, single monkeys in 70cm x 75cm x 77cm cages. Commercial dry food was provided at 7:30, supplemented with fruit at 15:00. Water was available ad libitum. Room temperature was maintained at 21°C, with a 12-hr light/dark cycle.

Statistical analysis was done with Student's t-test [1].

FIGURE 1. Venipuncture of a paired female rhesus monkey in her homecage with her companion.

RESULTS

Mean serum cortisol concentrations did not differ significantly in the three categories of animals during the first venipuncture at 13:15 ($P > 0.1$; Table 1). At 13:30, mean cortisol concentration was significantly higher in single monkeys venipunctured in the restraint apparatus than in paired monkeys venipunctured in their homecages ($P < 0.01$; Table 1). Mean cortisol concentration of single monkeys venipunctured in their homecages was intermediate and differences were not significant ($P > 0.1$; Table 1).

In single monkeys venipunctured in the restraint apparatus, cortisol concentrations were on average 50% higher at 13:30 than at 13:15; this elevation was significant ($P < 0.001$; Table 1). The magnitude of cortisol elevation was only 18% and 14%, respectively, in single and paired monkeys venipunctured in the homecage. These elevations were not significant ($P > 0.1$; Table 1).

TABLE I. Serum cortisol concentrations in female rhesus monkeys during venipuncture in homecage versus venipuncture in restraint apparatus

Housing condition	Place where venipuncture occurs	No. of animals	13:15 Cortisol (μg/dl) Mean	± SD	Range	13:30 Cortisol (μg/dl) Mean	± SD	Range
Single	Restraint apparatus	10	19.9	4.2	14.0–28.1	29.8	5.2	23.6–39.0
Single	Homecage	5	20.5	2.1	17.3–23.8	24.2	5.7	19.9–35.4
Paired	Homecage	10	19.5	2.9	15.3–23.7	22.2	6.4	13.5–31.7

Dominant and subordinate partners had equivalent cortisol concentrations. This was true during both the first venipuncture at 13:15 (19.4 ± 2.9 vs. 19.5 ± 3.0 [µg/dl, P > 0. 1) and the second venipuncture at 13:30 (23.3 ± 5.7 vs. 21.1 ± 6.8 µg/dl, P > 0. 1).

DISCUSSION

This study demonstrates that temporarily removing a rhesus monkey from its homecage for venipuncture constitutes an avoidable disturbance for the research animal.

Cortisol concentrations were comparable in all three categories of animals during the first blood collection, probably because the time from the moment the caretaker entered the room to the moment of actual venipuncture was too short to allow for a measurable cortisol response. After 15 minutes, however, the magnitude of response was significant in animals that were removed from their homecage for venipuncture but not in those venipunctured at home. This suggests that the improved venipuncture procedure is less disturbing for the animals than the conventional procedure. Pair housing apparently does not interfere with in-homecage venipuncture, and the equivalent cortisol concentrations of subordinate and dominant partners indicate that living with a compatible companion does not constitute a distressing situation.

The findings lead to the conclusion that training rhesus monkeys for inhomecage venipuncture offers an important methodological improvement for research protocols that require blood collection of undisturbed animals.

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