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Original scientific article

Does colour matter? Preference of mice for different colours of the house mouse igloo: an observational study

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Summary

It is argued that mice have poor colour vision and are insensitive to the colour red, which they in theory perceive as dark. Therefore, the red tinted Bio-Serv Mouse Igloo® is used to provide mice with a dark shelter while enabling humans to monitor the animals through the shelter without disturbing them. However, we do not know if mice like the colour red. Therefore, this study investigated whether mice prefer an amber or blue igloo over a red igloo, still making it possible for humans to see through the igloos. A preference test consisting of 3 cages each containing a different coloured igloo, connected to a barren central cage, was conducted for 11 h 20 min in 32 female B6 mice. The results showed that some female B6 mice did not like to use the Bio-Serv Mouse Igloo® as a shelter, regardless of colour. Almost half of the mice chose to stay outside the igloos, but when choosing an igloo the mice preferred the blue or amber igloo over the red igloo.

Introduction

As a nocturnal, burrowing species, mice have a different visual capacity to humans. Unlike humans, mice have two and not three types of cone photopigment with maximal sensitivities around 359 nm (UV cone) and 511 nm (M cone), respectively (Huberman and Niell 2011; Jacobs et al. 1991; Yokoyama et al. 1998). Also, mice are regarded as being insensitive to the colour red, which has wavelengths ranging from 622 to 780 nm (Huberman and Niell 2011; Key 2004; NASA 2017). In other words, it is believed that mice will perceive red as being dark.

The Bio-Serv Mouse Igloo™ can be used as a shelter, a running wheel-igloo and in the scoring of nesting behaviour (Howerton et al. 2008; Key 2004; Pedersen et al. 2014; Robertson and Rowland 2005; Sager et al. 2010). The igloo is transparent and is available in red, amber and blue. These igloos provide shelter for the animals and easy visibility for animal caretakers, and the red igloo is believed to be perceived as dark by mice. Therefore, a transparent red-coloured shelter seems ideal for laboratory mice. But do the mice like these types of shelter? Marques and Olsson (2007) found that mice stopped using a red coloured Tecniplast Mouse House® shelter for sleeping at the age of four to eight weeks. It is unknown whether the mice disliked the colour or the material of the Tecniplast Mouse House. However, in pilot studies to test the nest scoring method developed by Pedersen et al. (2014) we found a similar dislike for the red igloo. We assessed nests in 9 cages and noted that all the mice had built nests using the provided Nestlet nesting material (Ancare, USA), but in no case did the mice use the red igloo when making a nest. The Tecniplast Mouse House® and the Mouse Igloo™ have the same red tint. Our findings are supported by anecdotal experiences from our research facilities indicating that mice tend to dislike and avoid using the red mouse igloo. Furthermore, Sherwin and Glen (2003) showed that female mice preferred white, black or green cages over red spray-painted cages. Maybe the dark shade that mice perceive instead of red is a shade they do not like. And maybe mice prefer an amber or blue igloo when making a nest? It is likely that mice are capable of seeing colours close to red. The upper limit of the murine M-cone photopigment is close to the lower limit of the red wavelengths and this may allow the mouse to perceive colours approaching orange. Hence, it is possible that mice in fact do not perceive a red plastic shelter such as the Mouse House® or the red Bio-Serv Mouse Igloo™ as dark, as stated by e.g. Key (2004).

Therefore, in this study we investigated whether mice prefer an amber or blue igloo over a red igloo, whilst still making it possible for humans to see through the igloos. We hypothesized that mice prefer the amber or blue igloo over the red igloo.

Materials and methods

The experimental procedures were approved by the Danish Animal Experiments Inspectorate under the Ministry of Environment and Food in Denmark (License number: 2016-15-0201-00871). The study was carried out in accordance with EU animal welfare requirements (Directive 2010/63/EU), and reporting of the study follows the ARRIVE guidelines for reporting animal research (Kilkenny et al. 2010). Efforts were made to minimize the number of animals used.

Animals and housing

Thirty two 6-week-old C57BL/6 female mice (Taconic, Ejby, Denmark) were randomly allocated into 8 cages in groups of 4. The cages were of transparent standard Makrolon type 4 (Tecniplast, Buguggiate, Italy) (LxWxH: 540 x 320 x 180 mm) with a 70 mm raised lid. All cages contained Aspen bedding, Enviro-Dri paper nesting material, biting blocks (LxWxH: 50 x 10 x 10 mm) in aspen wood (all Tapvei, Harjumaa, Estonia), two cardboard tubes (LBS serving Biotechnology, Horley, United Kingdom) and a hemp rope (length 30cm, diameter 6mm) hanging from the lid in the centre of the cage (Fyns Kran Udstyr, Odense, Denmark). All cages were provided with food pellets (Altromin type 1324, Lage, Germany) and water *ad libitum*. The mice were maintained on a 12:12 h dark/light cycle with lights on from 06:00 h to 18:00 h. Room temperature was 20-22^{0}C with a relative humidity of 45-65%. Health monitoring was performed daily and the cages were changed once weekly.

Experimental setup

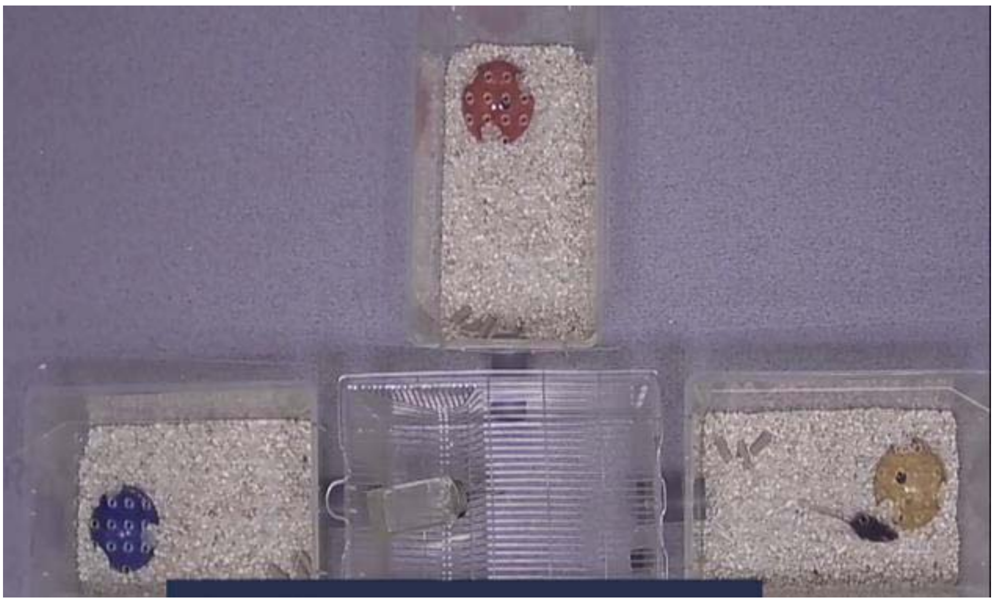

Figure 1. Experimental setup with three cages containing a different coloured igloo connected to a central barren cage.

Experimental procedure

The mice were allowed two weeks of habituation before testing. At 06.00 h two mice from the same cage were gently transported to the adjacent test room and allowed 30 min of habituation to the test room. The test room was maintained under the same conditions as the animal holding room. All testing was done during the light phase, the inactive period of mice. At 06.30 h the two mice were transferred to the experimental setup and the video cameras were turned on. All mice were tested individually to avoid the possible influence of another mouse on the igloo preference. At 17.50 h the cameras were turned off and the mice were marked with a permanent marker on the tail to make it possible to identify the remaining two mice from the same cage the following day. Each cage was tested over 2 days (4 mice in each cage, 2 mice tested per day). The experimental setup consisted of a central cage (without bedding) connected by grey plastic tubes (L 10 cm; Ø 40mm) to three preference cages (Figure 1). Each preference cage contained aspen bedding as floor substrate and *ad libitum* food placed in one corner (left corner closest to the connecting plastic tube). A water bottle was placed in the lid covering the central cage. The three preference cages were covered by transparent acryl covers with holes for ventilation. Each preference cage contained a different coloured Mouse Igloo (red, blue or amber) as shelter. Each day, the three coloured igloos were placed in a different preference cage (clockwise rotation) to exclude or minimize external stimuli such as light, sounds etc. affecting the shelter preference. Two sets of identical experimental setups were used, each filmed from above by a camera. The light intensity was approximately 40 lux 50 cm above the experimental setups. After each test session, the experimental setup was cleaned with 70% ethanol and the bedding was changed to remove bias caused by odours before testing the following day. Parameters assessed were: entries made into shelter, and time spent inside shelter. An entry was defined as the moment when the base of the tail was inside a shelter.

Finally, the transmission spectrum for each coloured igloo was established using an Aligent 8453 UV-visible Spectroscopy System (Aligent Technologies, California, USA).

Statistics

The study was performed as an observational study and no statistical analyses were applied. For each mouse, we deemed the igloo that was occupied for the longest time period as that individual's most preferred igloo and the igloo occupied for the shortest time period as the least preferred igloo. Occupancy of the barren central cage was rare and data thereof were excluded from the analysis.

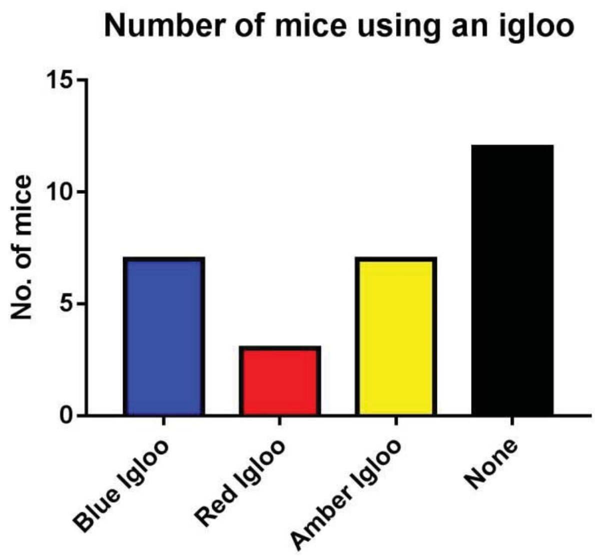

Figure 2. Preference of female B6 mice for igloos of different colours. 29 female B6 mice were included. 17 mice spent between 1 and 9 hours in an igloo while 12 mice used an igloo less than a minute or not at all.

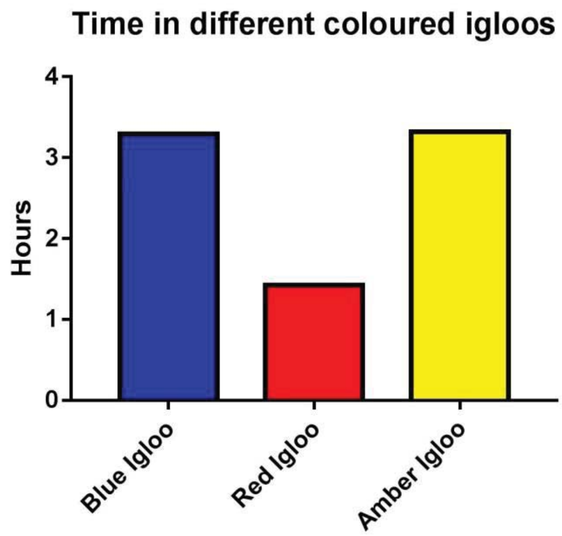

Figure 3. Average time spent in different coloured igloos for the 17 mice choosing to use an igloo during the test period.

Results

One mouse escaped from the experimental setup after 2 hours and was excluded from the analysis. Also, two mice never left the first cage they entered and were therefore excluded from the analysis. Altogether, 29 mice were included in the analysis.

During the 11 h and 20 min test period, 17 mice (58.6 %) spent between 1 and 9 hours in an igloo while 12 mice (41.4 %) spent less than 1 minute or no time at all in an igloo. Of the 17 mice using an igloo, 7 preferred the blue igloo, 7 preferred the amber igloo while 3 mice preferred the red igloo. Thus, among mice using an igloo, the majority chose either the blue or the amber igloo over the red igloo (Figure 2). The 17 mice choosing to use an igloo spent an average of 8.03 h in an igloo during the 11 h 20 min test time. This means that these mice spent 71 % of the test time inside an igloo; 3.29 h were spent in the blue igloo, 3.32 h in the amber igloo and 1.42 h in the red igloo (Figure 3). Hence 133 % more time was spent in the blue or amber igloo compared with the red igloo. Also, among the 12 mice not using an igloo at all, 5 mice chose to be in the cage containing the blue igloo and thereby in close proximity to the blue igloo, 5 mice chose the cage with the amber igloo while only 2 mice chose the cage containing the red igloo. The 17 mice using an igloo entered on average the blue igloo 60 times, the red igloo 57 times and the amber igloo 49 times.

The transmission spectra for the blue, red and amber igloos are depicted in Figure 4 together with the relative sensitivity of the two known murine photopigments. The transmission of the blue igloo peaks at 495 nm while the transmission of the red and amber igloos peaks at 657 nm and 604 nm, respectively (Figure 4). From Figure 4 it is clear that the red igloo is a visible object for mice, but the red igloo only reflects a small amount of light detectable by the murine photoreceptors.

Discussion

In this study investigating the preference of female B6 mice for different coloured igloos, almost half of the mice did not use an igloo as a shelter. Among mice choosing to stay in an igloo, the majority preferred the blue or amber igloo over the red igloo. Also, among mice choosing not to stay in an igloo, the majority preferred to stay in cages containing a blue or an amber igloo over the cage containing a red igloo.

The results from our study indicate that female B6 mice either choose to use the igloos for shelter for an extended time period or not use them at all. Almost half of the mice included in the analysis chose to stay outside an igloo even though they did not have any alternative shelter or nesting material. The other half stayed inside an igloo for an average of 8.03 h, and the majority of these mice preferred to stay inside the blue or amber coloured igloo. This indicates that mice using an igloo for shelter rarely chose the red igloo. Even though the mice preferred the blue or amber igloo over the red igloo, they still entered all igloos approximately the same number of times. Mice have an innate preference for dark enclosed spaces. In theory, the red igloo represents one as mice are regarded to be insensitive to red colours (Key 2004; Latham and Mason 2004). Therefore, mice are expected to like and prefer the red coloured igloo. Also, the igloo itself represents an enclosed area and this gives the mice an opportunity to escape the high intensity light and avoid damage to their retinas. However, our results show that the red igloo

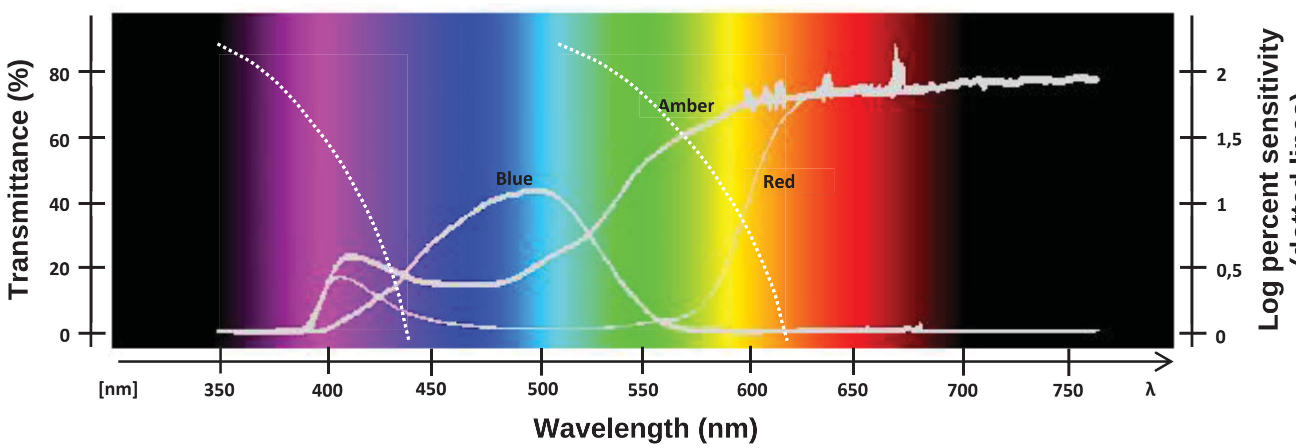

Figure 4. Transmission spectrum of the blue, red and amber igloos (solid lines) and the relative sensitivity of the known photopigments of the murine eye. UV cone with a λmax at 359 nm and M cone with a λmax at 509 nm (broken lines).

used in this study is in fact a visible object for the murine eye. Although, the red igloo reflects only a minor portion of light detectable by the murine photoreceptors. As mentioned, almost half of the mice in this study did not like the igloos, but when they chose an igloo, they chose the blue or the amber over the red igloo despite the common belief that mice like red shelters. A reason for this could be the small amount of light that mice detect through the red igloo. Maybe mice do not like the shades of red they are able to see. Also, it cannot be ruled out that the 30 min habituation period to the test room was too short and that the outcome therefore was affected by this even though we transported the mice gently and only to the adjacent room. It is a common statement that even short periods of transportation affect mice and that 24 h are needed for acclimatization to a novel environment. However, data supporting this are very limited. Also, reproducing the results of this study has not been attempted and it is therefore not possible to know whether the results of this study are completely reliable. This could be a goal for future studies.

Other studies have shown that mice prefer other types of shelters over a red plastic shelter and that mice prefer to be in a black or green cage rather than a red cage (Marques and Olsson 2007; Sherwin and Glen 2003; Van Loo et al. 2005). Moreover, a recent study by Wren-Dali et al. (2016) demonstrated that coloured tunnels serving as enrichment altered the melatonin levels in rats. Additionally, several other studies have shown that different coloured cages, and even a dim red light in the ceiling, affect hormone levels in rats (Dauchy et al. 2013a, 2013b, 2015; Wren et al. 2014*)*. It is difficult to know how mice perceive the colours at the upper limit of the murine M-opsin. However, according to previous studies (Marques and Olsson 2007; Sherwin and Glen 2003; Van Loo et al. 2005) it is likely that mice respond to various colours. In rats, which have approximately the same photoreceptors as mice (Lei and Yao 2006), a physiological response to altered characteristics of the cage (perceived by humans simply as colour changes) has been demonstrated (Dauchy et al. 2013a, 2013b, 2015; Wren et al. 2014). Therefore, researchers should remain aware of the possibility that mice and rats may be affected by colours in ways different from humans and in ways we are unaware. Therefore, to provide the optimal enrichment solution in a given study, investigators need to test the basic preferences of the animals. Moreover, it should be investigated whether coloured items such as the Bio-Serv Mouse Igloo® used in research affect the normal hormonal levels involved in metabolism and physiology.

In conclusion, more than half of the female B6 mice in this study choose to stay inside a Bio-Serv Mouse Igloo® for the majority of the inactive period tested. When choosing an igloo the mice preferred the blue or amber igloo over the red igloo.

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