The Use of the Techniplast Mouse House® in Four Strains of Mice

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Summary
To combine the good qualities of a dark shelter with the possibility of the staff to monitor the animals without disturbing them, a transparent plastic shelter with a red tint has been designed for mice. As the mice do not see red colours, but perceive them as being grey or dark, this design is thought to provide a dark shelter for mice. As humans do see red colours, the red transparent material allows for inspection of the animals while they are inside the shelter.
However, recent studies and anecdotes seem to indicate a preference in some strains for not using the red houses. Four strains of mice, C57BL/6/Bkl, Balb/c/Bkl, CBA/BomTac and Bkl:NMRI, were housed individually with access to a red house as the only shelter. The result showed that there were significant differences in the time spent in the red house and the number of entries between the strains with NMRI mice – and to some extent the Balb/c - spending less time and demonstrating a higher number of entries in the red house than the pigmented strains.

Introduction
Belonging to a family of nocturnal burrowers, both rats and mice are highly depending on senses other than vision. Nevertheless their vision is fairly well-developed. Mice and rats are adapted to a life in environments with low light intensity and therefore the retinas of these animals are mainly composed of rod cells. The retina of rats and mice contain only one class of cone with pigment sensitive in the visible spectrum. The visible spectrum ranges from 400 to 700 nm with this photo pigment having maximum sensitivity around 508 nm, the green part of the spectrum(Lucas et al., 2001). Therefore it is argued that mice have poor colour vision (*Jacobs et al., 2001; Lawlor, 1994; Shaaban et al., 1998*) Moreover, the rodent retina contains cones with an ultraviolet-sensitive photo pigment (*Jacobs et al., 2001*) that is maximally sensitive around 360 nm. Mice are thus regarded as being insensitive to the red (long-wave) end of the colour spectrum (Figure 1).
Shelters in transparent plastic with a red tint have been designed for mice. This design is thought to provide a dark shelter, as the mice do not see red,

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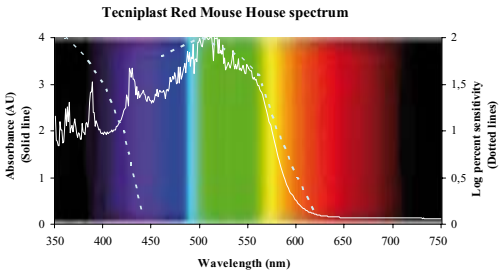

Figure 1. Absorbance spectrum of the Tecniplast Mouse House (TMH, solid line) and the relative sensitivity of the two known murine photopigments (UV cone opsin with λmax at 360 nm and Green cone opsin with λmax at 508 nm; dotted lines).

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but perceive the shelter as being dark. Equally important the red transparent material allows animal technicians to monitor the mice without disturbing them. Key & Hewett (*2002*) evaluated the shelter using solely Balb/c mice, an albino strain, and concluded that this cage "resulted in significant increases in several positive behaviours…"

However, a recent study showed that pigmented CBA female mice preferred white, green or black cages to red ones, and mice reared in red cages displayed elevated plus maze behaviour indicative of a higher level of anxiety than controls, indicating that the colours of the cages had an impact on animal behaviour (*Sherwin & Glen, 2003*). Marques & Olsson (*2007*) observed that a red coloured nest box was a less preferred location in pigmented C57BL/6J. Finally, anecdotal experience from Danish laboratory animal units indicated that pigmented mice (C57B/6J) tended to avoid the red mouse houses, whereas albino mice (NMRI and Balb/C) apparently preferred the red mouse houses. It is noticeable that the red mouse house was originally tested on Balb/c, which is an albino strain, whereas the studies and anecdotes all indicating an aversion towards the red mouse house relate to pigmented strains such as CBA and C57BL/6J.

The present study aims at evaluating possible differences between strains for a specific type of shelter with the above mentioned properties, namely the red-coloured Tecniplast Mouse House® (TMH). Such information is necessary in order to consider possible strain-differences when enriching the cages of laboratory mice and thus ensuring the most optimal enrichment.

Materials and Methods

Animals

Eight male mice of 4 strains, C57BL/6/Bkl (B6), Balb/c/Bkl (Balb/c), CBA/BomTac (CBA) and Bkl:NMRI (NMRI), were used. All mice were barrier-bred and health-monitored according to FELASA guidelines. At the start of the experiment, the mice were15-17 weeks of age. The Balb/c/Bkl, CBA/JBomTac and Bkl:NMRI mice were housed in groups of 8 individuals. C57BL/6/Bkl mice were housed in groups of 4. The animals were housed in plastic cages (Tecniplast 1291H: Eurostandard Type III H: 42.5 x 26.6 x 18.5 cm with a floor area of 800 cm^2, (Techniplast, Italy)) Each cage was provided with enrichment in accordance with the Danish legislation, namely aspen bedding, (Tapvei oy, Finland), nesting materials (Nestlets, Datesand, UK), woodwool (Tapvei oy, Finland), and cardboard shelters (Des.Res.™, Lillico Biotechnology, UK). The mice were supplied with food-pellets (Altromin 1324, Brogaarden, DK) and tap water *ad libitum*. The mice were housed and tested in the same room. In this room the temperature was maintained at 20° C +/- 1° C with a relative humidity of 55-80%. The light/dark cycle was 12 hours light and 12 hours dark with no natural light. The light was on from 6 a.m. to 6 p.m.

Experimental procedure

Prior to the test, the mice were weighed. During the experiment the mice were housed individually in modified plastic Eurostandard Type III H cages with aspen bedding, one Techniplast Mouse House® and one pad of Nestlets nesting material. The mice were supplied with food-pellets (Altromin 1324) and *ad libitum* tap water. The cages were covered by transparent acrylic covers with holes for ventilation. Behavioural parameters were measured between 06.40 and 17.50 using the method described below. The recordings were made with a Panasonic WV. BP330 camera and recorded with a Time Lapse video recorder to avoid human interference. All testing was done during daytime (lights on), the inactive period of the mice. The light intensity 50 cm above the test set-up was 44 lux. The total test time for each mouse was 12 hours from 6 a.m. to 6 p.m. Testing was done from June 7th to June 20th.

The behaviour of the mice were categorised as time spend inside the house when the base of the tail was inside the house. Time spend in the house and number of entries were measured and total time spend in the house and mean time spend in the house were calculated for each individual.

Statistic analysis

All statistical analysis was done using the Statistical Analysis System SAS (SAS Institute Inc., version 9.1). Data on bodyweight were not normally distributed and data were analysed using an ANOVA on ranked data. Pair-wise comparisons were done using the Differences of Least Squares means procedure.

Table 1. Statistics used for analysing the data.

Variable	Normality of data	Statistics
Total time spend in Mouse House	-	Ranked ANOVA
Number of entries	-	ANOVA
Mean time spend in Mouse House	+	ANOVA

Data relating the use of the house that were normally distributed (Table 1), were analysed using an ANOVA including strain, pigmentation and body weight of the mouse as fixed effects and cage and day of testing as random effects. For data that were not normally distributed, an ANOVA on ranked data was performed. Pair-wise comparisons were done using the Differences of Least Squares means procedure.

Results

All 40 mice participated in the trials, and no one had to be excluded because of illness or reduced welfare.

However, one Balb/C mouse was excluded from the behavioural dataset as he had turned the house upside down and therefore was unable to sleep inside the house.

Not surprisingly, the bodyweight of the animals were significantly different between the strains (Table 2). It is well known that NMRI mice are heavier than e.g. age matched Balb/c and B6 mice. As all animals were 15-16 weeks of age at test time, the genetics more than the age of the animals explains this strain difference in bodyweight. However, this difference emphasised the need for including bodyweight in the statistical analysis of behaviours in relation to the mouse house.

Table 2. Mean bodyweights of the four strains. Strains with different letters are significantly different.

Strain	n	Mean +/- std. deviation
Balb/C	8	27.7 +/- 1.2 a
B6	8	29.7 +/- 2.1 b
CBA	8	27.9 +/- 1.8 a
NMRI	8	40.8 +/- 2.1 c

The final statistical model for total time spent in the mouse house included strain as a fixed effect and day as a random effect. The strains spend significantly different amounts of time in the mouse house ($F=6.36$; $p=0.0031$) and using pair-wise comparisons, significant differences were demonstrated between NMRI mice and the pigmented strains as well as between Balb/C and CBA mice (Figure 2).

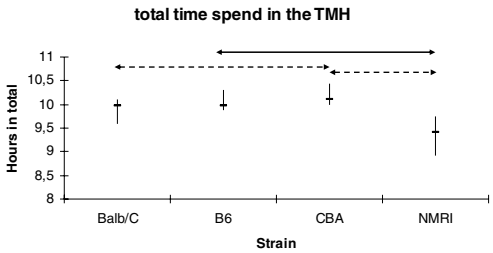

Figure 2. Total time (median, errorbars indicating 25% and 75% quantiles) spend in the TMH in the four strains. Dotted lines represent differences with $p<0.05$, straight lines indicate $p<0.01$.

The mean time spend in the mouse house also differed ($F=3.69$; $p= 0.023$) between strains. The final statistical model included strain as a fixed effect (Figure 3). Number of entries was significantly higher in the NMRI mice compared to the pigmented strains (Figure 4).

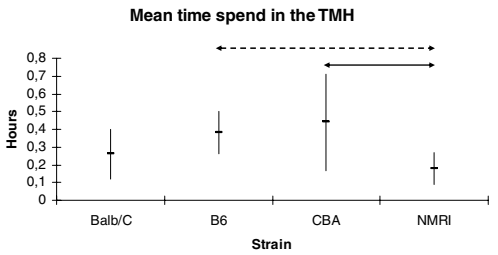

Figure 3. Mean time (time in hours, error bars indicating standard deviation) spend in the TMH when the mice visited the house. Dotted lines represent differences with $p<0.05$, straight lines indicate $p<0.01$.

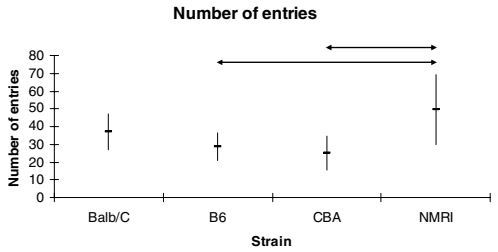

Figure 4. Number of entries (error bars indicating standard deviation) into the TMH during the entire test period. Dotted lines represents differences with $p<0.05$, straight lines indicate $p<0.01$.

Discussion

The result showed that there were significant differences in the time spent in the mouse house between the strains with NMRI mice – and to some extent the Balb/c - spending less time in the mouse house than the pigmented strains. However, this difference is in keeping with the result demonstrating a higher number of entries in NMRI mice. These findings could indicate the NMRI mice were more active, venturing in and out of the house to a higher degree than the pigmented strains, which would be in accordance with other studies demonstrating higher activity in Balb/c and NMRI mice compared to other strains (*Bolivar et al., 2001; Kalueff & Tuohimaa, 2005; Liu & Gershenfeld, 2003; Peeler & Nowakowski, 1987; Toth & Williams, 1999*).

Moreover, the difference in total time spend in the house is 1.61 h which equals 1 hour and 36 minutes out of 11 h 10 m. Whether this difference is also biologically significant is debatable. It does not seem reasonable to suggest that the welfare of the NMRI mice is at risk due to the provision of a non-preferred shelter. It seems more likely that these strains are in fact more active and thus simply spend less time resting in the Mouse House®.

When considering animal welfare, the mouse house apparently is a fair enrichment item; however, it is worth mentioning that studies have indicated that other types of shelters or bedding material in fact are preferred resources compared to the mouse house (*Marques & Olsson, 2007; Van Loo et al., 2005*). It is highly likely that the preferences for the mouse house depend not only on the genetics and age of the mouse, but also on the availability of resources that could positively substitute for the mouse house.

Acknowledgements

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