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Comparison of Toxicology Assessment Baseline Data Collected Using Two Restraint Methods in Nonhuman Primates: Restraint Chair/Pole and Collar vs. Procedure Cage

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INTRODUCTION

Physical or chemical restraint has been historically used in the laboratory setting to allow for humane and efficient handling of nonhuman primates (NHPs) while minimizing animal stress or distress. When stress occurs, there are alterations to the physiological homeostasis or psychological well-being of the animal. Among the several forms of restraint, restraint chairs have been shown to be the preferred method of restraint for research studies for NHPs. For this procedure, animals are fitted with a neck collar to which a pole can be attached and used to guide them from their home cage to the restraint chair termed pole and collar/ restraint chair for this study (PC/Restraint chair). This method allows for applying positive restraint techniques with limited use of negative reinforcement (NRT), which increases animal cooperation, reduces distress and use of anesthesia, and encourages increased cognitive stimulation. The other method of interest for this study, the procedure cage, relies primarily on physical (hand) contact and aspects of negative reinforcement training (NRT), where the animal moves into position for a procedure at the front of the cage and releases the squeeze-back as soon as the desired movement toward the cage front is achieved. This study aimed to investigate the differences in outcomes of using these methods on toxicological assessment in NHP studies and provide evidence for best practices in restraint in a laboratory setting.

MATERIALS AND METHODS

Animals and Animal Care

Test System: *Macaca fascicularis*, male and female

Source: Cambodia

Approval for Research: All animal-related procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Environmental conditions: Primary enclosure complied with the Animal Welfare Act and recommendations set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council 2011). Animals were housed in a temperature- and humidity-controlled environment with target ranges between 18 and 29 degrees Celsius, and 30 and 70%, respectively. A 12-hour light/dark cycle was set, and animals were kept in stainless steel metal cages.

Diet: PMI LabDiet® Fiber-Plus® Monkey Diet 5049 biscuits and water was provided *ad libitum*. Treats were provided daily, including fresh produce, marshmallows, raisins, juice, etc.

Approach

A retrospective analysis of acclimation data from six studies, 3 (83 males and 87 females that employed the procedure cage) and 3(45 males and 75 females that used the pole and collar and restraint chair) was performed. Animals were acclimated over at least six sessions to ensure that they were adequately adapted to the restraint method with the provision of visual access to restraint devices where applicable (i.e., chair and pole).

Clinical pathology values from hematology, coagulation, and serum chemistry and which are typically evaluated in toxicological assessments collected during the acclimation period, were compared between the two restraint methods.

EQUIPMENT

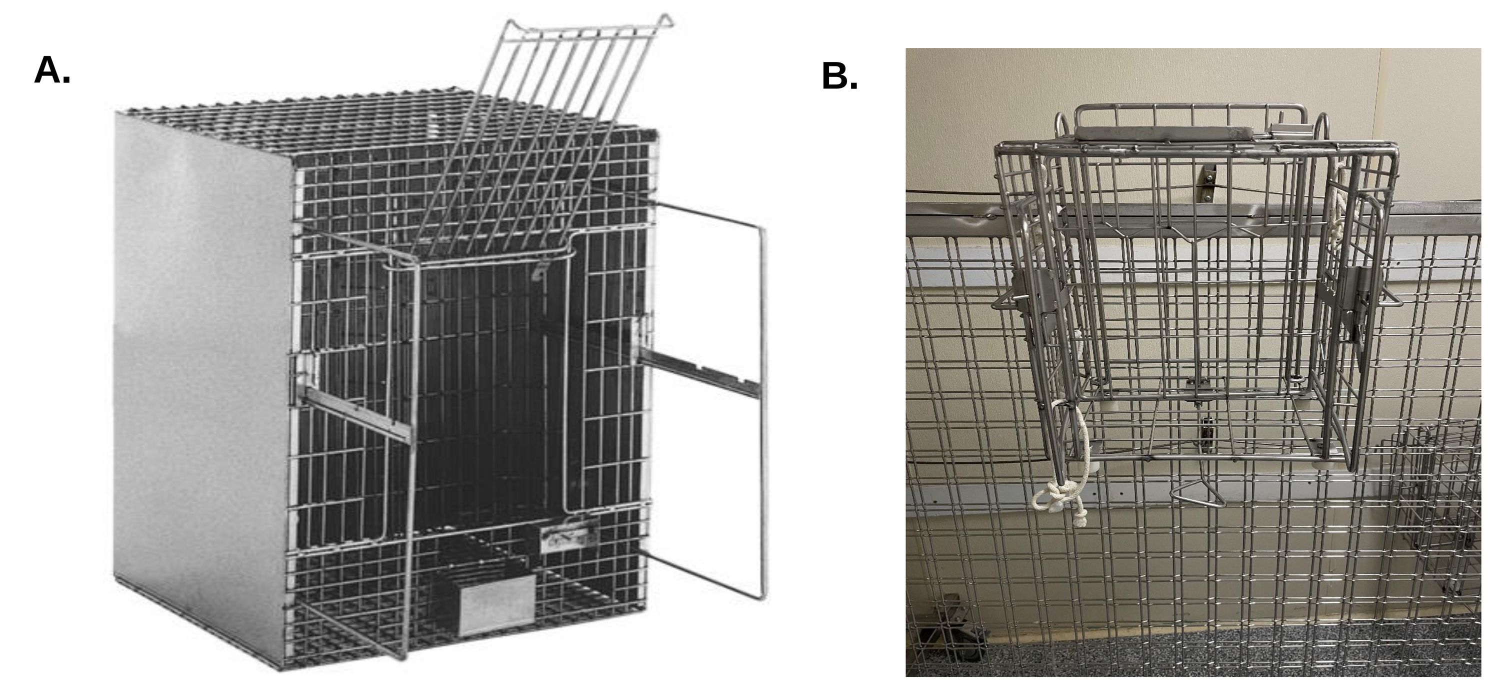


Figure 1. Procedure Cage

Cages are equipped with a special back panel that can be moved in such a way that the animal is forced to come to the front of the cage and tolerate being partially or completely immobilized.

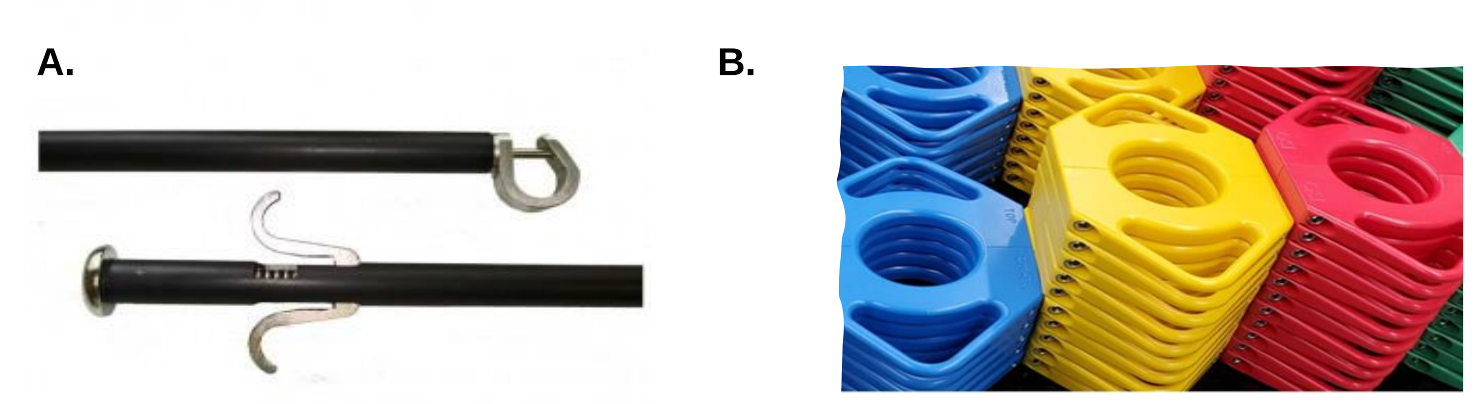


Figure 2. Pole and Collar

A. The primate capture pole is designed to work with the capture collar **B**. It has a trigger system that holds itself open and closes to a light touch providing a standardized method for handling nonhuman primates.

Figure 3. Restraint chair. A. Front B. Back

Restraint chairs maintain an animal in a sitting position, with restraint being affected by pillory-type attachments at the neck**. Insert** Demonstration of use.

RESULTS

Surrogate measures of stress (neutrophil, eosinophil, monocyte, and lymphocyte counts, i.e., stress leucogram), inflammation (albumin, fibrinogen, and prothrombin time), and muscle damage (creatine kinase) levels, compared between the two restraint methods are represented below.

A two-way ANOVA followed by the Šídák's multiple comparisons test was used to evaluate differences in these clinical pathology parameters for each restraint method.

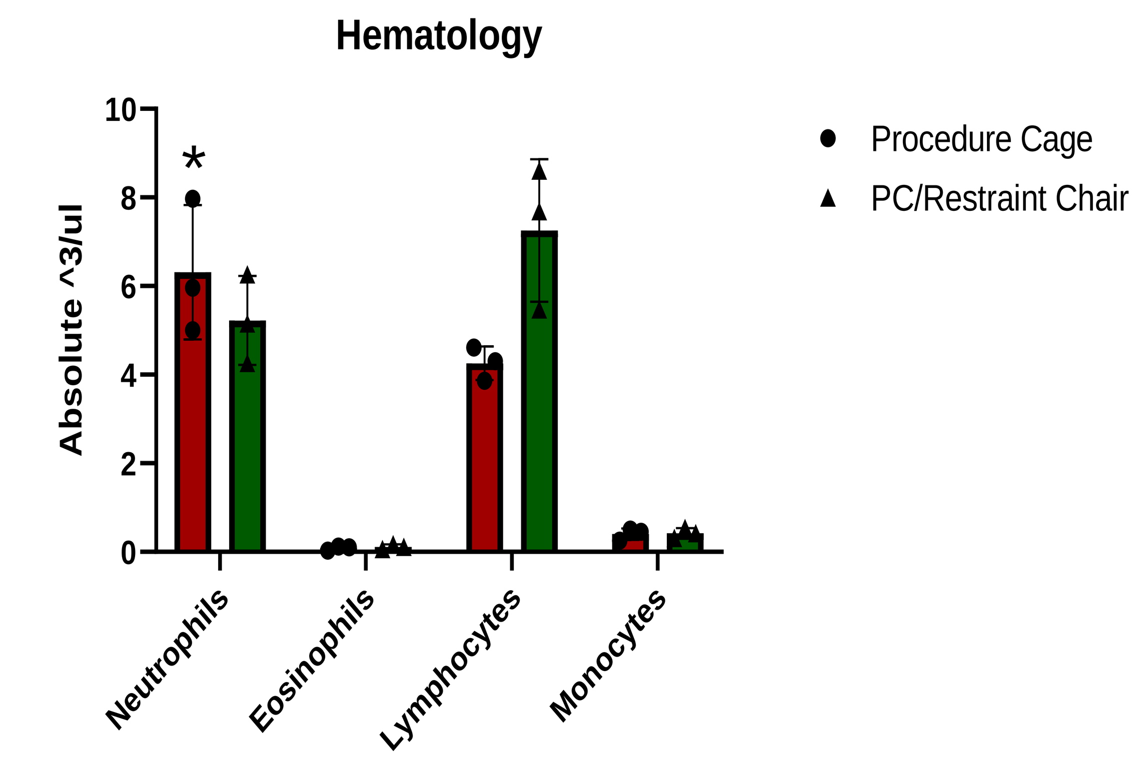
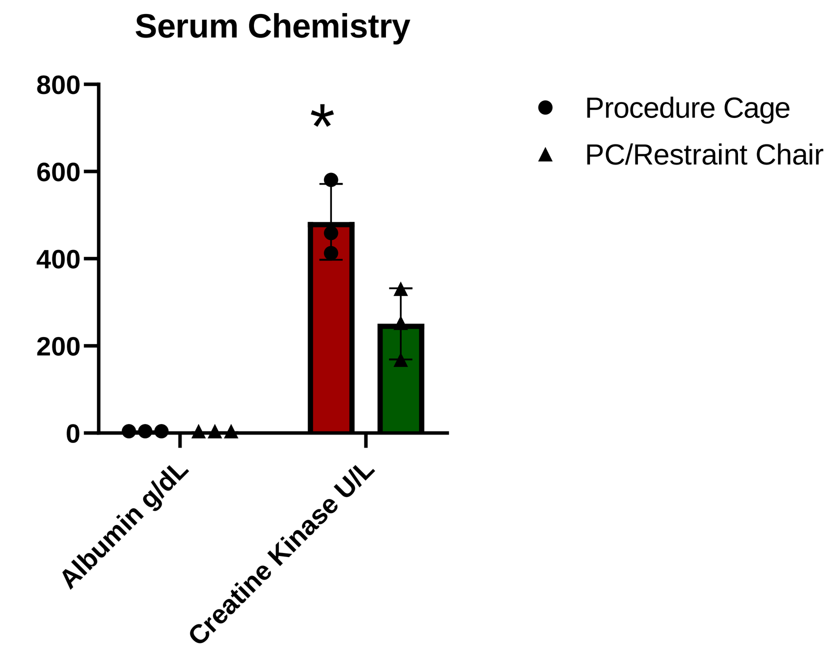
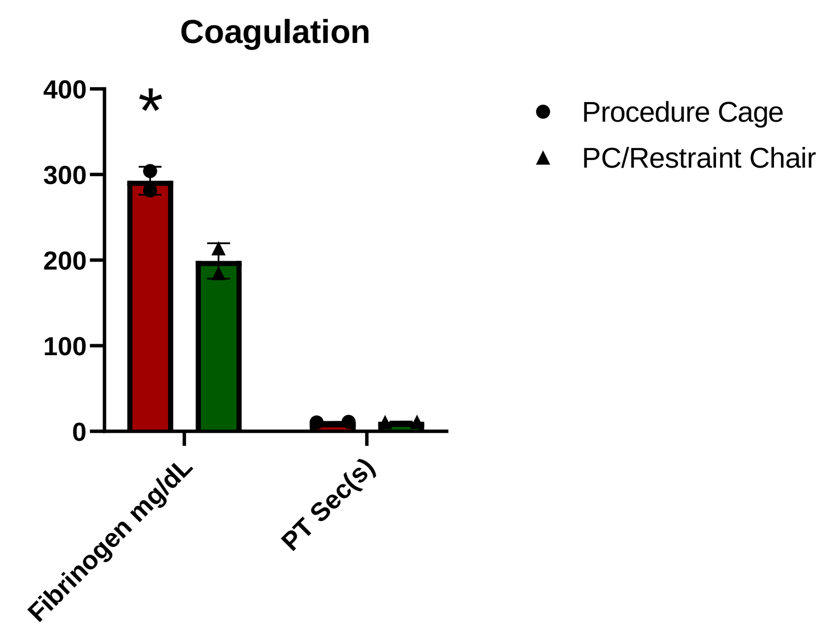


Figure 4. Neutrophilia ($p=0.4684$) and Lymphopenia ($p=0.0026$), indicative of the stress leucogram, were present when the Procedure cage was used compared to the use of the PC/restraint chair method.





Figures 5 and 6. Levels of creatine kinase and fibrinogen were significantly higher in Procedure cage vs. PC/Restraint Chair animals ($p=0.0027$) and ($p=0.0041$), respectively, suggestive of acute muscle damage and inflammation.

CONCLUSIONS

This study is a direct clinical evaluation of the Procedure cage vs. PC/Restraint Chair and provides evidence that the PC/Restraint Chair leads to less distress and inflammation, resulting in limited alterations to physiological homeostasis, translating to the psychological well-being of animals.
More published information is required on this subject. Institutions that use these restraint procedures need to work together to define best practices for using restraint chair/pole and collar.