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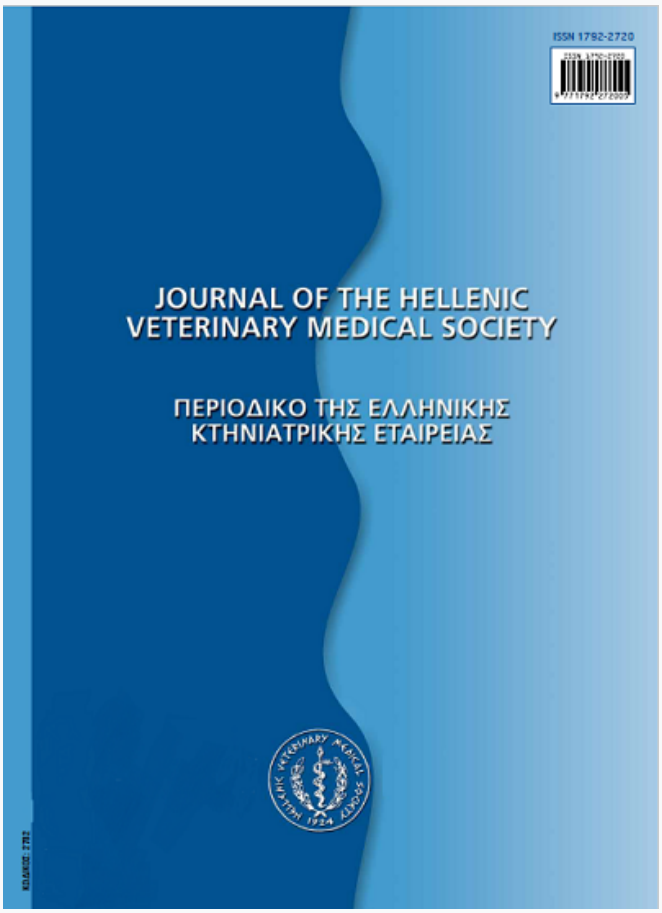

Evaluation of an environmental enrichment device used for laboratory rabbits

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Evaluation of an environmental enrichment device used for laboratory rabbits

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Αξιολόγηση μιας συσκευής εμπλουτισμού του περιβάλλοντος στέγασης των εργαστηριακών κονίκλων

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ABSTRACT. Improving the housing conditions of individually caged laboratory rabbits using environmental enrichment devices is a source of concern in the scientific community and is encouraged in the guidelines of the local and international laboratory animal associations and national regulations. In this report, we inform on the results of an investigation whose aim was to evaluate the use of a commercially available shelter as an enrichment device for six individually caged rabbits (mean body weight 2.5 Kg) during their resting period. After an acclimatization period of five days in an animal room, a commercially available enrichment device, the *Rabbit Hut*™ (BioServ, New Jersey, USA), was introduced into every cage. After a 3-day familiarization period, the interactions of the rabbits with the device were video recorded for 12 hours during the light phase (06:00 – 18:00) on five consecutive days. Each rabbit's interaction with the device was monitored and analyzed from the video recordings. We found that the rabbits used the enrichment device in different ways, which included passing through the device, staying under the device, gnawing the device, pulling and dragging the device using their teeth, and playing with the device. We also found that the rabbits mainly interacted with the device during the first two hours (06:00 – 08:00) and last four hours (14:00 – 18:00) of each 12-hour observation period of the 5-day investigation. Although the *Rabbit Hut*™ is marketed as an environmental enrichment item that could be used as a shelter for individually caged laboratory rabbits, our analysis of the interactions of individually caged

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laboratory rabbits with the device has generated new questions about the usefulness of the device and its benefit for the rabbits. However, further investigation is needed to clarify whether some of the expressed interactions indicate curiosity, interest or frustration with the item.

Keywords: caging, environmental enrichment, laboratory rabbit, shelter

ΠΕΡΙΛΗΨΗ. Η βελτίωση των συνθηκών στέγασης των ατομικά στεγαζόμενων κονίκλων που χρησιμοποιούνται στη βιοϊατρική έρευνα με τη χρησιμοποίηση αντικειμένων που εμπλουτίζουν το περιβάλλον στέγασης αποτελεί αντικείμενο ιδιαίτερου ενδιαφέροντος για την επιστημονική κοινότητα και υποστηρίζεται από σχετικές οδηγίες αντίστοιχων επιστημονικών οργανισμών αλλά και από τη νομοθεσία. Σκοπός της συγκεκριμένης εργασίας ήταν να αξιολογήσει τον τρόπο με τον οποίο ατομικά στεγαζόμενοι κόνικλοι αλληλεπιδρούν με ένα εμπορικά διαθέσιμο αντικείμενο που μπορεί να χρησιμοποιηθεί σαν φωλιά στη διάρκεια της περιόδου ανάπαυσης του ζώου. Για τη συγκεκριμένη εργασία χρησιμοποιήθηκαν εξι (6) αρσενικοί κόνικλοι, φυλής Νέας Ζηλανδίας και μέσου σωματικού βάρους 2,5 κιλών. Μετά από περίοδο εγκλιματισμού πέντε ημερών στο δωμάτιο στέγασης, μια φωλιά με την εμπορική ονομασία *Rabbit Hut*™, τοποθετήθηκε σε κάθε κλουβί. Μετά από περίοδο εξοικείωσης τριών ημερών με το αντικείμενο ξεκίνησε η καταγραφή των ζώων μέσω κάμερας, συνδεδεμένης με ηλεκτρονικό υπολογιστή, κατά τη διάρκεια της φωτεινής περιόδου (06:00 – 18:00) για συνολική περίοδο πέντε ημερών. Από την παρατήρηση προκύπτει ότι τα ζώα χρησιμοποιούσαν το αντικείμενο για διαφόρους λόγους όπως να περάσουν ή να καθίσουν από κάτω, να το δαγκώσουν, να το τραβήξουν καθώς και για άλλους χειρισμούς. Αυξημένη αλληλεπίδραση του ζώου με το αντικείμενο καταγράφηκε στην αρχή και στο τέλος της φωτεινής περιόδου. Συμπερασματικά, αν και η υπό έλεγχο συσκευή φαίνεται να είναι κατάλληλη προκειμένου να χρησιμοποιηθεί για τον εμπλουτισμό του περιβάλλοντος των κονίκλων που χρησιμοποιούνται για ερευνητικούς σκοπούς, εντούτοις περαιτέρω διερεύνηση απαιτείται προκειμένου να διευκρινιστεί αν ορισμένοι χειρισμοί που καταγράφησαν αποτελούν εκδήλωση περιέργειας, ενδιαφέροντος ή εκνευρισμού.

Λέξεις ευρετηρίασης: εμπλουτισμός περιβάλλοντος, κλωβός, εργαστηριακός κόνικλος, φωλιά

INTRODUCTION

The behavior of a single-housed rabbit in a laboratory cage is influenced by specific environmental parameters, such as cage size and design (Lockley, 1961; Batchelor, 1991; Gibb, 1993), the quantity and availability of food, the rabbit's age (Katsarou et al., 2011; Krohn et al., 1999), and the availability of objects and social contacts (Lidfors, 1997; Harris, 2001; Hansen and Berthelsen, 2000). In contrast to wild rabbits whose environment is free of any limitations, laboratory rabbits, which are usually housed in a barren cage system, may display stereotypic behaviors, such as gnawing of the metal bars of their cage and over-grooming due to stress (Morton et al., 1993; Love, 1994).

Environmental enrichment is defined as any modification in the environment of captive animals that improves their physical and psychological well-being by providing stimuli that meet their species-specific needs (Baumans, 2005). Several types of environmental enrichment for laboratory rabbits exist (Morton et al, 1993). A commonly used type of enrichment for rabbits is different food types, such as cereals or carrots, which may be provided in varying quantities (Krohn et al., Lidfords, 1997; Harris et al, 2001). Objects, such as wooden sticks, hay, or toys, can also be used to enrich a rabbit cage (Morton et al, 1993; Huls et al., 1991), and when used, only one object should be used when studying its effects on the caged rabbit's behavioral patterns (Lidfords, 1997).

Although it is fundamental to good animal husbandry that the caging environment should meet the needs of a caged animal and standard laboratory cages need to be enriched, not all enrichments or changes to husbandry benefit caged animals. Accordingly, it is proposed that enrichment devices or protocols should be validated in order to show that

(a) they are beneficial to the animals, (b) they do not cause any unexpected adverse effects on the animals, and (c) the enrichment does not jeopardize the experimental outcomes (Baumans et al., 2006). Hence, the aim of the present investigation was to evaluate the use of a commercially available shelter, as an enrichment device for individually caged rabbits during their resting period (light phase). The study parameters that were evaluated in the caged laboratory rabbits were the type and the frequency of their behavioral interactions with the device.

MATERIALS AND METHODS

The study was performed in the animal facility of the Center for Experimental Surgery of the Biomedical Research Foundation of the Academy of Athens. The facility is registered as a "breeding" and "experimental" facility according to the Greek Presidential Decree160/91 (Governmental Gazette, A, 64), which harmonizes national legislation with the European Directive 86/609 on the protection of animals used for scientific purposes (European Union, 1986)

Animals

The investigation comprised six male New Zealand White rabbits (body weight 2.5 (mean) ± 0.173 Kg (standard deviation (SD)) which were purchased from a conventional rabbit farm (Farma Trompetas, Megara, Attiki, Greece) and reported to be free of *Pasteurella multocida*, *Salmonella* spp., *Treponema cuniculi, Encephalitozoon cuniculi*, and *Eimeria* spp.

Husbandry

The six rabbits were housed and maintained in accordance with the European Commission Recommendations for the accommodation and care of animals used for experimental and other scientific purposes (European Commission, 2007).

Each rabbit was housed individually in a single cage (67 cm (width) x 67 cm (length) x 47 cm (height)) in a stainless steel rabbit cage rack (Tecniplast, Buguggiate, Italy) in a room at a room temperature of 21 ± 2°C, a relative humidity of 50 ± 5%, and with a 12:12 hour light:dark cycle in which the light period was set between 06:00 and 18:00. Each cage comprised a perforated plastic floor (no bedding material was included) and walls with metal bars in the front. The rabbits were fed *ad libitum* with a prepackaged certified fiber rabbit chow (12C, Pezzulo, Italy), and had unlimited access to water *via* an automatic watering system.

Environmental enrichment device

The environmental enrichment item that was evaluated in this investigation was a commercially available device, called *Rabbit Hut*™ (BioServ, New Jersey, USA). The device (279.4 mm (width) x 254 mm (height) x 304.8 mm (length)) is used as a shelter, is fabricated from high-temperature resistant and red transparent polycarbonate, and weighs 745 grams so that the rabbits can move it while playing with it (Fig. 1).

Video recordings of the rabbit's behavior

Video recordings of each rabbit's behavior during the light period were made for five consecutive days using an MSI StarCam Flip Webcam which was connected to a computer (NB200-13T, Toshiba). Since rabbits are less active during the light period than during the dark period (Gunn and Morton, 1995), a computerized closed circuit television surveillance camera system with motion detection capabilities (Cam Wizard) was used to capture the video feed from the webcam.

The video recordings were reviewed to transform the rabbit's interactions with the device and behavior into quantitative data. For this purpose, the

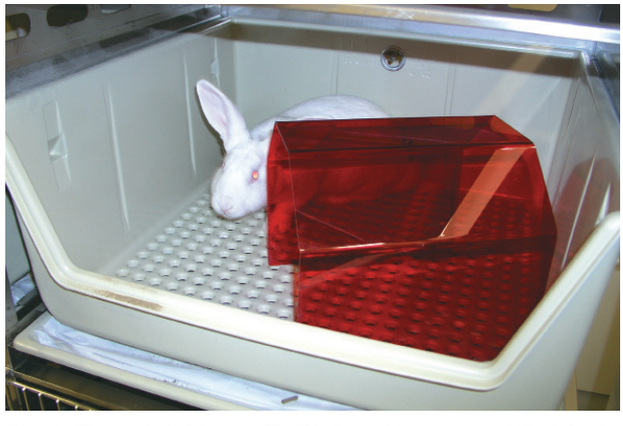

Fig. 1. The red *Rabbit Hut*™ (BioServ, New Jersey, USA) in the rabbit cage

number of times that the rabbit interacted with the device in a particular way was recorded during each 12-hour observation period for five consecutive days. Each type of interactions was then transformed and the transformed data were plotted in order to determine the frequency of occurrence of each interaction of each rabbit with the device.

Experimental design and data collection

Following a 5-day acclimation period of the rabbits in the animal facility, *Rabbit Hut*™ was introduced into each cage and the rabbits were given three days to become familiar with the device. No recordings of the rabbit's behavior and interactions with the device were made during the 3-day familiarization phase. At the end of the familiarization period, video recordings of each rabbit's interactions with the device were made for each 12-hour observation period for five consecutive days. The data were summarized using descriptive statistics (mean, SD, median, range, and 95% confidence intervals) using a computerized statistical software package (SPSS version 13.0, Chicago, Illinois, USA).

RESULTS

The main interactions between the rabbits and the device that were observed during the 5-day recording period were (a) passing through the device, (b) staying under the device, (c) gnawing the device, and (d) pulling and dragging the device using their teeth. The rabbits also played with device by pushing the shelter with their feet, sniffing the shelter, turning the shelter upside down, or sitting inside the shelter after turning it upside down, and these playful interactions were classified as "other interactions".

During the 5-day observation period, we registered 1,625 interactions with the shelter of which 401 (25%) were passing through the device, 274 (17%) were staying under the device, 273 (17%) were gnawing the device, 247 (15%) were pulling and dragging the device with their teeth, and 430 (26%) were playful or other interactions with it (Fig. 2).

Passing through the device

Passing through the device was a frequently observed behavioral interaction of the rabbits during the first two hours (06:00 – 08:00) and last four hours (14:00 – 18:00) of each 12-hour observation period of the 5-day investigation (Fig. 3).

Staying under the device

Staying under the device was observed during the first two hours (06:00 – 08:00) and last four hours (14:00-18:00) of each 12-hour observation period of the 5-day investigation (Fig. 4).

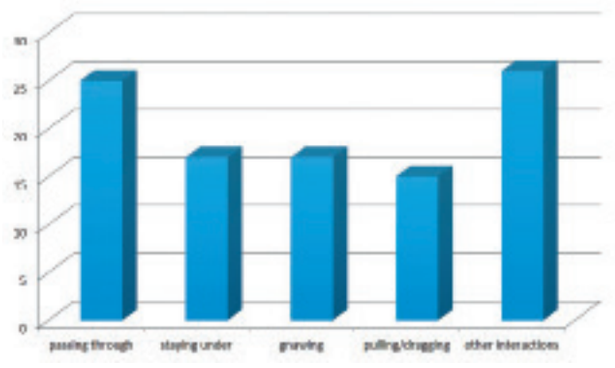

Fig. 2. The distribution of behavioral interactions (expressed as a percentage) between the rabbit and the environmental enrichment device, the *Rabbit Hut*™ (BioServ, New Jersey, USA) during the 5- day investigation. A complete explanation of each behavioral interaction is given in the Results section.

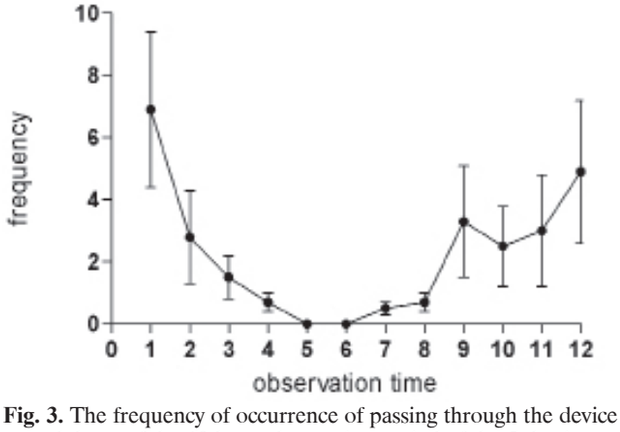

Fig. 3. The frequency of occurrence of passing through the device at each hour of the 12-hour observation period of the 5-day investigation. Each frequency is expressed as the mean number of interactions ± standard deviation. Each number on the x-axis represents the hours of observation of the 12-hour observation period on each day of the 5-day investigation, namely 1 = 06:00 to 07:00, 2 = 07:00 to 08:00, and 12 = 17:00 to 18:00.

Gnawing the device

Gnawing the device was observed during the first two hours (06:00 – 08:00) and last four hours (14:00-18:00) of each 12-hour observation period of the 5-day investigation (Fig. 5).

Pulling and dragging the device

Increased pulling and dragging of the device by the rabbits was observed every day during the first two hours of the observation (06:00 – 08:00) and the last three hours (15:00-18:00) of each 12-hour observation period during the 5-day investigation (Fig. 6).

Other interactions with the device

Other or playful interactions between the rabbits and the device were observed during the first two hours (06:00 – 08:00) and last four hours (14:00-18:00) of each 12-hour observation period during the 5-day investigation (Fig. 7).

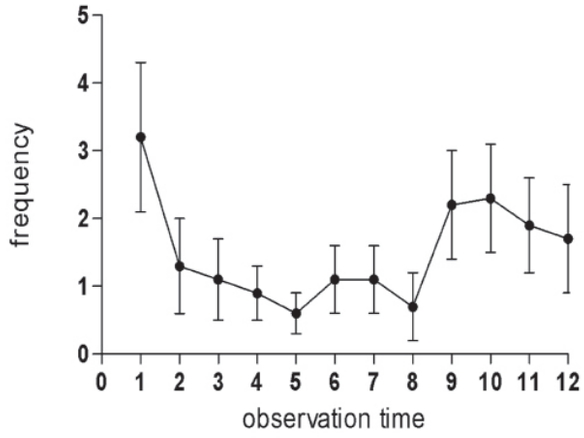

Fig. 4. The frequency of occurrence of staying under the device at each hour of the 12-hour observation period of the 5-day investigation. Each frequency is expressed as the mean number of interactions ± standard deviation. Each number on the x-axis represents the hours of observation of the 12-hour observation period on each day of the 5-day investigation, namely 1 = 06:00 to 07:00, 2 = 07:00 to 08:00, and 12 = 17:00 to 18:00.

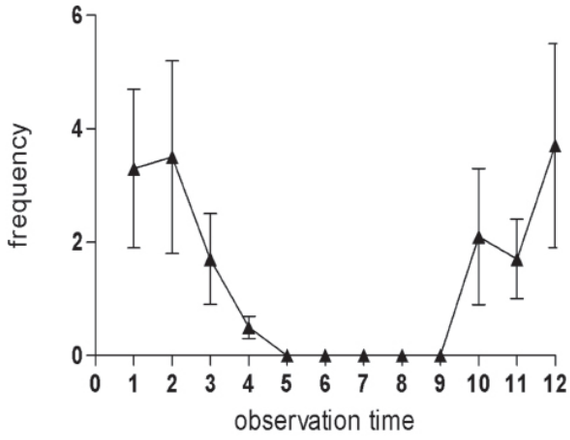

Fig. 6. The frequency of occurrence of pulling and dragging the device at each hour of the 12-hour observation period of the 5-day investigation. Each frequency is expressed as the mean number of interactions ± standard deviation. Each number on the x-axis represents the hours of observation of the 12-hour observation period on each day of the 5-day investigation, namely 1 = 06:00 to 07:00, 2 = 07:00 to 08:00, and 12 = 17:00 to 18:00.

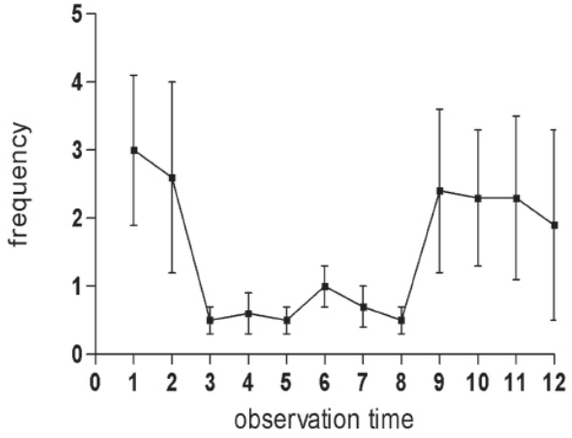

Fig. 5. The frequency of occurrence of gnawing the device at each hour of the 12-hour observation period of the 5-day investigation. Each frequency is expressed as the mean number of interactions ± standard deviation. Each number on the x-axis represents the hours of observation of the 12-hour observation period on each day of the 5-day investigation, namely 1 = 06:00 to 07:00, 2 = 07:00 to 08:00, and 12 = 17:00 to 18:00.

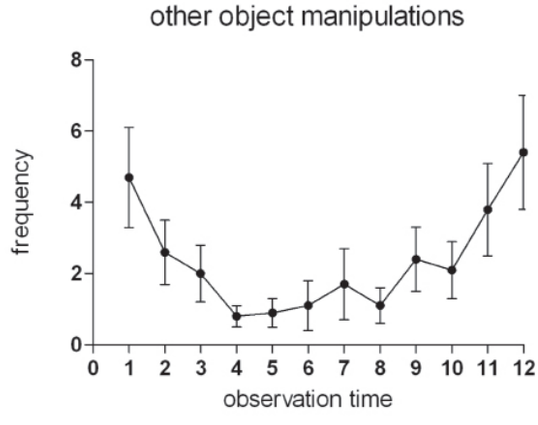

Fig. 7. The frequency of occurrence of other interactions with the device at each hour of the 12-hour observation period of the 5-day investigation. Each frequency is expressed as the mean number of interactions ± standard deviation. Each number on the x-axis represents the hours of observation of the 12-hour observation period on each day of the 5-day investigation, namely 1 = 06:00 to 07:00, 2 = 07:00 to 08:00, and 12 = 17:00 to 18:00.

DISCUSSION

Although rabbits are social animals and group housing is recommended, there are still circumstances where rabbits are housed individually with restricted or *ad libitum* amounts of food and water. Single housing of social animals can influence the expression of normal behavior, and in many cases, can be detrimental to their psychological well-being (Brooks et al., 1993). During the last decades, increasing trends on the use of environmental enrichment for improving the caging conditions of captive animals have been reported. Environmental enrichment is very important for an individually caged rabbit only for the validity of the scientific outcome. It is also important because it can protect them from boredom and safeguard their well-being and their right to live a life as similar as possible to that of their wild counterparts (Whary et al., 1993; Lidfords, 1997; Harris et al., 2001).

The validation of enrichment devices is very important and is recommended by the FELASA Working Group on Standardization of Enrichment (Baumans et al., 2006). Accordingly, the aim of our investigation was to monitor, evaluate, and if possible explain the interaction of individually caged rabbits with an enrichment device, *Rabbit Hut*™, which is marketed as a hiding place and a shelter. Hence, it was decided to monitor the rabbits during their resting period (light cycle), and the data which we present in this report are the preliminary results of an environmental enrichment project that we are conducting in our Institution.

According to our results, we found that most interactions with the shelter occurred early in the morning when the lights in the room in which the rabbits were housed were turned on and late in the afternoon before the lights were turned off. "Passing through" was a frequent interaction of the rabbits with the device, and we interpreted this interaction as an expression of the rabbit's exploratory need. We surmise that this device probably simulates objects in the wild, such as rocks, shafts, and nests, which are necessary for the survival of wild rabbits and helps them to be active and efficient.

Another frequently observed interaction was "staying under" the device in order to rest, hide, or sleep. It has been previously reported that rabbits do not use a nest box when it is used as an enrichment device or as a place for rest or sleep (Hansen and Berthelsen, 2000; Batchelor 1991) but only as a hiding place. Moreover, Whary et al. (1993) reported that rabbits use a shelter when placed in a cage as an enrichment device as a resting place for only 3% of the time during the day.

We found that "gnawing" the device was another common interaction of the rabbits with the device. We interpreted this interaction as an indicator of curiosity because the rabbits perceived the device as something new, stimulated their interest, and were willing to explore it. This interaction could be also interpreted as an indicator of stereotypy and frustration because gnawing at cage furniture and cage bars is thought to be common behavioral problem of captive animals (Morton et al., 1993; Love, 1994). Additionally, the "pulling and dragging" of the shelter by the rabbits using their teeth or the legs could also be considered to be an expression of the rabbit's exploratory need.

The other occurring interactions, which included sniffing, moving or turning the device over could also be considered as expressions of the rabbit's exploration activity. These interactions could also be considered to be an indicator of frustration with the item because its presence reduces the cage area and restricts the rabbit's ability to move.

CONCLUDING REMARKS

Although the *Rabbit Hut*™ is marketed as an environmental enrichment item that could be used as a shelter for individually caged laboratory rabbits, our analysis of the interactions of individually caged laboratory rabbits with the device has generated new questions about the usefulness of the device and its benefit for the rabbits. Thus, further investigations, which should also include the measurement of physiological parameters, are now needed to better explain and clarify whether some of the expressed interactions indicate usefulness, curiosity, interest, or frustration with the device.

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CONFLICT OF INTEREST STATEMENT

The authors of this article declare that they do not have any conflicting interests.

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