Cage position preferences of rats

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Introduction

Many animal husbandry procedures have evolved from a practical view, *i.e.* what is most feasible and inexpensive. Over time, these procedures have become standard accepted practice, and if there has been no obvious untoward signs in animal wellbeing, it has been assumed that there is no problem. Yet, in order to draw meaningful conclusions, crude morbidity and mortality data are not enough to assess animal wellbeing (*Richmond 2000*). Only recently some of these practices have been reassessed with systematic research.

Polycarbonate and stainless steel are commonly used cage materials for laboratory rodents. Both of these materials can be rated on a variety of factors such as hygiene, durability and unit logistics. Stainless steel is typically used for wire mesh and polycarbonate for solid bottom cages.

Only a few international reviews or recommendations take a stand on cage material. The Multilateral consultation lists some criteria for cage material: it should be easy to clean and allow proper inspection of the animals (*European Convention 1997*). The Berlin report states that polycarbonate or polypropylene should be used for the cage body construction, since stainless steel has inherent difficulties (*Brain et al. 1993*). The report does not list these difficulties, but generally stainless steel cages are perceived as inferior to polycarbonate or polypropylene cages because the material does not provide heat insulation, is heavy and noisy. The obvious problem here is that the users – rats and mice – have not been consulted.

There are studies comparing polycarbonate solid bottom cages to stainless steel wire mesh cages, but, to our knowledge, there is only one study comparing the materials as such, and it reported inconclusive result (*Kaliste-Korhonen et al. 1996*). The aim of this study was to assess the preference of rats between stainless steel and polycarbonate cage body in solid bottom cages.

Materials and methods

The study was carried out in two laboratory animal facilities: University of Kuopio and University of Oulu. The main environmental factors were similar in these facilities and the experimental procedures were carried out identically. However, there were differences between animal stocks, feed and cages.

Animal housing and care

Altogether, 64 conventionally housed male rats were used, 32 in each facility. The rats originated from two outbred stocks: in Kuopio, Wistar rats (WH, Hannover origin) and in Oulu, Sprague Dawley (Mol:SPRD). The study protocol was reviewed and approved by the Ethics Committees of the University of Kuopio and University of Oulu.

The temperature in the animal rooms was 21 ± 2 °C and the relative air humidity 55±10 %. The automatic light and dark cycle of the animal rooms was 12 hour light and 12 hours dark, lights on at 07.00 and off at 19.00 hours. Pelleted rat food (Kuopio: R36, Lactamin Ab, Stockholm, Sweden and Oulu: RM1, SDS, Essex, England) and tap water in bottles were available *ad libitum*.

Aspen bedding (Tapvei Oy, Kaavi, Finland) was used in both units. Because the types and sizes of the cages differed between the two facilities, the volume of bedding was calculated and equalized to 1.2 ml/cm^2 of cage floor area. Cages, bedding and water bottles were changed twice a week, and the recording period preceded every other change.

Cage and rack details

The experimental cages were made of either stainless steel with a false polycarbonate inner half (Kuopio) or polycarbonate with a false steel inner half (Oulu). Between these two materials there was a threshold in order to prevent bedding accumulation on either half. Figure 1 shows the cage design in Kuopio and Figure 2 that from Oulu.

In Kuopio the cage racks were kept in a cubicle room, in Oulu in an open animal room. Figure 3 shows the rack placement in a cubicle. The differences in rooms had some effect on light intensity inside the cages. During the dark period, light intensity was similar (Oulu: 3.8 - 5.9 lx, Kuopio: 3.8 – 12 lx) in all parts of the cage. During the light period in Kuopio the angle of incoming light illuminated the cage half facing the room center much less (11 lx) than the opposite half (72 lx). In the Oulu unit, the half facing the center of the room was, irrespective of steel half cage polarity, lighter (132-146 lx) than the opposite half (78-80 lx). This was mainly because the cage material itself was transparent (polycarbonate), the stainless steel half cage was five cm lower than the cage wall and because hopper end was at an angle.

During the recordings, the experimental cages were removed from an ordinary rack to a recording rack. In this rack, normal, empty cages were used above and adjacent to the experimental cages in order to achieve the desired normal lighting circumstances. Figure 4 shows the positions of the experimental cages during recording. After the recording the cages were moved back to their places in the ordinary rack and the next recording group was moved to the recording rack. Thus the animals were always allowed to adapt to the recording rack from 08.00 till 16.00 hours.

Allocation of animals to experimental groups

Before the experiment, from birth to weaning, the rats were kept either in stainless steel cages (Kuopio: 49 cm x 29 cm x 20 cm) or in polycarbonate cages (Oulu: 55 cm x 35 cm x 20 cm). At weaning (three weeks of age) the animals were allocated to four different experimental groups with two cages in each group. The animals were chosen from litters large enough to ensure one male to all the cages. Thus, the four experimental groups consisted of two cages with four rats in each cage and the groups had siblings from a total of eight female rats.

There were two different options for alignment of false cage halves and food hopper, and likewise two options in the way the end of the cage faced the wall. In total these added up to four different combinations both in Kuopio (Figure 5) and in Oulu (Figure 6).

Recording of the location in the cage

Rats were monitored with video cameras (Kuopio: Grundig® LC 295 SN, Grundig, Fürth, Germany, Oulu: Panasonic® WV-BL200, Japan). The recordings were started after a one-week habituation period to the new cage environment. Time lapse recording of one second in every minute was used. The recording started at 16.00 and ended at 01.30. During the dark period, low intensity red lights (2 x 25 W) were used to attain visibility. The rats in each cage were recorded at the age of four, five, six, seven and eight weeks, always during the same night of the week. Two cages from different groups were recorded at the same session.

Data processing and analysis

From video recordings, the animals' location in a cage was counted once a minute to yield the number of animals at both ends of the cage. Data from two facilities and for light and dark periods were processed separately and presented as pooled data from five consecutive weeks. Statistical analysis was done with ANOVA (SPSS for Windows, Release 8.0, SPSS Inc., Chicago, IL, USA) using alignment of food hopper to false inner cage half and direction to the wall as the main effects. Statistical significance was set at $p < 0.05$.

Results

All processed data are presented as number of rats (mean ± SD) counted in the stainless steel side of

Figure 1. Illustration of cages with stainless steel body and false inner polycarbonate half in Kuopio.

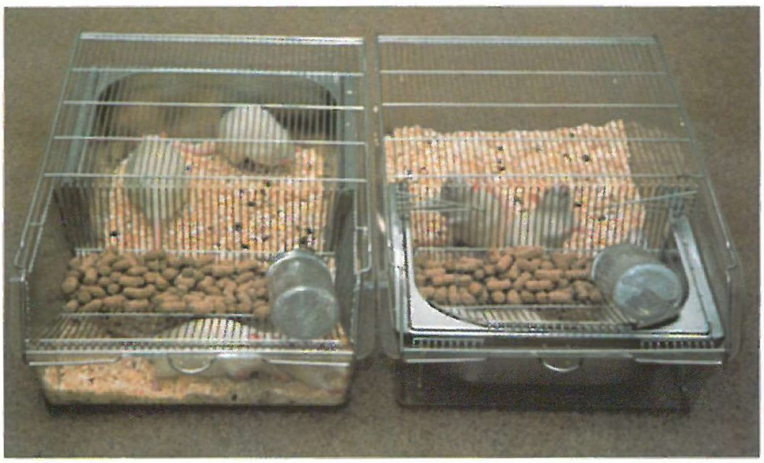

Figure 2. Illustration of cages with polycarbonate body but inner stainless steel half cage (Oulu) and two variations of alignment between food hopper and inner half.

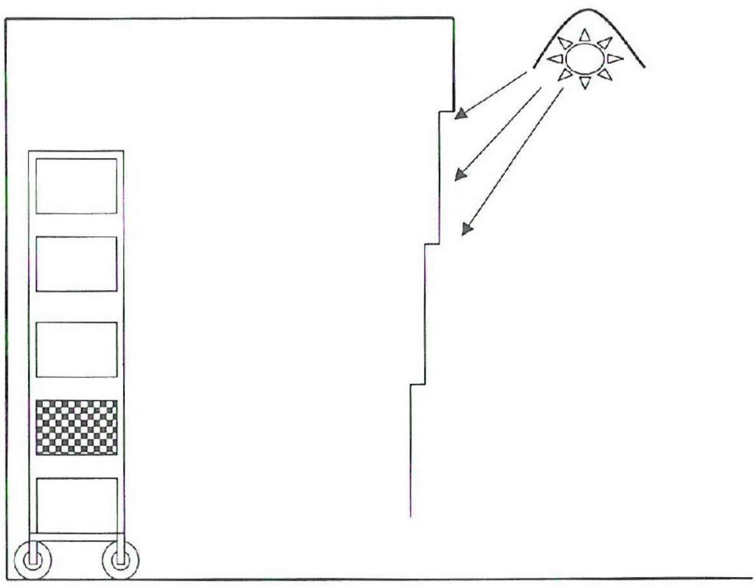

Figure 3. Side view of recording rack in a cubicle (Kuopio) and direction of light into the cubicle through transparent gliding front panels.

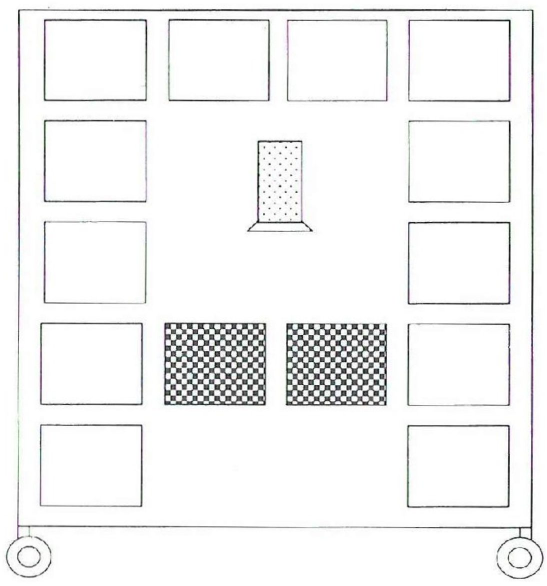

Figure 4. Front view of recording rack showing the cages being recorded (checkered) and the video camera above the cage.

FIGURE 5

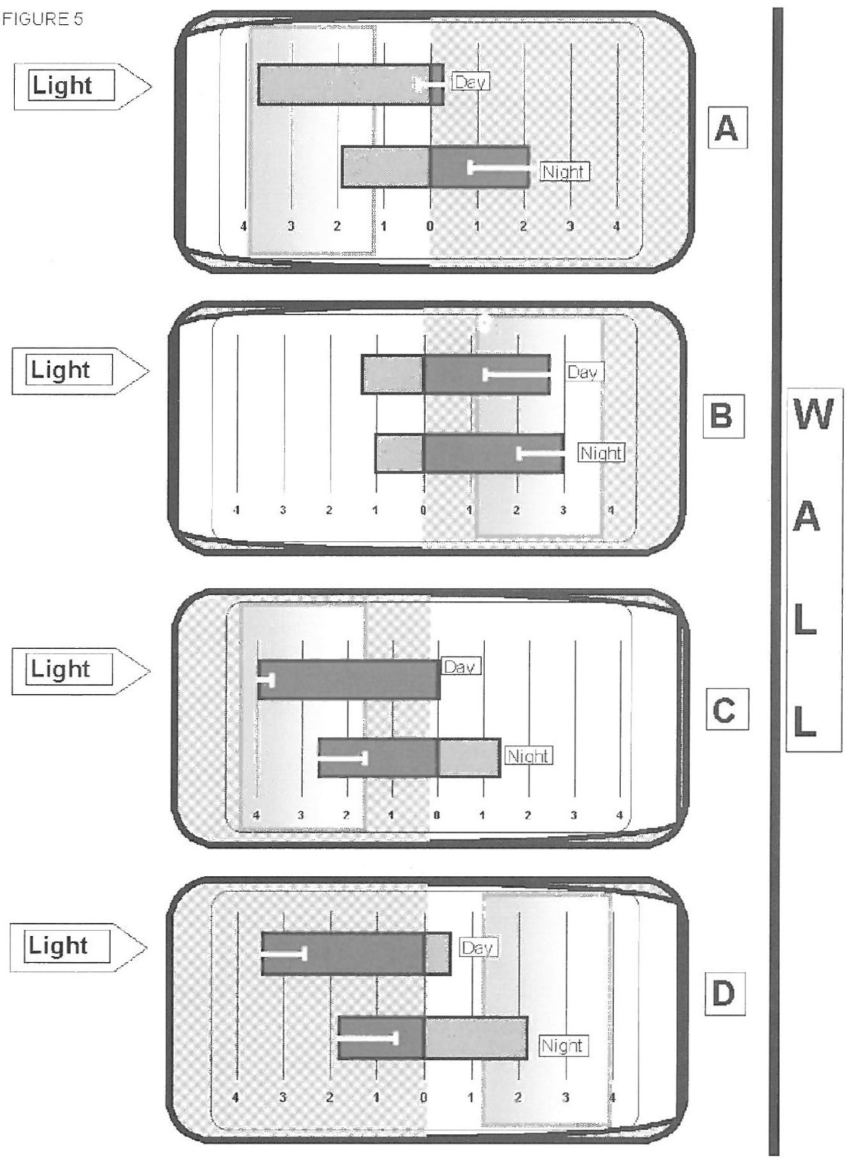

Figure 5. Mean ± SD of all recordings in each of the four groups with stainless steel body and polycarbonate half cage inside. There were always four rats in a cage. A significant ($p < 0.001$) two-way interaction between alignment of food hopper to inner half cage was detected both during the day and the night.

FIGURE 6

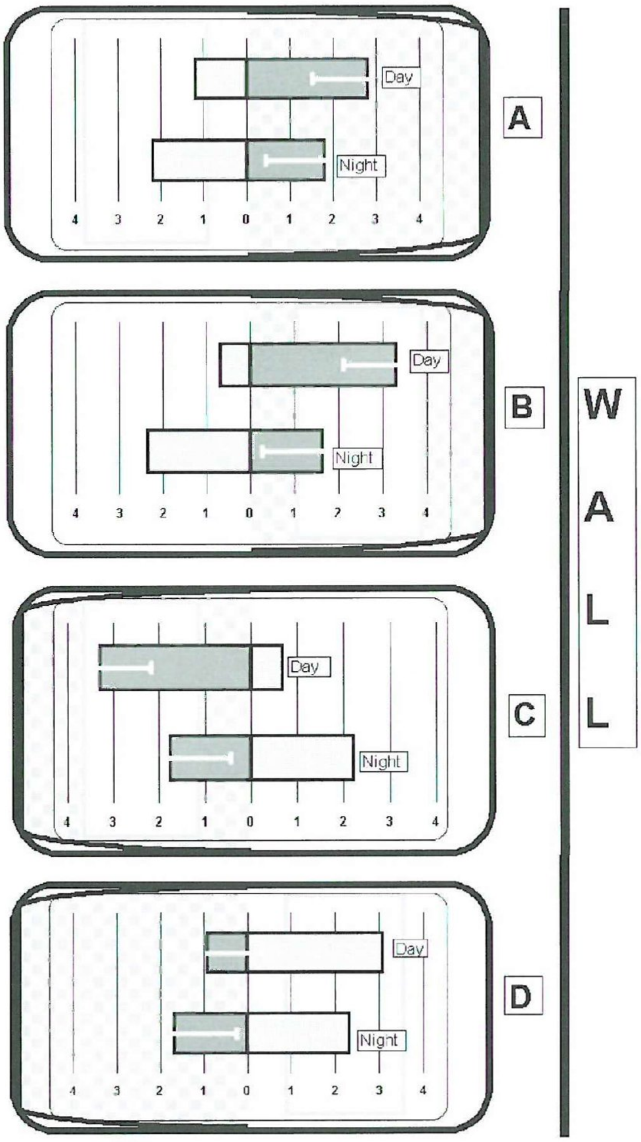

Figure 6. Mean ± SD of all recordings in each of the four groups with polycarbonate body and stainless steel half cage inside. There were always four rats in a cage. A significant ($p < 0.001$) two-way interaction between alignment of food hopper to inner half cage was detected both during the day, but during the night only hopper location was significant ($p < 0.001$).

the cage. The mean for the opposite side is simply four (numbers of animals in each cage) minus the mean. All these are illustrated in Figures 5A-5D for Kuopio and 6A-6D for Oulu.

Stainless steel body with polycarbonate false half cage

When the lights were on the cages with stainless steel body and polycarbonate inner half cage (Kuopio) there was a significant ($p < 0.001$) two-way interaction between the main effects, *i.e.* between the alignment of the food hopper with inner half cage and relation of the end of the cage to the wall. When the food hopper was at the darker end of the cage (Figure 5A and 5C), the rats preferred this end irrespective of the cage material. Accordingly, when the food hopper was at the lighter end of the cage (Figure 5B and 5D), the steel side was clearly favored by the rats.

When the lights were off, there was also a significant two-way interaction ($p < 0.001$) between the main effects. In practical terms, it did not make any difference which end of the cage faced the wall, as can be seen in the almost similar means of Figures 5A to 5 D, and 5B to 5C. The combination steel and food hopper at the same end (Figures 5B and 5C) increased the presence of rats by 50 % compared to almost equal presence at both ends of the combination of polycarbonate and food hopper.

Polycarbonate cage body with steel false half cage

When the cage body was made of transparent polycarbonate and the inner half cage made of stainless steel (Oulu) and the lights were on, there was a significant ($p < 0.001$) two-way interaction between the two main effects. Alignment of food hopper and steel half cage (Figures 6B and 6C) was the favored combination no matter which end of the cage faced the wall. With the alignment of polycarbonate half cage and food hopper (Figures 6A and 6D) it made a difference which end of the cage was against the wall, *i.e.* the darker side was favored.

When the lights were off, only the alignment of the hopper to the inner half cage had a significant effect ($p < 0.001$). The combination polycarbonate and food hopper (Figures 6A and 6D) was marginally more attractive than steel side and hopper (Figures 6B and 6C).

Discussion

Many aspects of animal care and management are based on practical procedures, which have been shown to work. Only recently have some of these routines, often called best practice, been reassessed with systematic research. Various approaches have been used to compare solid bottom to grid floor (*Manser et al. 1995, Manser et al. 1996, van de Weerd et al. 1996*), to search for optimal cage size and height (*Blom et al. 1995*), and to evaluate enrichment (*Kaliste-Korhonen et al. 1995, Blom et al. 1996, Eskola & Kaliste-Korhonen 1998, Eskola et al. 1999*).

Cage materials are considered as inert materials, which could not result in interference of research in the same way as residues and natural components in the diet or bedding (*Nevalainen & Vartiainen 1996*). The cage material has been traditionally combined with floor type: stainless steel with a grid floor and polycarbonate with a solid bottom. A study on cage material preference offered the choice between two cages, one made of polycarbonate and one of stainless steel (*Kaliste-Korhonen et al. 1996*). The choice between cages necessitates cumbersome connectors, which may be difficult for animals to use, thus hindering the evaluation of preference test results.

This study focuses on within-cage location of rats in a tailor-made cage, where half of the floor and walls were made of either polycarbonate or stainless steel as shown in Figures 1 and 2. The study design consists of two rat stocks with different breeding cage material and study cages with an added false half cage made of the other material than breeding cage and actual cage. This was done in order to improve the applicability of the results and to take into account the fact that earlier experiences have an impact on preference tests (*Broom & Johnson 1993*).

In a traditional solid bottom cage, there are several determinants, which could be anticipated to have an impact on where the rats are located. This study identified light intensity in cage, food hopper location and cage material, and in the statistical evaluation these parameters were used as the main effects. The light intensity in the cage with polycarbonate as the actual cage material was much higher than the stainless steel proper cage because of the cage transparency. Furthermore, light entrance into the polycarbonate cage in an open animal room is much less unidirectional and less sensitive to cage polarity than is the case with a steel cage in a cubicle.

It may be argued that rats could move bedding to a preferred location within the cage. During videotape viewing or visual observation before and after recordings no indication of this possible bias was seen.

Stainless steel body with polycarbonate false half cage

In cages with stainless steel body and polycarbonate false half cage darkness created by the actual cage and food hopper attracted almost all of the activity to that end during the daytime irrespective of cage wall and floor material (Figures 5A and 5C). It appears that this quite shaded area of the cage is a good place to escape from high light intensities.

When cages were rotated 180 degrees, and shaded areas provided by the front wall and the hopper were at opposite ends (Figures 5B and 5D), the stainless steel side was favored over polycarbonate, especially so when the steel side was towards the lights (Figure 5D). Even though it has been suggested that a stainless steel wall is inferior to polycarbonate because of the mirror effect and inability to be seen through *(Weihe 1987)*, this study suggests the opposite even though the difference is small. Mirror effect of the stainless steel walls was minimal because cages were not electropolished, the rats were accustomed to living in the same stainless steel and the light intensity was low.

During the dark period, the light direction and resulting shaded areas are much less obvious, which is verified since we obtained practically the same values irrespective of hopper position or cage direction. With polycarbonate and a feed hopper the rats were evenly distributed, but when the feed hopper was placed in the steel side three of four rats were found at the steel end. Hence, there was an obvious preference for stainless steel compared to polycarbonate cage walls. Again this may be partly a reflection of the fact that these animals had always been housed in stainless steel cages (*Broom & Johnson 1993)*.

Polycarbonate cage body with steel false half cage

The light intensities in cages made of polycarbonate with steel half cage inside were more complicated and higher than in stainless steel cages, especially during the daytime. The half devoid of false cage was illuminated from three directions through the wall and from above through the wire top. For technical reasons, the stainless steel inner half was only 15 cm high while the cage was 20 cm. Moreover, in Oulu the racks were in an open room while they were in cubicles in Kuopio.

The combination of stainless steel half cage and hopper was most popular during the daytime regardless of cage direction (Figures 6B and 6C). It is suggested that nontransparent stainless steel wall and hopper provide the best-shaded area, and this attracts the animals. Shade may be a necessity since the sense of sight of albino animals may be vulnerable to normal room light intensities (*Sharp & La Regina 1998*).

When the hopper was at the polycarbonate end of the cage facing the room center (Figure 6A), this end was less occupied than the stainless steel end. Rotating the cage 180 degrees (Figure 6D) shows that occupancy is sensitive to cage direction. This difference can be explained with difference in light intensities, with the half facing the room center being about twice as popular as the half facing the wall.

When the lights were turned off, only the hopper location had a significant effect. The mean occupancies were quite similar with a minor trend favoring polycarbonate. This may be explained by the fact that these rats had earlier been raised in similar polycarbonate cages (*Broom & Johnson 1993*).

Conclusions

The results of this study are rather complicated, and interpretation of calculated interactions is laborious. During daytime, location of rats in nontransparent steel body cages is largely governed by the light intensity created by the cage walls and hopper, but stainless steel was clearly preferred in three of four possible combinations. A preference of this magnitude is unlikely to be due to previous housing experience in stainless steel cages. During the night, the element of light direction becomes less important, but again hopper and steel combination was more attractive than hopper and polycarbonate combination.

In polycarbonate cages with steel inner half cages, the light intensity difference has a less prominent role. However, during the day, the hopper alignment with steel revealed preference to steel. This occurred despite the previous experience of the rats living in pure polycarbonate cages. During the night, the even distribution is indicative of a slight preference for the familiar wall material, polycarbonate.

In conclusion, the interplay of the alignment of inner half cage to hopper and the direction of the cage in the room is a complex phenomen, but allows one to draw some conclusions. In both study sites stainless steel was favored in three out of four possible combinations during the day. During the dark, when transparency of the material is of less value, in rats accustomed to stainless steel body cages, steel was superior to polycarbonate, but in rats brought up in polycarbonate cages no clear preference was detected for one material over the other.

Summary

Polycarbonate and stainless steel are commonly used cage body materials for laboratory rodents. The aim of this study was to assess preference of rats for the cage material. Altogether 64 male rats were used, 32 in two different facilities. The study cages were made of either stainless steel with a polycarbonate false inner half (Kuopio) or polycarbonate with a steel false inner half (Oulu). There were four different options for alignment of false cage halves and food hopper, and likewise two options for which end of the cage faced the wall. A video camera with time lapse recording of one second each minute was used. Weekly recording started at 16.00 and ended at 01.30, and each cage was recorded when the rats were aged four, five, six, seven and eight weeks. The results were processed separately for both facilities and for day and night. Statistical analysis was done with ANOVA using alignment of food hopper to false inner cage half and direction to the wall as the main effects. During the daytime the location of the rats in nontransparent steel body cages is largely governed by the light intensity created by cage walls and hopper, but stainless steel was clearly preferred in three of four possible combinations. During the night the element of light direction became less important, but again hopper and steel combination was more attractive than the hopper and polycarbonate combination. In polycarbonate cages with false steel inner half cages, the light intensity difference had a less prominent role. However, during the day, placing the feed hopper with steel was preferred. During the night, the even distribution was indicative of a slight preference to the familiar wall material, polycarbonate. In conclusion, in both study sites stainless steel was favored in 3 out 4 possible combinations during the day. During the dark, when transparency of the material was less critical, animals accustomed to a stainless steel cages preferred steel over polycarbonate, but for animals raised in polycarbonate cages neither steel nor polycarbonate was favored.

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