Effect of Crinkle Paper Nesting Material on the Ability of Animal Care Workers to Perform Cage Checks

College of Veterinary Medicine
University of Missouri

NC STATE UNIVERSITY College of Veterinary Medicine

Jacob D. Simpson[1&2], Daniel J. Davis[1], Elizabeth C. Bryda[1]

[1]Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, MO

[2]North Carolina State University, College of Veterinary Medicine, Raleigh, NC

ASLAP The American Society of Laboratory Animal Practitioners

Abstract

The Guide for the Care and Use of Laboratory Animals recommends environmental enrichment for all laboratory animals to promote species specific behavior and deter abnormal stress behaviors. Nesting material for lab mice has been shown to eliminate ambient cold stress, improve reproduction, and decrease stereotypical behavior. The University of Missouri has instituted the use of 8 grams of crinkle paper in addition to a nestlet as enrichment for mice. It has been suggested that this additional paper material may compromise the ability to visualize animals while evaluating their health status. Our objective was to determine the effect crinkle paper has on worker ability to assess animals during routine cage checks. Three separate trials were completed with eight individuals evaluating twenty-one cages of mice per trial. Cages were divided into three enrichment groups: 8 grams of crinkle paper and a nestlet, 4 grams of crinkle paper and a nestlet, and a nestlet with no crinkle paper. Each group contained eight simulations of common issues found during routine cage checks with each appearing once per group over all trials. Time to perform individual cage checks and ability to identify simulation conditions were recorded for each evaluator. An approximate 11% increase in cage check time for cages with 8g of paper, no significant time difference between 4g and nestlets alone, and a trend of better simulation identification with less paper material were found. We recommend the use of 4g of paper for enrichment instead of 8g and for future studies to explore this reduction's measurable physiological impact on mice.

Objective and Hypothesis

Objective: To determine the effect crinkle paper nesting material has on worker ability to assess animals during simulated cage checks.

Hypothesis: The addition of crinkle paper enrichment will not significantly impact individual timing or ability to assess mouse cages.

Experimental Design

Trial 1	1	2	3	4	5	6	7
B	Empty						
C	n = 3	DEAD n = 1	STRAIN n = 2	RED LINE n = 2	n = 3	n = 2	n = 2
		DEAD	STRAIN	RED LINE			
D	n = 2	LITTER n = 2	BALD n = 3	n = 3	SEX n = 3	n = 2	n = 2
		LITTER	BALD		SEX		
E	n = 2	n = 2	n = 2	NO F/W n = 3	n = 2	n = 3	TOO FEW n = 2
				NO F/W			TOO FEW
F	Empty						

Trial 2	1	2	3	4	5	6	7
B	Empty						
C	n = 2	BALD n = 3	n = 2	DEAD n = 1	n = 2	n = 3	n = 2
		BALD		DEAD			
D	NO F/W n = 3 NO F/W	n = 2	n = 3	n = 2	n = 3	LITTER n = 3 LITTER	SEX n = 3 SEX
E	n = 3	RED LINE n = 3 RED LINE	n = 3	n = 3	STRAIN n = 2 STRAIN	TOO FEW n = 2 TOO FEW	n = 2
F	Empty						

Trial 3	1	2	3	4	5	6	7
B	Empty						
C	n = 3	n = 2	n = 2	n = 3	n = 2	DEAD n = 1 DEAD	NO F/W n = 3 NO F/W
D	LITTER n = 2 LITTER	n = 2	RED LINE n = 3 RED LINE	STRAIN n = 3 STRAIN	n = 3	n = 3	SEX n = 3 SEX
E	n = 2	BALD n = 2 BALD	n = 2	n = 2	TOO FEW n = 3 TOO FEW	n = 2	n = 2
F	Empty						

Key	
8G Crinkle Paper	4G Crinkle Paper
Nestlet Only	Simulation Cage
Red Line = Red Line On Abdomen	Too Few = Fewer Mice Than Indicated
Dead = Deceased Mouse in Cage	No F/W = No Water In Cage
Litter = Litter Indicated, But Not Present	Bald = Mouse w/ Alopecia
Sex = Wrong Sex In Cage	Strain = Wrong Strain In Cage
n = # Of Mice In Cage According To Cage Card	

Figure 1. Experimental Design. For each trial, a single individual ventilated cage rack held 21 cages of mice. Each row (C,D,E) contained a different enrichment group (8G of crinkle paper with a nestlet, 4G of crinkle paper with a nestlet, and a nestlet with no crinkle paper). Each group contained 8 different simulations with each simulation appearing only once per enrichment group over all three trials. Location of simulation and number of mice per cage were determined using a random number generator. Evaluators were tasked to assess cages and verbally state any abnormal findings they found. Time taken for each individual cage assessment and correct simulation identification were recorded.

Enrichment Groups

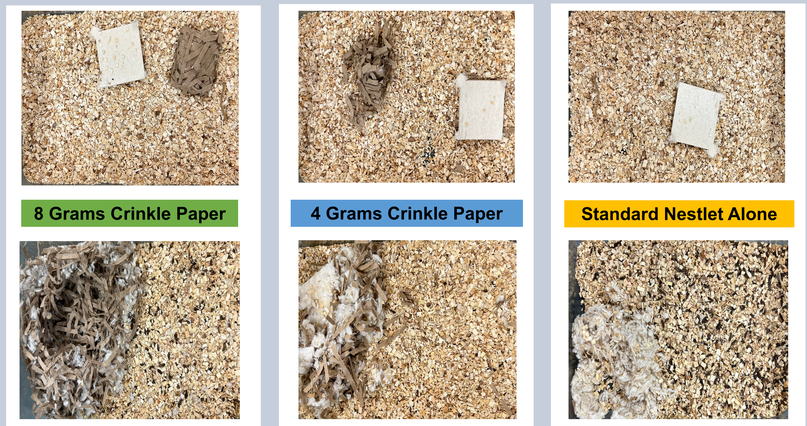

Figure 2. Enrichment Groups. The top row of images represent the enrichment groups provided to the mice during each trial setup. The bottom row of images represent the enrichment conditions evaluators may encounter during their assessments. Mice were given a minimum of 48 hours to interact with enrichment before a trial began.

Results

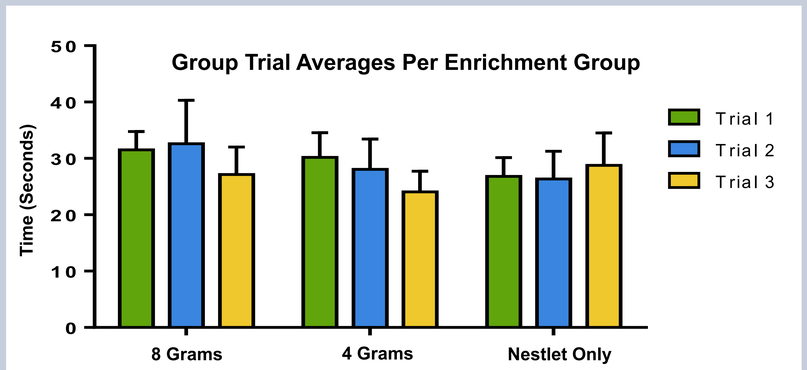

Figure 3. Group Trial Averages Per Enrichment Group. The time taken to assess each cage was recorded for each evaluator. Their individual average for each enrichment group per each trial was calculated. These individual averages were then averaged together to calculate a group average in seconds of the required time to perform a simulated check per enrichment group for each trial. No significant differences (significance $p<0.5$) were noted using a two-way ANOVA with nesting material as one factor and trial number as the second factor.

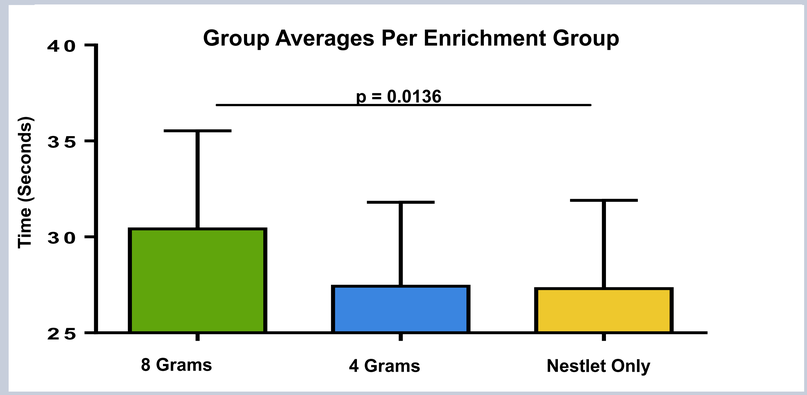

Figure 4. Group Averages Per Enrichment Group. The group trial averages per enrichment group were averaged together for each specific enrichment group to determine the average time in seconds taken to complete a simulation cage check. A one-way ANOVA with Dunnett's Post Hoc Test determined cages with 8 grams of enrichment took significantly longer time ($p = 0.0136$) to check compared to nestlet only control. No significant difference was noted for 4 grams of material compared to nestlet only control.

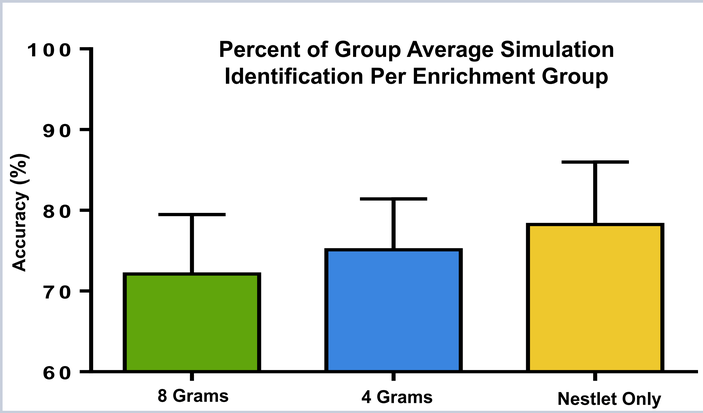

Figure 5. Percent of Group Average Simulation Identification Per Enrichment Group. The correct identification of the 8 simulations was recorded for each evaluator across all three trials. Individual percent accuracy averages were then calculated for each enrichment group. These averages were then averaged with the other evaluator averages to determine the percent accuracy per enrichment group. No significant differences (significance $p<0.5$) were noted using a one-way ANOVA.

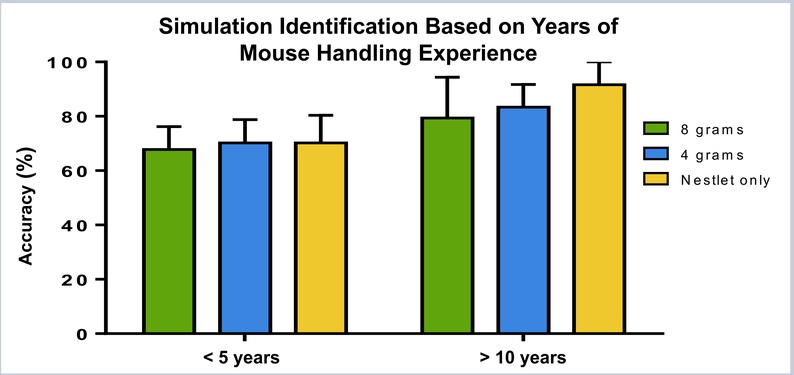

Figure 6. Simulation Identification Based on Years of Mouse Handling Experience. Upon evaluation, evaluators were asked to quantify their years in mouse handling experience. Evaluators were broken into groups of less than 5 and greater than 10 years experience. Individuals in each group had their percent accuracy averages for all trials averaged together to determine the percent accuracy per enrichment group for each experience group. No significant differences (significance $p<0.5$) were noted using a two-way ANOVA with experience level as one factor and nesting material as the second factor.

Conclusions

- ❑ There was an approximate 11% increase in time to perform cage checks with 8g of crinkle paper compared to other enrichment groups.
- ❑ There was no significant time difference for cage checks between 4g of crinkle paper and cages with nestlets alone.
- ❑ A trend for accurate simulation identification corresponded with less paper enrichment within cages and increased years of mouse handling experience.

Future Directions

- ❑ The use of 4g of crinkle paper with a standard nestlet for enrichment protocols.
- ❑ Future studies should evaluate the physiological impact on mice.
- ❑ Repeat studies should explore the potential for an evaluator learning curve and the identification of legitimate health concerns.

Acknowledgments

Thank you to the ASLAP Foundation and IDEXX BioAnalytics for student support, the Bryda Lab for funding, and cage evaluators Yuksel Agca, Jim Amos-Landgraf, Talia Elliott, Britt Lister, Zach McAdams, Leif McAllister, James F. McNew, Payton Oswalt, and Kirstin Smith.