Avoiding Undue Cortisol Responses to Venipuncture in Adult Male Rhesus Macaques

Viktor Reinhardt, Doug Cowley, Steve Eisele, Joan Scheffler
Wisconsin Regional Primate Research Center,
University of Wisconsin,
1223 Capital Court, Madison, WI 53715, USA

SUMMARY

Six single-caged adult male rhesus monkeys were venipunctured in their homecage and, on a different day, away from their cage in a restraint apparatus. The animals were habituated to both procedures and readily presented a leg for blood collection without being mechanically immobilized. The time required to draw a blood sample was less than 2 minutes for both procedures.

Serum cortisol concentrations were equivalent in blood samples collected at 1200 h when the animals were in the restraint apparatus (mean = 15.3±4.4 µg/dl) and when the animals were in their homecages (mean = 15.7±2.4 µg/dl; $p>0.1$). Cortisol concentrations during a second venipuncture at 1215 h were significantly higher during blood collection in the restraint apparatus (mean = 23.2±4.7 µg/dl) than during blood collection in the homecage (mean = 17.7± 5.0 µg/dl; $p < 0.0\ 5$). The magnitude of cortisol increase during the 15 minutes was significant (52%; $p<0.025$) when the males were venipunctured in the restraint apparatus but not (13%; $p>0.1$) when they were venipunctured in the homecage.

It was concluded that venipuncture *per se* was not a physiologically distressing event for the males. It became distressing only when it was associated with a temporary removal from the homecage. In-homecage venipuncture is therefore recommended as an alternative to venipuncture in a restraint apparatus for those research protocols that require blood samples from undisturbed experimental monkeys.

INTRODUCTION

A simple technique has recently been described for training adult male rhesus monkeys to actively cooperate during in-homecage venipuncture[1]. It was argued that data-confounding distress reactions are eliminated by this new technique because the experimental subject remains in its familiar cage. The present investigation further examines this argument.

Blood samples were collected from adult male rhesus monkeys that were both:
a) habituated to actively cooperate during venipuncture away from the homecage in an apparatus specially designed for blood collection;
b) trained to actively cooperate during in-homecage venipuncture.

Serum cortisol concentrations were taken as parameters for evaluating distress[2-7].

METHODS

Six healthy male rhesus monkeys (*Macaca mulatta*) were subjects of this study. The animals ranged in age between 9 and 13 years with an average of 10.3±1.2 years. They were housed singly in 85 x 85 x 85 cm squeeze-back cages. Commercial dry food was provided at 0730 h, supplemented with fruit at 1500 h. Water was available *ad libitum*. Room temperature was maintained at 21ºC, with a relative air humidity of 50% and a 12-h light/dark cycle.

Blood samples were collected from each of the six males according to two procedures. In the **conventional** procedure, a male was removed from his homecage to a transport box and transferred to a restraint apparatus located in another room. A blood sample was drawn by puncturing a saphenous vein, after which the animal was returned to its cage and rewarded with favoured food. The six males had been habituated to the conventional blood drawing procedure for several years. 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 restraint apparatus since they actively presented a leg for venipuncture.

In the new procedure, the animal was not removed from his homecage during blood collection. All six males actively presented a leg behind or through the party opened cage door and displayed no resistance during saphenous venipuncture. Using the squeeze-back mechanism, the cage space was reduced 50-75% during this procedure, but the monkey had enough room to move around comfortably. The animals had been trained to cooperate during in-homecage venipuncture in July 1990.

Blood samples (1 cc) were collected by the attending caretaker with whom the animals were familiar. Each monkey was bled using the conventional and the new procedure on different days in August 1990 at 1200 h and again at 1215 h. The monkeys were subjected to no external disturbances for 1 hour prior to the venipuncture at 1200 h. The 1200 sample was used to assess basal cortisol concentrations, the 1215 sample to evaluate the magnitude of cortisol response 15 minutes after venipuncture.

Blood samples were centrifuged at 2,000 rpm for 10 minutes and the serum stored at -20C within 30 minutes of collection. Subsequent analysis for cortisol was done in duplicate with a Clinical Assays Gamma Coat Cortisol Kit (Dade, Baxter Travenol Diagnostics, Cambridge, MA, USA).

Statistical analysis was done with the Mann Whitney test[8].

RESULTS

After entering an animal room, a caretaker required 77.5±27.4 seconds to venipuncture a monkey in its homecage, and 72.2±24.7 seconds to venipuncture a monkey in the restraint apparatus. The difference was not significant ($p>0.1$).

Serum cortisol concentrations of the six animals did not differ significantly between the first venipuncture (1200 h) in the homecage and the first venipuncture in the restraint apparatus ($p>0.1$; Table 1). Cortisol concentrations were significantly higher ($p < 0.05$), however, during the second venipuncture (1215 h) in the restraint apparatus than during the second venipuncture in the homecage (Table 1).

When venipunctured in the homecage, the males showed cortisol concentrations at 1215 h that were, on average, 13% higher than at 1200 h. This endocrine response was of no statistical significance ($p>0.1$; Table 1). When venipunctured in the restraint apparatus, however, the males showed cortisol concentrations at 1215 h that were, on average, 52% higher than those at 1200 h. The magnitude of this response was statistically significant ($p< 0.025$; Table 1).

DISCUSSION

The present investigation demonstrates that venipuncture does not need to be a disturbing experience for adult male rhesus monkeys.

In both venipuncture techniques, the experimental subjects were not immobilized, displayed no resistance, and actively presented a leg for blood drawing. Also, the time required to puncture a subject's vein was the same for both techniques. The time was probably too short to allow for a measurable cortisol response to the 1200 venipuncture. This explains why cortisol concentrations during the first venipuncture at 1200 h were equivalent for both techniques. During the 1215 venipuncture, however, the males showed cortisol concentrations that were significantly higher when they were removed from their homecage than when they were allowed to remain in their familiar cage during blood collection. The magnitude of cortisol response was statistically significant only when the males were venipunctured in the restraint apparatus but not when they were venipunctured in their familiar homecages. This indicates that venipuncture *per se* was not a distressing event. It became distressing only when associated with a temporary removal from the homecage. Similar findings have been reported for female rhesus monkeys[9].

Venipuncture is probably the most frequent procedure to which experimental rhesus monkeys are subjected for the collection of research data. The scientific validity of such data may be confounded seriously if the animal shows physiological signs of disturbance such as a significant rise in serum cortisol concentration, that are not accounted for in the experimental protocol. This study has demonstrated the data-confounding effect of removing a male rhesus monkey from its familiar cage for the purpose of blood collection. The study has tested in-homecage venipuncture as an alternative that can be recommended for research protocols that call for blood collection from undisturbed experimental subjects.

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