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Snuggle Wrap-based Restraint Method for Continuous Infusion in Juvenile Nonhuman Primates

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ABSTRACT

With the increase of pediatric pharmaceuticals in preclinical studies, the ability to adapt to the use of a younger and smaller test system is necessary to ensure process evolution and to reduce stress on the research animals. The routine method for infusion restraint at our facility includes the use of a procedure cage. This method would have been highly stressful for animals under a year of age and was not considered suitable for small primates. The alternative “snuggle wrap” was developed to allow young animals to remain immobile while still feeling comforted by a hugging-like sensation. Given that social pairs need to remain in proximity, the “snuggle board” was also developed. The boards hold four snuggled animals closely together while keeping social pairs in visual and auditory contact during dose administration. The animals were visually monitored, provided treats, and manipulated throughout the dosing period. This snuggle method was successfully utilized in two 13-week studies with intravenous infusions in animals of 6 to 9 months, 12 to 15 months, and 40 to 48 months of age. The data sets collected using the snuggle method or the procedure-cage method were pooled and utilized for comparison. Based on the data, there are no apparent differences between the two restraint methods. Therefore, both the procedure-cage and snuggle methods can be successfully utilized based on the age and size of the animals, with the snuggle method being the most suitable for younger primates.

INTRODUCTION

With the increase of pediatric pharmaceuticals in preclinical studies, the ability to learn and to adapt the use of a younger and smaller test model is necessary to ensure process evolution and stress reduction on animals. The cynomolgus macaque is commonly used in non-human primate (NHP) research. Our onsite team has an expansive variety of non-human primate experience, cynomolgus macaque, and others, with subjects ranging from newborn to geriatric. Method development for a repeated infusion dose in juvenile non-human primates required a united effort to create a concise study and equipment design, with animal welfare at the forefront of all considerations. The snuggle method was successfully utilized in the study that involved animals of various age ranges (6 to 9 months, 12 to 15 months, and 40 to 48 months of age), as well as in 13-week repeat-dose studies, during which animals transitioned from the snuggle wrap to the procedure cage with age.

STRATEGIES

- Create a cohesive design that promotes functionality and comfort, provides the necessary equipment/supplies, and allows for long duration of care and support
- Select designated procedure room
- Itemize possible stress factors and reduction plan
- Utilize design with 6 training animals under current SOPs and IACUC approvals
- Train technical staff with training animals

METHODS

- Startup and development of the juvenile restraint procedure involved oversight by a multi-disciplinary group of professionals.
- The development and refinement of all portions of the procedure was an ongoing process; we started with 6 age-appropriate juvenile cynomolgus macaque with previous history of human interaction as training animals.
- Snuggle acclimation training occurred for 2 weeks every other day, with increased snuggle-wrap time at each training session. Positive reinforcement was provided during and following each session.
- Body temperature taken at intervals ensured animals did not overheat during procedures.
- In repeat-dose studies, the animals transitioned from snuggle boards to procedure cages in a stepwise fashion. As animals reached an appropriate age and/or weight/size, two cage-mates were restrained in the same procedure cage next to each other to decrease procedure-related stress. Over time, the animals were individually restrained in the procedure cages as they became acclimated to the procedures.

DESIGN

Figure 1.
Small-hole procedure cages were used for transport from study room to designated procedure room.

Figures 2 and 3.
Receiving blankets were used as a base wrap prior to the use of the unique customized snuggle wraps.

Figures 4 and 5.
Six different snuggle wrap sizes were utilized depending on the size of the animal. Wraps were used to expose one arm for either the cephalic vein or saphenous vein for catheter placement rotation.

Figure 6.
Restraint boards held four monkeys per board. Socialized pairs were able to remain close. Infusion pumps were in proximity for vein accessibility.

Figure 7.
Color-matching cage cards and Velcro® were used to avoid cross-contamination. Fabric items were washed daily after use.

Figures 8 and 9.
Twenty-four animals were monitored, treated, and cared for during weekly dosing by trained staff with assessable equipment.

BODY TEMPERATURES

No changes in average body temperatures were noted prior to or after snuggle, confirming that animals were not stressed during the procedure.

Average Juvenile Cynomolgus Macaques	Pre-Snuggle Average	30 Minutes Post-Snuggle Average
37.0 °C - 39.5 °C	38.5 °C	38.8 °C

CLINICAL PATHOLOGY VALUES

Animal Numbers	Age (months)	Sex	Glucose (mg/dL)	Absolute Neutrophil Count (10^3/µL)	Absolute Lymphocyte Count (10^3/µL)	Absolute Monocyte Count (10^3/µL)	Absolute Eosinophil Count (10^3/µL)
1	7 - 9	Male	77	4.13	7.25	0.25	0.07
2		Male	74	4.53	6.2	0.35	0.05
3		Male	71	6.99	5.72	0.46	0.23
4		Male	74	5.64	10.55	0.92	0.07
5		Male	80	6.89	4.5	0.46	0.06
6		Male	74	4.32	9.69	0.71	0.09
7		Female	82	2.53	1.06	0.22	0.01
8		Female	74	5.53	5.99	0.46	0.06
9		Male	89	5.47	10.37	0.41	0.25
10		Male	72	5.14	7.35	0.22	0.02
11		Female	72	3.9	3.74	0.23	0.14
12	12 - 15	Male	76	10.25	4.46	0.57	0.05
13		Male	69	4.06	7.63	0.35	0.03
14		Male	62	3.93	3.77	0.19	0.01
15		Male	74	9.57	5.36	0.39	0.65
16		Male	94	5.12	5.69	0.47	0.13
17		Male	83	4.47	3.18	0.27	0.05
18	40 - 48	Female	76	7.08	4.67	0.52	0.15
19		Female	65	5.88	5.63	0.33	0.05

Clinical pathology parameters comparison between various age groups:

- The table compares the data between various age groups being restrained via snuggle method (7 to 9 months) and the procedure-cage method (12 to 48 months).
- Data suggests lack of significant changes in the various stress-indicating parameters.

EQUIPMENT AND SUPPLIES

Items	
Separate procedure room	Restraint boards/snuggle boards
Small-hole procedure cage for transfer	Colored Velcro® straps
Receiving blankets	Color projector
RFID implants	Fruits, veggies, pasta, sweet treats
Transferable ID card	Juice
Velcro® strips	Enrichment toys
Soft cloth ties	Reusable diapers

CONCLUSION

Drawing on the knowledge and expertise of our team members, we implemented a stress-reduced dose infusion administration method for juvenile non-human primates (NHPs). We applied the snuggle method to weekly 1-hour infusions for an initial 52-week study in 6-month-old animals before switching to our standard procedure-cage method. The snuggle method can be additionally used on similarly aged or younger NHPs for standard IV bolus or shorter infusions. Several chronic toxicology studies in juvenile NHPs for which standard restraining methods do not apply also successfully employed this method.