Journal of the American Association for Laboratory Animal Science
Copyright 2020
by the American Association for Laboratory Animal Science

Vol 59, No 4
July 2020
Pages 356–364

Behavioral Effects of Cage Size and Environmental Enrichment in New Zealand White Rabbits

Kathleen A Coda,[1,*] Jeffrey D Fortman,[1] and Kelly D García[1]

One of the goals of environmental enrichment is to encourage species-typical behaviors, while discouraging abnormal behaviors or stereotypies. Assessing the effectiveness of various enrichment modalities can be challenging, particularly for prey species such as rabbits that exhibit freezing responses in the presence of people. In this study, we housed rabbits in 3 different sized cages and observed their behaviors. The 3 cage sizes were our standard rabbit housing cage, a medium sized cage, and a large run. Based on analysis of the recordings, ethograms were constructed and behaviors were quantified. The rabbits in large runs spent more time performing active, exploratory behaviors (431 ± 74 s) than rabbits in the standard cages (184 ± 55 s). However, space constraints inside research facilities often make it impractical to house rabbits in large runs. Therefore, we decided to explore if enrichment devices could promote the expression of active behaviors, similar to those displayed by rabbits housed in the large runs. We selected 3 devices: a hanging toy, a destructible device, and a dig bin. All 3 enrichment devices promoted more time spent performing active, exploratory behaviors (389 ± 48, 463 ± 50, and 420 ± 44 s, respectively), compared with control rabbits housed without an enrichment device (226 ± 53 s). We also analyzed the fecal glucocorticoids of rabbits after shipping or surgery to determine if enrichment devices could mitigate the physiologic impact of these stressors. We found no significant differences in fecal glucocorticoid levels between rabbits that experienced the stressor and rabbits that did not, or between rabbits with or without enrichment devices. Overall, the provision of larger caging and/or addition of enrichment devices encouraged a broad spectrum of active, species-typical rabbit behaviors, suggestive of improved animal welfare.

Abbreviations: NZW, New Zealand White; FRAP, frenetic random activity periods

DOI: 10.30802/AALAS-JAALAS-19-000136

A primary goal of laboratory animal medicine is to improve the welfare of the animals used in research. However, our ability to meet this goal is complicated by the challenge of distinguishing true improvements in animal welfare from perceived improvements. Social housing, environmental enrichment, and cage size have all been identified as ways to potentially improve animal welfare.[4,11,15,19,26] However, the use of any particular strategy may not be applicable to all species, as some animals may be threatened by housing with conspecifics, intimidated by overly complex environments, or feel vulnerable in large open spaces.[2,4,6] Thus, we sought to determine which specific components of enrichment programs truly improve the animal's welfare. Methods that are commonly employed to evaluate animal welfare include the display of species-typical behavior, the reduction of abnormal behaviors, and the assessment of levels of physiologic markers of stress such as glucocorticoids.[1,2,4,9,10,18] The overarching goal of this project was to test the hypothesis that increased cage size and/or the provision of various enrichment devices could improve the welfare of laboratory rabbits, as evidenced by an increase in species-typical behaviors, a reduction in abnormal behaviors, and decreased fecal glucocorticoid levels.

Received: 11 Sep 2019. Revision requested: 11 Oct 2019. Accepted: 2 Jan 2020.
[1]Biologic Resources Laboratory, University of Illinois at Chicago, Chicago, Illinois
[*]Corresponding author. Email: kcough@uic.edu

Materials and Methods

All work on rabbits was conducted at the University of Illinois at Chicago (UIC), a fully AAALAC-accredited institution, and was reviewed and approved by UIC's IACUC. Rabbits were acquired under alternate IACUC-approved protocols, used for the present noninvasive study, and subsequently transferred back to the original protocol. Purpose-bred male and female New Zealand White (NZW) rabbits (Charles River), aged 3 to 4 mo (1.4 to 2.3 kg) were brought into the facility and singly housed upon arrival. All rabbits except those on Study 3 were acclimated to the facility for at least 2 wk prior to participating in these studies. They were fed a commercial diet (Teklad 2031 High Fiber Rabbit Diet), provided ad lib city of Chicago water through an automatic watering system or water bottles, and exposed to a 14:10-hr light:dark cycle. Pans were changed twice weekly and cages were sanitized every other week. Standard enrichment consisted of a hard dumbbell device (Lab Supply, K3224) for chewing that was changed out and cleaned with cage change as well as food enrichment (such as fruits, vegetables, or hay) 3 times weekly.

Study 1. Ethogram Construction and Cage Size Evaluation. The objective of the first study was to construct an ethogram of the behaviors performed by laboratory rabbits and to evaluate any behavioral changes seen when rabbits were housed in various-sized cages. To accomplish this, 6 adult NZW rabbits (2.4 to 3.6 kg, 3 males and 3 females or 3M/3F) were removed from their home cage and placed in a clean test cage with no

enrichment material. The test cages consisted of a standard housing cage (stainless steel cage with interior dimensions of 63.5 × 75 × 40.5 cm (depth x width x height)), a medium-sized cage (stainless steel cage with interior dimensions of 71 × 114 × 68.5 cm), and a large cage (run with dimensions of 165 × 178 × 244 cm) (Figure 1). A rubber mat was added to the floors of the medium and large cages to facilitate grip. Water was available; however, rabbits did not drink during the observation periods when in new clean cages.

The rabbits were left alone and their activity was video recorded for 120 min (T0 to T120 min), after which they were returned to their home cage. The activity of each rabbit was recorded in each cage size on different days. Initially, 2 rabbits were randomly assigned to each test cage size. The rabbits were then cycled through the remaining cage sizes in the following days. All recording sessions took place in the early afternoon (approximately 1300 to 1500).

After a general evaluation of the video recordings, an ethogram of behaviors was constructed (Figure 2). Once the ethogram was established, videos were analyzed for behaviors using the constructed ethogram as a metric. Behaviors were quantified either as timed duration of a behavior or counted instances of a behavior. Three behaviors were quantified based on time spent performing the behaviors; these were exploring, grooming, and resting, as defined in Figure 2. The rabbits were considered to be performing one of these 3 behaviors at all times. Three other behaviors were quantified as number of individual occurrences of the behavior; these were rearing, digging, and frenetic random activity periods (FRAP, also known as "binkies"), as defined in Figure 2. Both the time spent grooming and individual instances of grooming were measured. To determine differences in activity and behavior, we analyzed 2 5-min periods of time in the recording window. We analyzed behaviors displayed at T10 to T15 min and at T115 to T120 min. These timepoints were selected based on observations of the initial 2-h recordings, which revealed that these 2 intervals provided a good representation of the full spectrum of laboratory rabbit behavior.

Study 2. Enrichment Evaluation. The goal of the second study was to evaluate behavioral outcomes when various enrichment devices were provided to rabbits in standard housing cages. To accomplish this, adult NZW rabbits (2.2 to 3.6 kg) were given various novel enrichment devices in their home cage and video recorded for 120 min. Four groups were tested (3 different enrichment devices and a control group with no device), and 8 rabbits (2M/6F) were recorded in each group. The first device was a wire ball with a bell (Pet's Warehouse, SKU 007136) that contained Timothy hay (Oxbow Animal Health) and was hung at the top of the cage door ("hanging toy", Figure 3 A). The second device was a plastic bin (ULINE, S-16278BLU) that was filled with clean corncob bedding (Teklad 7097) with Timothy hay scattered within the bedding ("dig bin", Figure 3 B). The third device was a hand-constructed destructible paper origami box (approximately 8 × 8 × 8 cm) that was filled with Timothy hay ("destructible device", Figure 3 C). The standard enrichment was removed from the cage and rabbits were presented with the novel devices immediately before the recording session. After the recording session, the novel devices were removed and standard enrichment replaced. All of the novel devices were either cleaned or replaced between recording sessions and refilled with fresh Timothy hay and/or bedding. All recording sessions took place in the early afternoon (approximately 1300 to 1500). The videos were analyzed as described previously, with an additional counted behavior of device interactions, which was defined as a purposeful touching of any part of the novel enrichment device. During the enrichment study, drinking or eating were considered in the "exploring" category/definition. Defecation was not counted or analyzed.

Figure 1. Test cages used for this study included (A). Standard rabbit housing cage with interior dimensions of 63.5 × 75 × 40.5 cm (depth × width × height), (B). Medium sized stainless-steel cage with interior dimensions of 71 × 114 × 68.5 cm, and (C). Large cage or run with dimensions of 165 × 178 × 244 cm.

Study 3. Enrichment and Fecal Glucocorticoid Evaluation after a Stressor. The primary goal of the third study was to determine if enrichment devices could reduce physiologic markers of

Behavior	Definition
Exploring	Animal is actively moving around in the enclosure, sniffing or chewing, standing on four legs, eating, drinking, digging, rearing, playing, FRAP, but not grooming
Grooming	Animal is licking or lightly chewing at its fur, rubbing or scratching itself with its paws, or otherwise performing self-directed cleaning behavior
Resting	Animal is sitting still, laying down, may be sleeping, but is not moving around or grooming
Digging	Animal is using forepaws to scratch at ground or object
Rearing	Animal is up on both hind legs, neither foreleg is touching the ground
FRAP (binkies)	Animal exhibits a "frenetic random activity period", hops or jumps into the air, runs around, may twist body or head around, quickly moves from a standstill

Figure 2. Ethogram of rabbit behaviors seen in our facility with included definitions of each specific behavior.

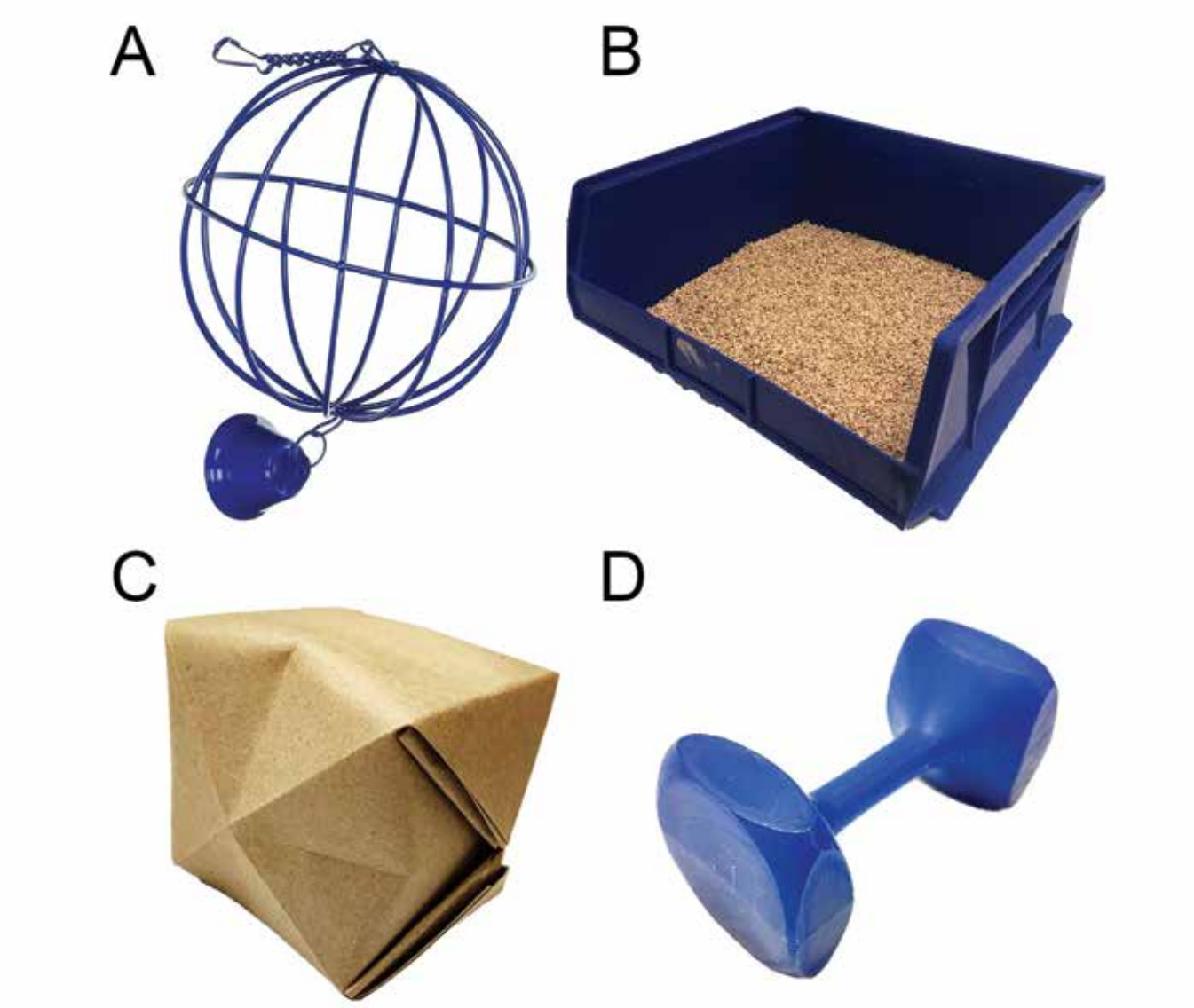

Figure 3. Enrichment devices used for this study included (A) Hanging toy, (B). Dig bin, (C). Destructible device, as well as (D). Standard enrichment dumbbell device.

stress. After experiencing shipping, an event known to be stressful,[23] rabbits were provided with enrichment devices. We tested the hanging toy and destructible device because results from Study 2 demonstrated that rabbits spent significantly less time grooming with these devices as compared with rabbits with no enrichment device. These devices were also simple to construct and less labor-intensive than the dig bin and thus more likely to be implemented on a wide scale. A secondary goal was to determine if device usage decreased over time as the devices became familiar to the animal.

Sixteen adult NZW rabbits (1.4 to 2.1 kg) were given different enrichment devices upon entering the facility after shipment

from the vendor (Day 1 = day of arrival). They were housed in standard caging and were exempt from receiving the standard enrichment dumbbell device; however, all rabbits did receive standard food enrichment of fruits, vegetables, or hay 3 times weekly. Three groups were tested (hanging toy $n = 6$, 1M/5F; destructible device $n = 5$, 1M/4F; and control with no enrichment device $n = 5$, 2M/3F). Rabbits had access to the novel enrichment devices at all times and the devices were refilled with fresh hay (hanging toy) or replaced (destructible) daily. Video recording and analysis took place as described previously on Day 2 and Day 6 to evaluate device usage as the devices become less novel to the animals. Fresh fecal samples were collected from the pans on Days 1, 3, 5, and 7. To collect the samples, a new pad was placed in the floor of the pan. Fecal samples were collected from the fresh pad 2 h later and stored at -80 °C until glucocorticoid analysis was performed.

We also elected to evaluate changes in fecal glucocorticoid levels associated with a controlled stressful event, as fecal samples could not be collected either before or during shipment. Eleven NZW rabbits (1.9 to 2.2 kg) were housed in standard housing and received the standard enrichment. Two groups were tested ($n = 5$, 5M/0F, rabbits undergoing a surgical procedure under another approved protocol and $n = 6$, 4M/2F, rabbits not undergoing a surgical procedure). Fecal samples were collected every day as described above, starting one week before surgery and continuing for one week after surgery. All fecal samples were sent to the St Louis Zoo Endocrinology Department for analysis as described below.

Fecal hormone extraction. Approximately 0.5 g of wet fecal material was weighed and then shaken overnight in 5 mL of a modified phosphate-saline buffer containing 50% methanol.[22] Liquid extracts were decanted and solids were removed through centrifugation at 4000 × g. Supernatants were then frozen at -80 °C until assay. Fecal material was placed in a drying oven overnight at 100 °C.

Glucocorticoid assay. Fecal glucocorticoid levels were determined using a commercially available corticosterone radioimmunoassay (DA Corticosterone kit, ICN MP Biomedicals). The lower detection limit of the assay was 0.26 ng/mL and upper detection limit was 20 ng/mL. The assay was performed according to manufacturer's protocols, with the exception that standard diluent was added to the fecal extracts and fecal extraction buffer (containing 50% methanol) was added to the standards. Concentrations were determined as ng/mL, and then divided by the dry weight of extracted feces to give the results as ng/g feces. All samples were assayed in duplicate. Mean intraassay variation of duplicate samples was 8.8%; mean interassay variation of 2 quality control pools was 6.7%.

Assay validation. Fecal extracts were tested for linearity by performing serial dilutions of 5 samples that contained high levels of fecal glucocorticoids by 1/2, 1/4, 1/8, and 1/16 with extraction buffer. Serial dilutions of fecal extracts measured an average of 91 ± 2% of expected values for corticosterone.

The accuracy of the assay was assessed by adding a known amount of corticosterone to 5 fecal extracts containing low values of fecal glucocorticoids. Addition of known amounts of hormone at 3 dosage levels resulted in recovery of 102 ± 3% of added corticosterone.

Statistics. For statistical analyses, the raw data were assessed for normal distribution, after which the data were compared between groups using one-way ANOVA with Tukey-Kramer or Dunnett posthoc tests applied as needed. All statistical analyses were conducted by using JMP 14.3 software (SAS Institute, Cary, NC). Summary data are expressed as mean ± SEM and differences with a P value of less than 0.05 were considered significant.

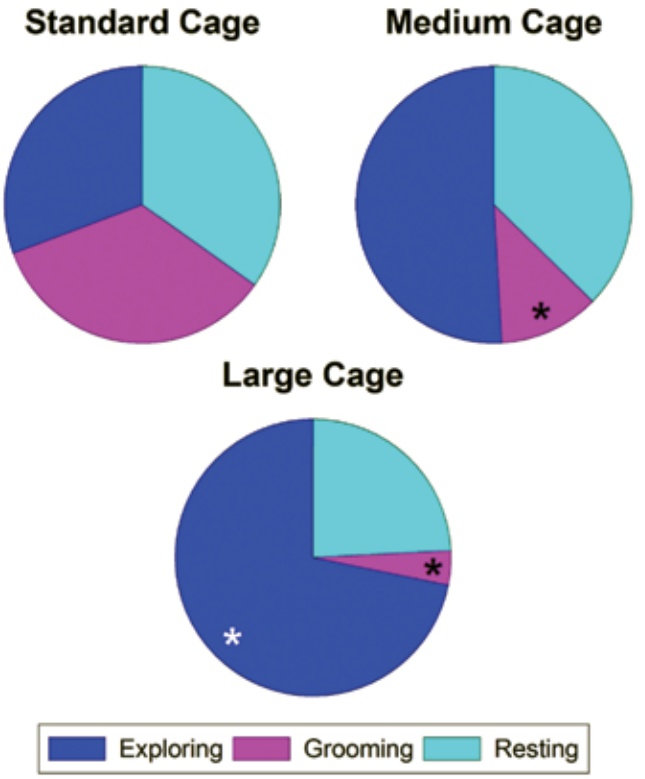

Figure 4. Average proportions of time rabbits spent performing exploring, grooming, or resting behaviors while in a standard sized cage, medium cage, or large cage. Asterisk (*) indicates a statistically significant difference in amount of time spent performing that behavior compared with the amount of time spent performing the same behavior in the standard size cage ($P < 0.05$).

Results

Study 1. Ethogram Construction and Cage Size Evaluation. The rabbits showed a number of species-typical behaviors (Figure 2) that are similar to those seen in wild rabbits.[5] Rabbits were considered to be performing one of 3 timed behaviors (exploring, grooming, or resting) at all times. The individual instances of rearing, digging, FRAP, and grooming behaviors were counted (defined in Figure 2).

Rabbits in the large cages spent significantly more time exploring (431 ± 74 s, $n = 6$) than did rabbits housed in the standard cages (184 ± 55 s, $n = 6$; $P < 0.05$) (Figure 4). The rabbits in both the medium (71 ± 18 s, $n = 6$) and large cages (24 ± 9 s) spent significantly less time grooming than did the rabbits in the standard cages (207 ± 37 s, $P < 0.05$) (Figure 4). The amount of time spent resting did not differ significantly between the 3 cage sizes. The differences between groups in number of instances of rearing (standard cage 8.7 ± 4.6; medium cage 7.5 ± 2.6; large cage 15.7 ± 6.2); FRAP (1.8 ± 1.2; 4.8 ± 3.6; 6.5 ± 3.1 respectively), digging (0 ± 0; 2.2 ± 1.9; 1.8 ± 1.3 respectively); and grooming (8.8 ± 2.1; 6.0 ± 1.1; 3.7 ± 1.2 respectively) were not statistically different (n=6 per group) (Figure 5). No significant differences were seen between sexes in any group (data not shown).

Study 2. Enrichment Evaluation. The rabbits given the hanging toy ($n = 8$, 389 ± 48 s), the destructible device ($n = 8$, 463 ± 50 s), and the dig bin ($n = 8$, 420 ± 44 s) all spent significantly more time exploring than control rabbits that did not have an

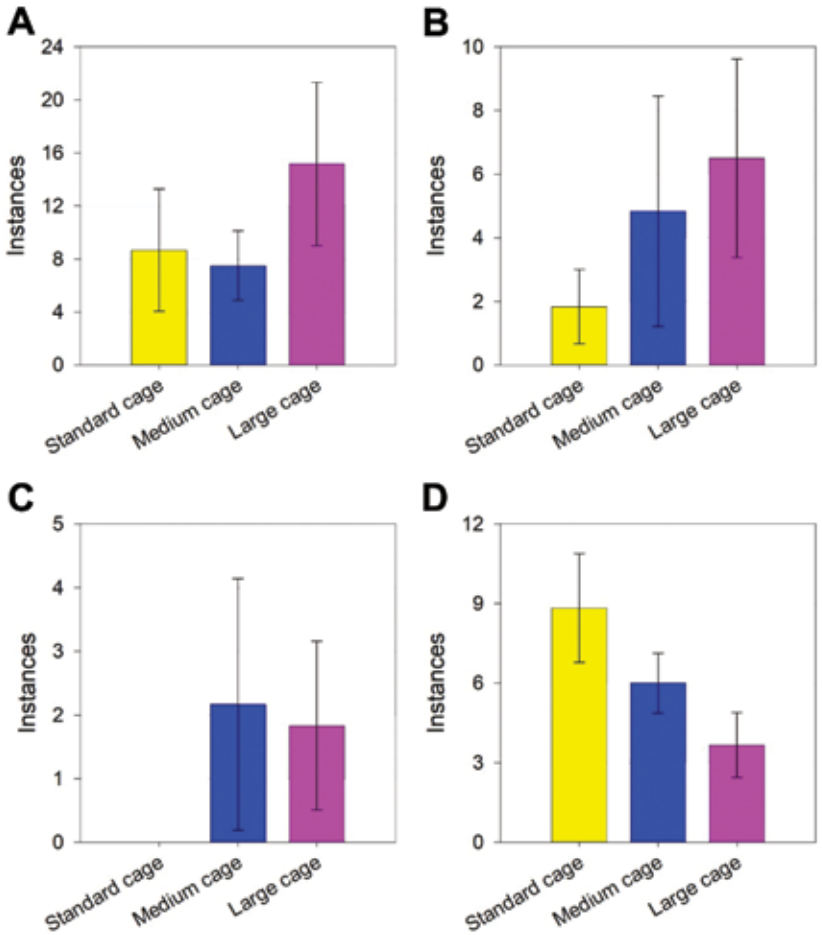

Figure 5. Average number of instances ± SEM of each of the specific behavior (A) rearing, (B) FRAP, (C) digging, and (D) grooming performed by the rabbits in the various sized cages.

enrichment device ($n = 8$, 226 ± 53 s, $P < 0.05$) (Figure 6). Rabbits given the hanging toy (20 ± 11 s) and destructible device (15 ± 13 s) also spent significantly less time grooming than did control rabbits (146 ± 51 s, $P < 0.05$) (Figure 6). Comparison of rabbits that received one of the 3 different enrichment devices (hanging toy, destructible device, and dig bin) showed no significant differences in the amount of time spent performing any of the 3 timed behaviors (exploring, grooming, and resting) (Figure 6). The time spent resting did not differ significantly between any of the groups, including the control group without an enrichment device (Figure 6).

The number of instances of grooming was significantly lower in each of the enrichment device groups (hanging toy 2.5 ± 1.1, destructible device 1.9 ± 1.6, dig bin 4.8 ± 2.2) compared with the control group (13.3 ± 2.8, $P < 0.05$) (Figure 7). The number of instances of grooming did not show any statistically significant differences between the rabbits that received the hanging toy, destructible device, or dig bin (Figure 7). The number of instances of rearing, digging, and FRAP did not differ significantly between any of the groups, including the control group (Figure 7).

The rabbits showed significantly more interactions with the destructible device (33 ± 4) than with either the hanging toy (20 ± 5) or the dig bin (22 ± 4, $P < 0.05$), with no significant difference in number of interactions between the hanging toy and the dig bin. No significant differences were seen between sexes in any group (data not shown).

Study 3. Enrichment and Fecal Glucocorticoid Evaluation with Stressor. The primary goal of this study was to analyze fecal glucocorticoids to determine if enrichment devices could mitigate the physiologic effects of a stressful event. However, fecal glucocorticoid levels did not differ significantly between any of the groups at any time point, or between animals that had recently arrived to the facility and animals conditioned to the facility (data not shown). This negative finding prompted us to evaluate fecal glucocorticoid changes surrounding an intense stressor - a surgical procedure performed under another protocol. No significant differences in fecal glucocorticoid levels were detected between rabbits that underwent surgery and rabbits that did not receive surgery at any time point or between time points within groups (data not shown).

The secondary goal of this study was to determine if device usage would change over time as the devices became more familiar to the animals. Rabbits undergoing the first part of this study (with the shipping stressor) were video recorded and analyzed on Day 2 and Day 6 after arrival. The number of device interactions did not differ significantly between the 2 time points within either of the groups (Figure 8). No significant differences were found between sexes in any group (data not shown).

Discussion

Providing laboratory animals with environmental enrichment is recognized as a strategy to improve their wellbeing and is encouraged by the *Guide*.[12] Environmental enrichment may entail altering cage size or complexity, adding sensory

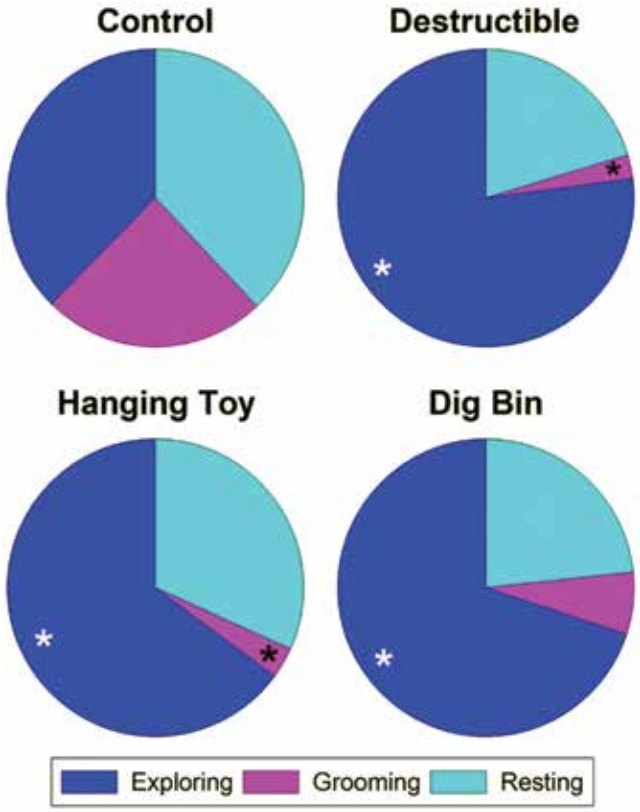

Figure 6. Average proportions of time rabbits spent performing exploring, grooming, or resting behaviors with no enrichment device (control), hanging toy, destructible device, or dig bin. Asterisk (*) indicates a statistically significant difference in amount of time spent performing that behavior compared with the amount of time spent performing the same behavior in the control group ($P < 0.05$).

stimuli or manipulanda, and/or maintaining social animals with conspecifics.[6,11,19,27] The provision of any environmental enrichment requires a commitment of labor, space, and/or resources,[2,9,10] which heightens the importance of providing enrichment that is appropriate for the species. However, determining the effectiveness of the enrichment can be challenging.

The goal of appropriate animal husbandry is enhancing animal welfare, which includes the opportunity to perform species-typical behaviors that are expressed by the species in the natural environment.[4,9,10,12,18] Stereotypic behaviors, on the other hand, are abnormal behaviors with no appreciable purpose, and can be a sign of reduced wellbeing or increased stress.[4,12,27] Some examples of stereotypic behavior include self-directed behaviors such as hair plucking, overgrooming, and self-injury.[4,9] If stereotypies cannot be measured, an alternative method to evaluate animal wellbeing is to measure glucocorticoid levels, which have been shown to increase with stress.[1,8,16,20,21]

We began our study by determining the types of behaviors that laboratory rabbits express in the laboratory setting and then compared these behaviors to published reports of behaviors of rabbits in the wild.[5,9] We accomplished this by creating and evaluating an ethogram. Next, we evaluated multiple enrichment strategies and measured the impact of these strategies on the rabbits' behavior. First, we evaluated differences in behaviors expressed by the rabbits in various sized cages. We found that rabbits in the larger cages spent more time performing active, exploratory behaviors, as do rabbits in the wild, supporting the premise that cage size affects behavior and potentially, animal welfare. However, housing rabbits in large runs is not always practical in the laboratory setting. Thus, we developed multiple enrichment devices that could be added to standard sized rabbit cages and evaluated whether these devices encouraged the expression of species-typical behaviors.

Wild rabbits spend a large amount of time performing active, exploring behaviors such as foraging for food, avoiding predators, socializing, finding mates, and raising young. They also spend time performing sedentary behaviors such as resting and grooming.[5] Rabbit behaviors may be quantified as frequency of occurrence or duration of action – for example, one grooming event might last for several minutes. Therefore, some behaviors were quantified in duration of time, whereas others were quantified in frequency of occurrence, with one behavior (grooming) quantified in both.

Our study found that laboratory rabbits in larger cages spent more time performing active exploratory behavior than did rabbits in the standard sized cages. We subsequently found that the provision of enrichment devices to rabbits in standard sized cages produced active, exploratory behavior similar to that seen in rabbits housed in the large cage. Greater activity has been shown to have multiple health benefits for cage-housed rabbits, decreasing the risks of osteoporosis and gastrointestinal stasis.[24] Thus, increasing active exploring behavior can be considered a method of improving welfare.

Wild rabbits rear onto their hind legs while foraging for food or scouting for predators.[3] Similarly, our rabbits exhibited rearing when housed in all cage sizes and when provided any enrichment devices. Although rabbits with no enrichment device and rabbits with the hanging toy had high numbers of rearing events, those with the device would stay in the reared/vertical position for long periods of time as they interacted with the device, while the instances of rearing from the rabbits with no device was brief (subjective observation). An improvement to this study could be to evaluate the amount of time spent in the rearing position to determine if the effects of the hanging toy were significant as compared with the control rabbits. Rabbits housed with other devices had fewer rearing events, likely because they were interacting with floor-level devices. Wild rabbits will occasionally perform a distinct rearing behavior called "telescoping," which is rearing up on their hind legs in an extreme vertical position to scout for predators or other threats.[3] Telescoping was only seen in the rabbits housed in the largest cage and thus was not specifically defined in our ethogram of rabbit behaviors (data not shown). Overall, the highest number of instances of rearing occurred in rabbits housed in the large cage, as its dimensions gave the rabbits the vertical space necessary to rear to their full height.

Frenetic random activity periods (FRAP) or "binkies" is a phenomenon that has been documented in many mammals, including dogs and rabbits.[3] FRAP is thought to be a behavioral expression of excitement and has been documented in both wild and domesticated rabbits.[3] Overall, our laboratory rabbits exhibited FRAP behaviors in all conditions, and the total number of instances was not significantly greater than what was seen in the standard housing cages. FRAP did not increase with the enrichment devices, perhaps because the animals were instead interacting with the devices. An improvement to this study could be to evaluate the time spent performing FRAP behavior, as the rabbits housed in a larger area performed FRAP longer than did rabbits with less space available.

Some behaviors are considered species-typical when expressed at a frequency or duration similar to animals in their natural environment but are deemed stereotypic when overexpressed by animals in confinement. While wild rabbits will

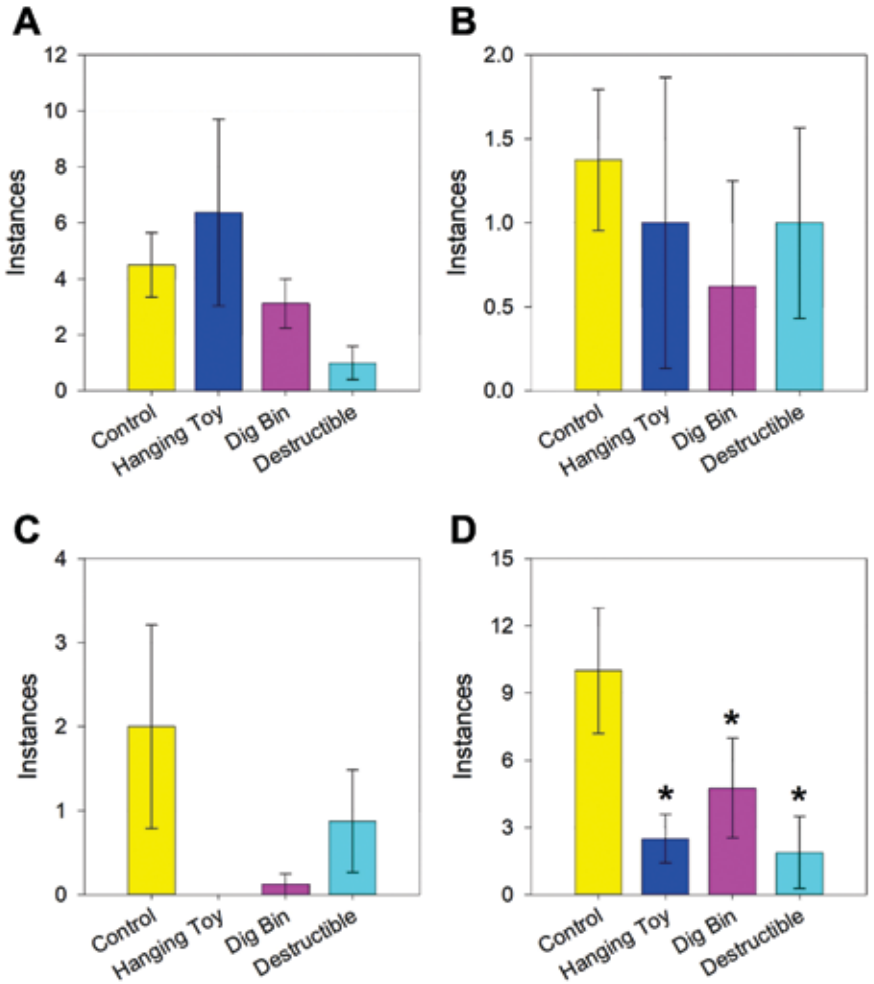

Figure 7. Average number of instances ± SEM of each specific behavior (A) rearing, (B) FRAP, (C) digging, and (D) grooming performed by the rabbits with the various enrichment devices. Asterisk (*) indicates a statistically significant difference in the number of instances of behavior compared with the number of instances of the same behavior in the control group ($P < 0.05$).

dig burrows, as well as dig to find roots and other vegetation for food, digging has also been referred to as a stereotypic or abnormal behavior in laboratory or farmed rabbits.[14,25] This is especially true when there is no substrate available for them to express this behavior, or when they dig to the extent that they acquire self-induced injuries to the forepaws. Our rabbits did not spend a large amount of time digging at the rubber floor mat or injure themselves performing this behavior, and we considered digging to be species-typical rather than abnormal behavior. For the most part, our rabbits did not exhibit many instances of digging behavior (maximum averages around 2 events), even when provided with enrichment designed to encourage that behavior.

Grooming is another species-typical behavior that can become stereotypic if done excessively.[25] However, without obvious signs of hair loss or skin lesions, determining the difference between normal and abnormal grooming is difficult. Overall, both larger cage sizes and provision of enrichment devices decreased the frequency and duration of self-directed grooming behavior, with no changes in overall appearance or health of the rabbits. Less grooming could reflect improved animal welfare, as the rabbits spent more time performing active exploratory behaviors yet still spent an appropriate amount of time grooming.

One of the concerns with the provision of enrichment devices is that the animals will lose interest in the devices over time. To explore this possibility, rabbits in the first part of the third study were video recorded and their behavior analyzed on Day 2 and Day 6 after arrival. Despite having the same type of device constantly available to them (refilled with fresh hay or replaced daily), our rabbits did not show a decrease in number of device interactions between the 2 time points. This indicates that the rabbits remained interested in the devices and did not become bored with their presence over the time period analyzed. An improvement to this study would be to determine if number of device interactions changed over a longer period of time.

Along with the improvement of animal welfare comes the assumption of a reduction of stress. Stress causes increases in the production of glucocorticoids, such as cortisol and corticosterone in most mammals.[17] Glucocorticoids are partially excreted in bile through the gastrointestinal system and therefore can often be measured using fecal samples.[1,2,18] The time period for fecal glucocorticoid deposition depends on intestinal transit times, but typically falls within hours to days.[7,13] In rabbits, glucocorticoid deposition typically occurs around 12 h after an acute stressor.[16]

We elected to measure fecal rather than blood glucocorticoids, as fecal samples were easily acquired from the pan of the cages without the need to handle the rabbits. We originally used shipping to the facility as the major stressful event for our rabbits. However, we did not see any differences in

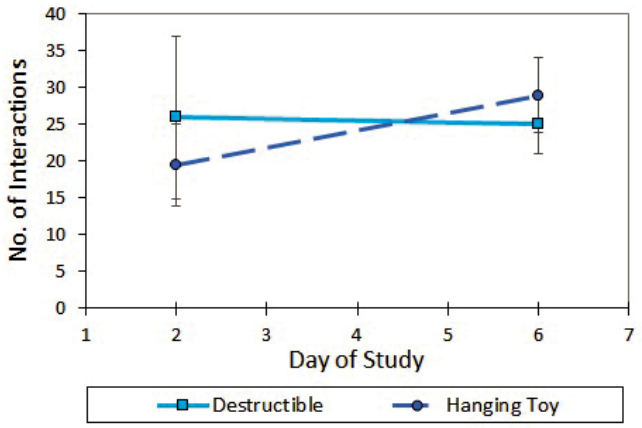

Figure 8. Average number of device interactions ± SEM on Day 2 and Day 6 post-arrival in rabbits housed with the hanging toy or the destructible device. There were no statistically significant differences between number of interactions on Day 2 compared with Day 6 in either group.

fecal glucocorticoid levels between rabbits that were given enrichment devices and those that were not. Furthermore, we did not see any increase in fecal glucocorticoids within the expected timeline after shipment, nor a subsequent decrease in levels as animals acclimated to the facility. To explore this unexpected fecal glucocorticoid result, we analyzed fecal samples of rabbits before and after the surgical implantation of a telemetry device, as well as in rabbits that did not have a surgical procedure. As with the previous study, we did not identify a reliable increase in fecal glucocorticoids in rabbits after surgery, nor any differences between control rabbits and those that had experienced a surgical procedure (data not shown).

The lack of meaningful data from the fecal glucocorticoid tests could be due to a variety of reasons. Stressed rabbits often exhibit decreased appetite,[3] which results in reduced fecal output, making collection of fecal samples challenging. Complex rabbit gastrointestinal physiology may also confound the reliability of fecal glucocorticoids as an indication of stress in this species. Rabbit intestinal transit times can vary widely, from 4 to 48 h,[7,13] making it hard to directly correlate the time of the stressor to the excretion of glucocorticoids in feces. In addition, rabbits are coprophagic,[3] and the ingestion of their own feces may skew or alter the timeline of the glucocorticoid excretion in the samples collected. Overall, using fecal glucocorticoid values as a measure of acute stress in laboratory rabbits proved to be challenging, and we would not recommend this as a method of evaluation of welfare in this species.

In conclusion, we found that increasing cage sizes leads to an appreciable, positive change in laboratory rabbit behavior. Rabbits spend more time performing active, species-typical behaviors and less time performing self-directed grooming behaviors when they are provided a larger than standard housing space. Likewise, we determined that the provision of enrichment devices in standard sized cages resulted in the same positive behavioral changes that occurred in rabbits housed in larger cages. Therefore, if increasing cage sizes is not feasible, a practical method to improve laboratory rabbit welfare is through the provision of enrichment devices in their home cages. After completing this study, we included the hanging toy as part of the standard enrichment at our facility and provided the destructible device on a rotating basis. The food enrichment is now often provided inside either the hanging toy or destructible device.

Acknowledgments

This study was funded in part by the Animal Welfare Institute's Refinement Grant to Improve the Welfare of Animals in Research. The authors thank Stephanie Tomeczko for her expertise and assistance with video recording and enrichment construction for the rabbits. We would also like to thank Dr Corinne Kozlowski and the St Louis Zoo Endocrinology Department for their expertise and assistance with analyzing fecal glucocorticoid metabolites.

References

1. **Baias A, Bodnariu A, Nichita I, Cristina RT.** 2012. Stress in laboratory juvenile rabbits: physiological indicators. J Anim Sci Biotechnol **45:**142–145.
2. **Baumans V.** 2005. Environmental enrichment for laboratory rodents and rabbits: Requirements of rodents, rabbits, and research. ILAR J **46:**162–170. https://doi.org/10.1093/ilar.46.2.162.
3. **Bays TB.** 2012. Behavior of small mammals, p 545–556. In: Quesenberry KE, Carpenter JW, editors. Ferrets, rabbits, and rodents: clinical medicine and surgery, 3rd ed. St Louis (MO): Elsevier.
4. **Chamove AS.** [Internet]. 2019. Environmental enrichment: A review. [Cited 30 July 2019]. Available at: https://awionline.org/content/environmental-enrichment-review
5. **Díez C, Pérez JA, Prieto R, Alonso ME, Olmedo JA.** 2005. Activity patterns of wild rabbit (*Oryctolagus cuniculus*), under semi-freedom conditions, during autumn and winter. Wildl Biol Pract **1:**41–46.
6. **DiVincenti Jr L, Rehrig AN.** 2016. The social nature of European rabbits (*Oryctolagus cuniculus*). J Am Assoc Lab Anim Sci **55:**729–736.
7. **Gidenne T.** 1992. Effect of fibre level, particle size and adaption period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. Br J Nutr **67:**133–146. https://doi.org/10.1079/BJN19920015.
8. **Goldschlager GB, Gillespie VL, Palme R, Baxter MG.** 2013. Effects of multimodal analgesia with low-dose buprenorphine and meloxicam on fecal glucocorticoid metabolites after surgery in New Zealand White rabbits (*Oryctolagus cuniculus*). J Am Assoc Lab Anim Sci **52:**571–576.
9. **Gunn D, Morton DB.** 1995. Inventory of the behaviour of New Zealand White rabbits in laboratory cages. Appl Anim Behav Sci **45:**277–292. https://doi.org/10.1016/0168-1591(95)00627-5.
10. **Hansen LT, Berthelsen H.** 2000. The effect of environmental enrichment on the behaviour of caged rabbits (*Oryctolagus cuniculus*). Appl Anim Behav Sci **68:**163–178. https://doi.org/10.1016/S0168-1591(00)00093-9.
11. **Harris LD, Custer LB, Soranaka ET, Burge JR, Ruble GR.** 2001. Evaluation of objects and food for environmental enrichment of NZW rabbits. Contemp Top Lab Anim Sci **40:**27–30.
12. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
13. **Jilge B.** 1982. Rate of movement of marker particles in the digestive tract of the rabbit. Lab Anim **16:**7–11. https://doi.org/10.1258/002367782780908841.
14. **Krohn TC, Ritskes-Hoitinga J, Svendsen P.** 1999. The effects of feeding and housing on the behaviour of the laboratory rabbit. Lab Anim **33:**101–107. https://doi.org/10.1258/002367799780578327.
15. **Lidfors L.** 1997. Behavioural effects of environmental enrichment for individually caged rabbits. Appl Anim Behav Sci **52:**157–169. https://doi.org/10.1016/S0168-1591(96)01141-0.
16. **Monclús R, Rödel HG, Palme R, Von Holst D, de Miguel J.** 2005. Non-invasive measurement of the physiological stress response of wild rabbits to the odour of a predator. Chemoecology **16:**25–29. https://doi.org/10.1007/s00049-005-0324-6.
17. **Palme R.** 2005. Measuring fecal steroids: Guidelines for practical application. Ann N Y Acad Sci **1046:**75–80. https://doi.org/10.1196/annals.1343.007.

18. **Poggiagliolmi S, Crowell-Davis SL, Alworth LC, Harvey SB.** 2011. Environmental enrichment of New Zealand White rabbits living in laboratory cages. J Vet Behav **6:**343–350. https://doi.org/10.1016/j.jveb.2010.12.001.
19. **Princz Z, Radnai I, Biró-Németh E, Matics Z, Gerencsér Z, Nagy I, Szendrő Z.** 2008. Effect of cage height on the welfare of growing rabbits. Appl Anim Behav Sci **114:**284–295. https://doi.org/10.1016/j.applanim.2008.01.006.
20. **Scarlata CD, Elias BA, Godwin JR, Powell RA, Shepherdson D, Shipley LA, Brown JL.** 2011. Characterizing gonadal and adrenal activity by fecal steroid analyses in pygmy rabbits (*Brachylagus idahoensis*). Gen Comp Endocrinol **171:**373–380. https://doi.org/10.1016/j.ygcen.2011.03.002.
21. **Sheriff MJ, Bosson CO, Krebs CJ, Boonstra R.** 2008. A non-invasive technique for analyzing fecal cortisol metabolites in snowshoe hares (*Lepus americanus*). J Comp Physiol B **179:**305–313. https://doi.org/10.1007/s00360-008-0314-4.
22. **Shideler SE, Ortuño AM, Morán FM, Moorman EA, Lasley BL.** 1993. Simple extraction and enzyme immunoassays for estrogen and progesterone metabolites in the feces of *Macaca fascicularis* during non-conceptive and conceptive ovarian cycles. Biol Reprod **48:**1290–1298. https://doi.org/10.1095/biolreprod48.6.1290.
23. **Swallow J, Anderson D, Buckwell AC, Harris T, Hawkins P, Kirkwood J, Lomas M, Meacham S, Peters A, Prescott M, Owen S, Quest R, Sutcliffe R, Thompson K.** 2005. Guidance on the transport of laboratory animals. Lab Anim **39:**1–39. https://doi.org/10.1258/0023677052886493.
24. **Thurston S, Burlingame L, Lester PA, Lofgren J.** 2018. Methods of pairing and pair maintenance of New Zealand White rabbits (*Oryctolagus cuniculus*) via behavioral ethogram, monitoring, and interventions. J Vis Exp **133:**e57267. https://doi.org/10.3791/57267.
25. **Verga M, Luzi F, Carenzi C.** 2007. Effects of husbandry and management systems on physiology and behaviour of farmed and laboratory rabbits. Horm Behav **52:**122–129. https://doi.org/10.1016/j.yhbeh.2007.03.024.
26. **Whary M, Peper R, Borkowski G, Lawrence W, Ferguson F.** 1993. The effects of group housing on the research use of the laboratory rabbit. Lab Anim **27:**330–341. https://doi.org/10.1258/002367793780745615.
27. **Young RJ.** 2003. UFAW animal welfare series: Environmental enrichment for captive animals. Ames (IA): Blackwell Publishing.