Cage Material and Food Hopper as Determinants in Rat Preference Tests

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Summary

Polycarbonate and stainless steel are commonly used cage materials for laboratory rodents. The aim of this study was to assess within-cage preference of rats for cage material, when the effect of illumination was eliminated. Altogether 64 male rats were used in two different facilities. The cages were made of either stainless steel with a polycarbonate false inner half with or without a false food hopper (Kuopio) or transparent or non-transparent polycarbonate with a steel false inner half (Oulu). A video camera with time lapse recording of one second per min was used and the positions of the rats were recorded. Once each week 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 each facility and for day and night. Statistical analysis was carried out with repeated measures ANOVA. In cages with a stainless steel body and a polycarbonate false half, the rats chose always the cage half with the food hopper, irrespective of the cage material. Thus, the food hopper is more important to rats than the material of the cage; but when the rats were allowed to choose between those two materials, both with a hopper, they favoured steel. In cages with a polycarbonate body and a steel false inner half, the combination of food hopper with low illumination was favoured during light time. In conclusion, this study shows that rats, when given a choice, prefer low illumination and cage material may be of less importance.

Introduction

Pertinent recommendations and guidelines on laboratory animal housing contain specifications on space allocation and enrichment approaches (*Brain et al., 1993*, *Council of Europe, 2007; European Union, 2007; Jennings et al., 1998; National Research Council, 1996*). Only a few of these deal with the cage material as such (*Brain et al., 1993*; *Jennings et al., 1998*).

Traditionally, only two cage materials have been widely used in laboratory rodent housing: stainless steel and polycarbonate. Since stainless steel cages usually have a grid floor and polycarbonate cages have solid bottom enclosures, all possible choices have not been utilized. The real, valid comparison of cage materials should be made with the same floor type.

Until the present time, material choices have been based on practical aspects. Both the Berlin report and Rodent Refinement Working Party recommend polycarbonate as the preferred rodent cage material (*Brain et al., 1993*; *Jennings et al., 1998*). Steel

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cages are perceived as durable and autoclaveable but with non-transparent walls making animal observation more laborious. Furthermore, routine housing and care procedures are somewhat noisier with steel cages (*Voipio et al., 2006*). Polycarbonate cages provide insulation, are lighter to handle and make observations easier.

However, especially for albino animals, the light in polycarbonate cages may be excessive (*Schlingmann et al., 1993*). Albino rats can't control levels of incoming light because there is no pigment in their irises, so the retina is overpowered by light. This inability to control levels of incoming light, the scattering of light inside the eye, and gradual retinal degeneration lead to very poor visual acuity (*Prusky et al., 2002*). Even though polycarbonate cages are more popular, the true refinement outcome of these housing alternatives necessitates assessment of the animals' preference.

Comparisons of solid bottom to grid floor have been made by some groups (*Manser et al., 1995*; *Manser et al., 1996*; *Van de Weerd et al., 1996*) and mice preference for different cage types was assessed by (*Baumans et al., 1987*). The effect of cage type has also been combined with the use of certain enrichment objects (*Eskola & Kaliste-Korhonen, 1998*). In another study by the same group, rats' preference to polycarbonate or stainless steel was compared, though there seemed to be no clear preference for either of the materials (*Kaliste-Korhonen et al., 1996*).

In our earlier study, rats could choose between stainless steel and polycarbonate cage halves. In most cage options, the rats seemed to prefer stainless steel, irrespective of the cage material where they were born and raised, but the position of the food hopper and illumination had an effect on the choice (*Heikkilä et al., 2001*). The purpose of this study was to further assess the choices when the effect of illumination was excluded.

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 cages, animal stocks and feed.

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 (WH, Hannover origin) and in Oulu Sprague Dawley (Mol:SPRD). The study protocol was reviewed and approved by the Animal Care and Use Committees of both Universities.

The temperature in the animal rooms was 21±1ºC 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: RM3, SDS, Essex, England) and tap water in bottles were available *ad libitum*.

Aspen bedding (Tapvei Oy, Kaavi, Finland) was used in both units. Since 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

Stainless steel body with polycarbonate false half cage

In Kuopio, the study cages were made of stainless steel having a false polycarbonate inner half. In order to make both sides of the cage similar in their inside space and form, a false food hopper was built into half of the cages. The false hopper was at the opposite end of the top to the proper food hopper, with both hoppers having the same dimensions. No food was kept in the false hopper. In all of the cages, there was a low threshold between the two material sides in order to prevent bedding accumulation on either half. Figure 1 shows the experimental cages and illustrates the false food hopper used in Kuopio.

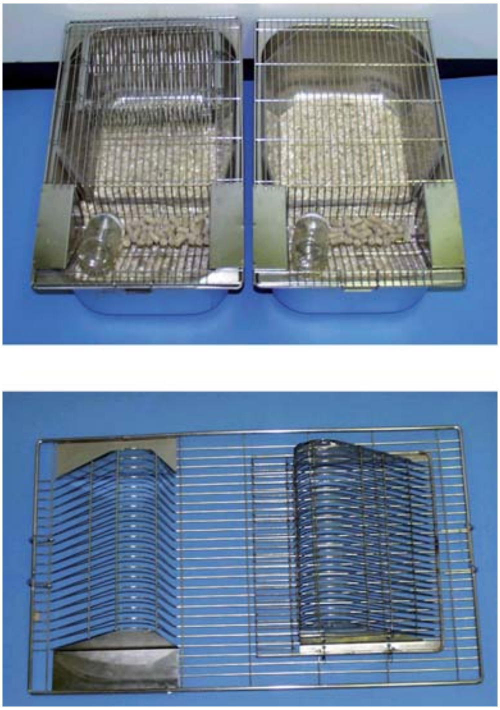

Figure 1. Illustration of cages with stainless steel body and polycarbonate inner half (Kuopio). In the left side cage, there is a cover with a false food hopper. The figure below shows the structure of the cover with the original and a false food hopper.

The cage racks were kept in a cubicle. To ensure similar light intensity in all parts of each cage, the cages were placed longitudinally with the back wall in the racks. During daytime, light intensity inside the cages was 19-22 lx under the food hopper and 35-43 lx at the opposite end. When there was a false hopper in the cage, the light intensity under it was 24-27 lx. During the night, the light intensity in all parts of the cage was always below 1 lx. Light intensities inside the cages are shown in Figure 3A-D.

Polycarbonate cage body with steel false half cage

In Oulu, the experimental cages were made of polycarbonate having a false steel inner half. In half of

Figure 2. Illustration of cages with polycarbonate body and inner stainless steel half cage (Oulu), one of the cages has a non-transparent plastic covering.

the cages, the outer surface of the transparent polycarbonate was covered with a black plastic tape in order to control the effect of light (Figure 2). Half of the cages were control cages with no covering. Again, there was a low threshold between the two materials.

The racks were in an open animal room. The cages were kept longitudinally along the back wall in the rack, which ensured equal illumination from light tubes to both halves of the cages. Light intensities in the cages during recording are shown in Figure 4A-D. During the dark period, the light intensity in all parts of the cages was about 1 lx in all cage types. In both units, the light intensities were measured with a luxmeter (Unitest digital luxmeter, BEHA, Germany) at about 3 cm above the bedding, at the level of the rats' eyes.

In both units, during the recordings, the experimental cages were removed at 8.00 from an ordinary rack to a recording rack, kept in the longitudinal position. In this rack, normal, empty cages were used above the experimental cages, in order to achieve the desired normal lighting circumstances. On the next morning 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.

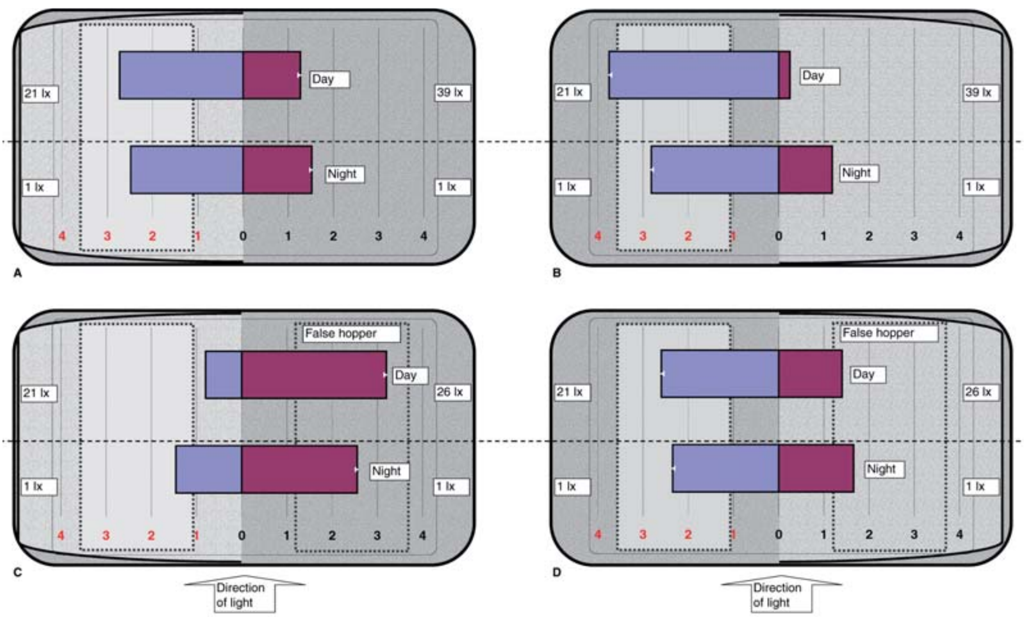

Figure 3A-D. Bird-eye view of cage interior combinations used showing mean ± SEM of number of rats present in respective cage half from all recordings during five weeks in each of the four groups with stainless steel body and polycarbonate half cage inside, with a false food hopper in half of the cages (Kuopio). Unlike in this figure, all the cages were placed as a single row with side walls of cages against the back wall of the room (opposite to light direction shown). There were always four rats in a cage. A significant ($p < 0.001$) interaction between the alignment of the food hopper and the false food hopper with the inner half cage was detected both during the day and the night. Light intensities during the light and dark time are shown in the cage halves.

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 the weaning age of three weeks, 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 that one male could be allocated to four cages, one to each experimental group. Thus, the four experimental groups consisted of two cages with four rats in each cage, and the groups had brother-pups from a total of eight female rats.

The experimental design resulted in four combinations both in Kuopio and Oulu. In Kuopio, the food hopper was placed either in the steel or polycarbonate part of the cage. In half of the tops there was a false food hopper. The experimental groups are shown in Figures 3A-D. In Oulu, half of the cages had the black plastic tape cover and the food hopper was placed either on the steel or plastic side of the cage, see Figures 4A-D.

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 peri-

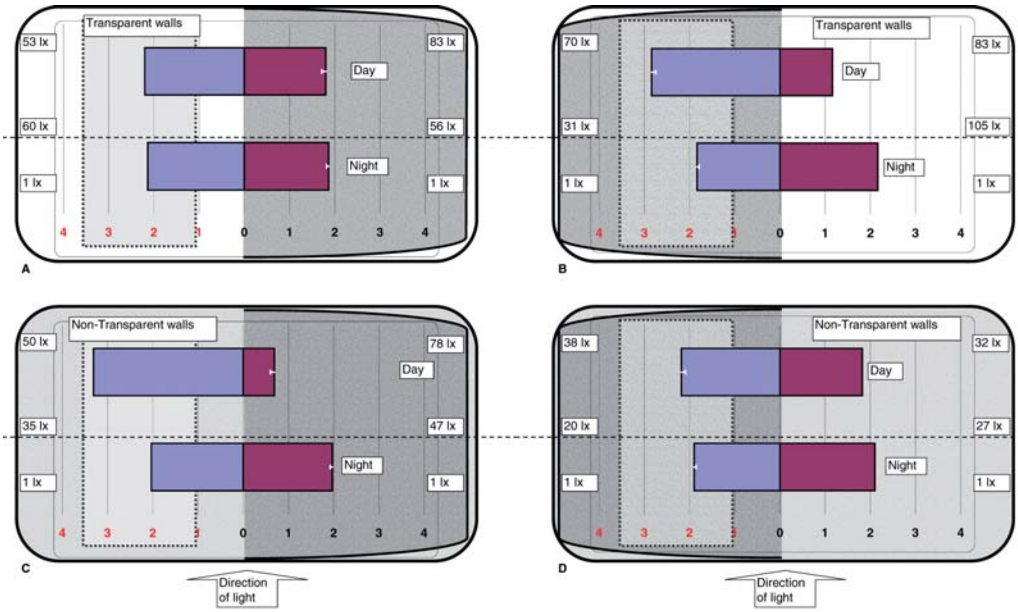

Figure 4A-D. Bird-eye view of cage interior combinations used showing mean $\pm$ SEM number of rats present in respective cage half from all recordings during five weeks in each of the four groups with polycarbonate body and stainless steel half cage inside, with non-transparent plastic cover in half of the cages (Oulu). Unlike in this figure, all the cages were placed as a single row with side walls of cages against the back wall of the room (opposite to light direction shown). There were always four rats in a cage. A significant ($p = 0.01$) two-way interaction between alignment of food hopper to the inner half cage was detected during the day. Light intensities during the light and dark time are shown in the cage halves.

od to the new cage environment. Time lapse recording of one second per minute was used. The recording started at 16.00 and ended at 01.30 on the same 24-h period. During the dark period, low intensity red lights (2 x 25 W) were used to ensure 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 min to yield the number of animals, and then averaged to number of animals per ten min, at both ends of the cage. Data from the 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 repeated measures ANOVA (SPSS 14.0 for Windows, SPSS Inc., Chicago, IL, USA) using alignment of food hopper to false inner cage half and presence of false hopper (in Kuopio) or alignment of food hopper to false inner cage half and presence of non-transparent plastic tape (in Oulu) as the main effects. Statistical significance was set at $p < 0.05$.

Results

All processed data is presented as number of rats (mean ± SEM) counted in the stainless steel side of the cage. The mean for the opposite side is simply four (numbers of animals in each cage) minus the mean. All these values are illustrated in Figures 3A-D for Kuopio and 4A-D for Oulu.

Stainless steel body with polycarbonate false half cage

When the lights were on, in the cages with stainless steel body and polycarbonate inner half cage (Kuopio) there was a significant ($p < 0.001$) interaction between the main effects, *i.e.* between the alignment of the food hopper and the presence of the additional false food hopper. In the cages with no false food hopper (Figure 3A-B), the rats preferred the half with the food hopper, irrespective of the cage material, but more so with stainless steel. In the cages with the false hopper (Figure 3C-D), the steel half was always favoured by the rats.

When the lights were off, there was also a significant two-way interaction ($p < 0.001$) between the main effects; the trends were the same as during the light period, but with less deviation from an even distribution within the cage.

Polycarbonate cage body with steel false half cage

During day time, in cages with a polycarbonate body there was a significant ($p = 0.01$) interaction between the two main effects. In cages with the food hopper in the transparent polycarbonate half, the rats spent equal time in both cage halves, while changing the walls to make them non-transparent made that the preferred end (Figure 4A and C). When the hopper was at the steel end, decreasing light with a non-transparent cover decreased the attractiveness of the hopper/steel combination (Figure 4B and D). When the lights were off, no significances were found.

Discussion

The laboratory animal cage is the primary enclosure where laboratory rats spend their entire life. At the moment much of the interest on welfare aspects is focused on cage furniture, *i.e.* enrichment, as if we had fact based evidence on what would be an optimal cage material. Practical aspects like cage weight and durability of the cage and ease of animal observation are important to us but not necessarily to the animals and their welfare. Consequently, it seemed logical to ask the clients, *i.e.* the rats, if they had a preference for their cage material over several weeks in order to avoid novelty or age related effects.

There are several recommendations about cage space (*Council of Europe, 2007; European Union, 2007*), and some of these recommendations mention also cage material (*Jennings et al., 1998*) though these are based mainly on practical aspects. The animal's preference for a cage material, with the same bottom type, is virtually unexplored.

In our recent study, the rats had the opportunity to choose between two ordinary cage materials, stainless steel and polycarbonate, with both materials in one cage (*Heikkilä et al., 2001*). However, the interfering effects of food hopper and illumination could not totally be excluded, yet the essential conclusion was that there was no evidence favouring polycarbonate as better than steel. This present study was designed to further assess the combined effects of the position of a food hopper and cage material excluding light as a determinant.

The initial assumption was that cage material would make less of a difference during the active, dark period, and *vice versa*. Indeed, from Figures 3 and 4 it appears that this is the case.

Light intensities measured from the cages show that aligning the long side of the cage with the wall yields more, albeit not completely, even illumination in cages with non-transparent walls. Residual differences are attributable to the food hopper, where it was necessary to have some food and water bottle, as shade from these items was unavoidable.

Stainless steel body with polycarbonate false half cage and a false food hopper

Alignment of the cages along the wall excludes the position effects of food hopper set either towards the room or wall. Thus, the possible combinations of cage material with location of food hopper are only two: food hopper above the steel or polycarbonate end. Furthermore, the assessment of the effects of food hopper element without food and water bottle (false food hopper) is possible (Figure 3). If one ranks the preference for combinations of material and real or false food hopper, the order for both day and night is as follows, starting from the most attractive:

1. Steel with real food hopper above without false hopper (Figure 3B)
2. Steel with false hopper above (Figure 3C)
3. Polycarbonate with real food hopper above without false hopper (Figure 3A)
4. Steel with real hopper above while false hopper is present (Figure 3D)

This is indicative of a preference for stainless steel as cage body material, albeit only one of the night results (Figure 3B) are sufficiently large enough to be of practical value. Since rodents are nocturnal animals, with their main activities like eating, drinking and exercise taking place during the dark period, it can be assumed that both cage halves are needed to allow the rats to enjoy these activities; and hence no preference is seen.

Albino rats, such as those used in the study, have a tendency to prefer dark parts of the cage (*Blom et al., 1995*). During the light period there was about a 10-20 lx difference between the halves under the food hopper and the opposite end, the hopper half being darker. In the cages with a false food hopper, it was about 5 lx darker under the original hopper than under the false one, due to the shadows from the food and water bottle. Thus, during the daytime it is surprising that the false hopper, with its somewhat higher illumination and nothing to eat or drink, on the steel side is favoured over the real food hopper at the opposite end (Figure 3C). The rats in this group were born in steel cages, which might have some effect on their choice.

In both cages without the false food hopper, the lower part (*i.e.* the space under the food hopper) was favoured. The reason seems to be obvious: it has been shown that rats prefer shelters: boxes or nest boxes (*Manser et al., 1998*, *Townsend, 1997*) or box-like enrichment tubes (*Eskola et al., 1999*). Furthermore, the cage height under the food hopper was 11.5 cm, which seems to be close to the dimensions of tunnels and nest chambers used by wild Norway rats: tunnels 7.5. cm, and nest chambers 14.5 cm high (*Calhoun, 1962*). This study suggests that during the inactive period the space underneath the food hopper is always preferred, but the combination with steel and false hopper is even more attractive than the combination of polycarbonate and hopper with food and water (Figure 3C).

Polycarbonate cage body with steel false half cage

In a transparent or non-transparent polycarbonate cage body with steel false half cage, the dark period results reveal an even distribution; during the active time either side will suffice (Figure 4A-D). When the lights were on, the top two combinations are:

1. Polycarbonate side with hopper above and wall made non-transparent (Figure 4C)
2. Stainless steel side with hopper opposite end with transparent walls (Figure 4B)

The remaining two combinations more or less represent an even distribution (Figure 4A and D).

In this cage design, before the walls were made non-transparent, the polycarbonate end was much brighter. Thus, it is logical that the hopper on the steel side is more favoured than hopper on polycarbonate side (Figures 4A and B). It has to be borne in mind that, for technical reasons, the stainless steel half cage did not provide full height walls.

If the difference in light intensity would be the reason for the animals' preference, increasing darkness with non-transparent tape should tempt animals from steel to the space under the hopper, and this seems to be the case (Figure 4A vs. 4C). When the hopper is above steel, it is apparent that making walls non-transparent increases the attractiveness of the polycarbonate half (Figure 4B vs. 4D).

The cage type with transparent walls under food hopper was one of the cages used also in our previous study. In that study, the rats favoured slightly more the steel half than was the case in the present study (*Heikkilä et al., 2001*). Although the results cannot be directly compared, the reason for the discrepancy may be illumination: in this study the cages were kept in a transverse position and the light coming from lighting tubes gave equal illumination to both parts of the cage. In the previous case, only the plastic half was towards the lighter room space.

Similar to our earlier study (*Heikkilä et al., 2001*) this study assessed the effects of possible determinants of rat location in a cage. These present comparisons permit us to draw conclusions about ranking the order of these determinants.

In cages with a stainless steel body with a polycarbonate false half, it is evident that the rats always chose the cage half with the food hopper, irrespective of the cage material, all day around (Figure 3A and B). Thus, the food hopper is more important to rats than the material of the cage. However, when the rats are allowed to choose between these two materials, both with a hopper, they do favour steel.

In cages with a polycarbonate body with steel false half, the most favoured combination was that of low light intensity, hopper and polycarbonate during light time.

In conclusion, this study shows that rats, when given a choice, prefer low illumination and that the cage material may be of less importance. The location of the food hopper attracts the rats, and during the day a false hopper is good enough for them even though it does not contain diet, water or shade.

Acknowledgements

The authors wish to thank the personnel of Department of Biology, University of Oulu, especially Jari Ylönen and Jari Mäntykenttä, for providing the recording equipment and their kind help with installation and use.

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