Shifting shadows: navigating the challenges and benefits of reverse photoperiod rooms in rodent behavioural studies

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Background

A consistent and regular photoperiod is an important variable to control in research with animal models. Light has a major influence on both physiology and behaviour in mammals, especially regulating circadian rhythms.

At the Crick, reverse light cycle rooms are used in behavioural studies as mice are nocturnal. This allows researchers studying behaviour and neuroscience to test and train animals in the dark when mice are most active, during normal working hours. In these rooms red lighting is used because of rodent insensitivity to long wavelength light, allowing staff to see while mice respond as if in darkness. Interruption of darkness with light at night (LAN) can disturb biological and circadian rhythms, so restricting this is also especially important. However working in the dark can also impact staff welfare affecting human circadian rhythms. This poster explores the challenges and advantages of these rooms, including opportunities for refinement and the adjustments we have made.

Introduction: the importance of light

- Consistent photoperiods are vital to maintain circadian rhythms in mammals. Light has been shown to have a substantial influence on the physiology and behaviour of laboratory mice.[1]

- The lighting conditions of holding rooms are significant to Animal Welfare and experimental reproducibility. Light intensity wavelength and photoperiod should all be considered.

Light at night (LAN) disturbs biological rhythms and can affect stress levels, immune response, depression like response and even result in an increased risk of cancer.[2] Even 15-minute bursts of light can cause increased stress levels in laboratory mice. During periods of darkness, effort should be taken to minimise any light exposure.[3,4,5]

Visual sensitivity of mice vs humans

- Mice have poor vision (qualifying as legally blind by human standards).
- Mice rely on sight for many different functions: simple object detection, movement detection and detecting changes in environmental light levels.[1]
  - Differences in visual pigments result in different sensitivities to different wavelengths of light.
  - Humans are maximally sensitive to red, green and blue light.
  - Mice are maximally sensitive to ultraviolet and green wavelength light.
  - Therefore mice are less sensitive to long wavelength light (red) than humans (Figure 1).

At 600nm (red light) humans are 12 times more sensitive than mice.[1]

Red light can be used for mouse behavioural work where mice respond as if in darkness but researchers are able to see. However the lighting must be of sufficient dimness as mice can still see under red light but at a reduced sensitivity.

Most lighting is measured and regulated using lux, a unit based on perceived brightness weighted to the sensitivity

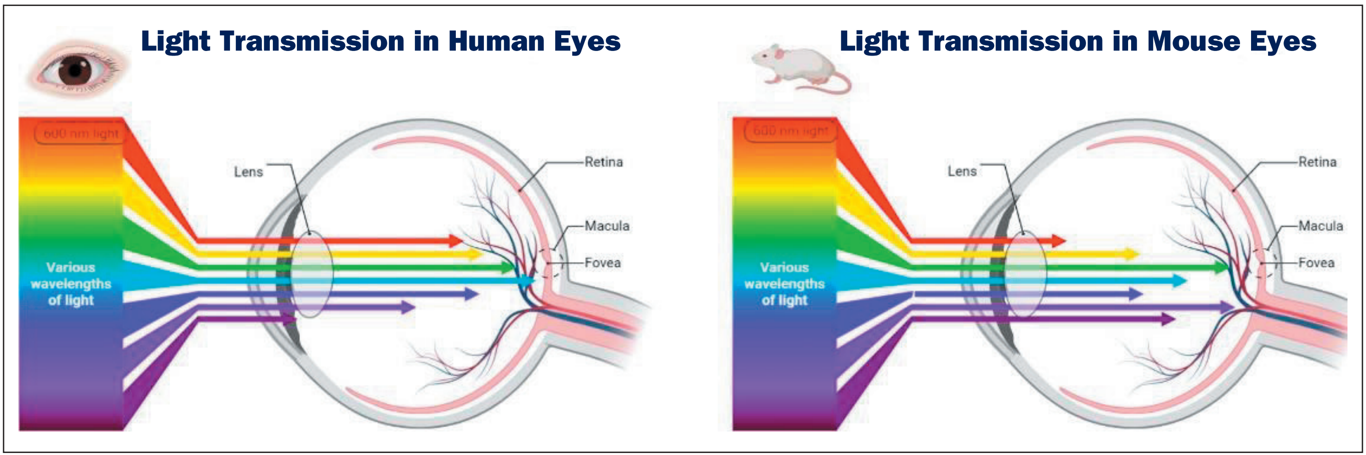

Figure 1. Schematic representation of human vs mouse sensitivities to different wavelengths of light. Made using BioRender.

of the human visual system. This is not relevant to non-human species which possess a different spectral sensitivity to light.

Instead a spectrophotometer could be used although these tend to be much more expensive.

Role of reverse lighting

- Mice are used as a model to study human physiology and disease but most research is carried out when the mice are inactive/asleep during the day.
- Studying mice under a reverse light cycle may produce more reliable and translatable data.
- Reversing the light cycle means the mice can be tested in the dark during the normal working day.
- Light plays a critical role in many non-visual responses, including regulating circadian rhythms, sleep, pupil constriction, heart rate, hormone release and learning and memory.
- Reverse lighting is often preferred for housing animals in behavioural testing experiments.

Getting the most out of red light

All holding room lights gradually turn on/off over a period of approximately 30 minutes with a dusk and dawn function avoiding a sudden change in darkness and mimics the natural effect of a sunrise/sunset. This gradual change means we can avoid the stress response animals experience from LAN.

Animal facilities generally use white laboratory light which is provided by white LED lights or fluorescent strip lighting that is usually measured in lux.

Lighting is regulated under ASPA in lux however this is more to do with the local regulations that are required for staff to work under than for Animal Welfare.

Calculating the wavelengths present in white light involves taking measurements with a spectrophotometer or power meter and complex calculations. Knowing the intensity of the wavelengths that make up white light is not a requirement in the Code of Practice.

Making sure that the light in reverse lighting rooms is of an appropriate intensity has been convoluted. Measurements have been taken in lux and although the intensity reads 0, the rooms are not under complete darkness but low intensity red light. There is still enough light for (limited) human sight, but not for mice.

It has been suggested that the spectral power distribution of light sources should be reported to ensure reproducibility. Knowing the lighting type and the lux measurements can provide a general idea of the content of the white light in holding rooms.

A free toolbox created by Stuart Peirson www.ndcn.ox.uk/team/stuart-peirson has been used here to display an approximation of the Crick spectral power distribution (Figure 2) in a holding room during daylight hours with fluorescent light at 363 lux.

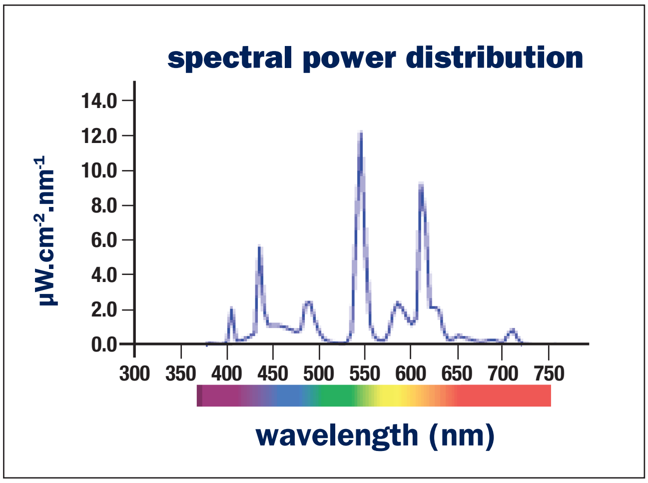

Figure 2. Graph showing the spectral power distribution of normal Crick holding rooms.

Reverse light rooms at the Crick

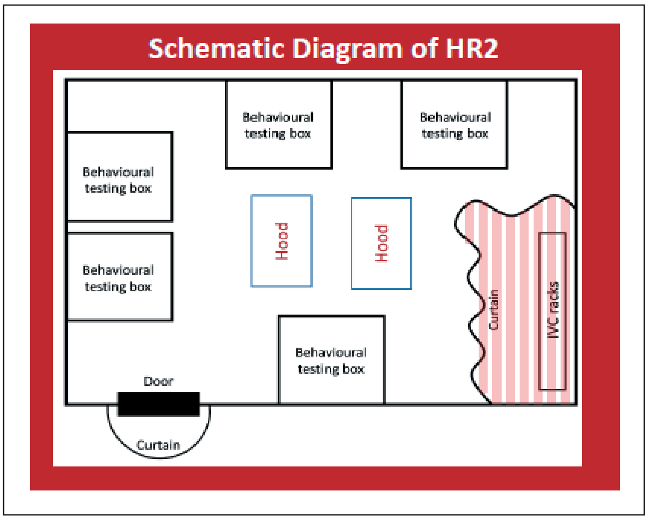

Figure 3. Example of a reverse light holding room.

Researchers can adjust the lighting in the behavioural area (Figure 3) while the lighting behind the curtain where mice are housed, is on a fixed timer.

Crick Institute light cycles:

Normal photoperiod	**07:00 to 19:00 hours**
Reverse photoperiod	**22:00 to 10:00 hours**

Animal and staff welfare provisions

The room consists of:

- Behavioural area lights can manually be changed between white and red lighting.
- Hoods can be changed to white or red light.
- IVC rack area behind a blackout curtain on an automatic timer.
- A light proof curtain around the outside of door stops light entering when the door is opened.
- This allows for researchers to work under normal lighting, even during dark hours.

Minimum mouse acclimatisation times at the Crick
Minor and terminal procedures = 2 days
Surgical procedures = 7 days

Acclimatisation periods

- Non-standard lighting cycles have been repeatedly shown to hasten death in animals.[6]
- Studies have shown that phase advance (earlier onset of light) is more detrimental than phase delay in both humans and rodents[7].
- One study suggests that for each hour of acute phase advance, the molecular clock requires 1 entire day for expression to shift to, e.g. 6-hour phase shift = 6 days of acclimatisation.[7]

More research is needed on the ideal acclimatisation period, and increasing these times is a key refinement opportunity.

Impacts on animals, technicians and scientists

Animals

- Welfare checks in dim light may result in indicators of ill health being missed.
- In a surgery heavy room (cranial implants, craniotomies), blood may be hard to spot.

Technicians

- Difficult to work in darkness. Work must be completed early, which can impact the distribution of work across the facility.
- Extended periods working in red light may result in eye strain.

Scientists

- Spending a significant amount of time in dim lighting can affect the human circadian rhythm and cause problems sleeping.
- Transferring animals to reverse lighting required organisation and more planning.

"Working under red light affected my sleep cycle. It shifted 3 to 4 hours later, and I couldn't sleep until 03:00 to 04:00 which left me feeling exhausted."
MG, a scientist who works under reverse lighting conditions for several hours daily.

Challenges and opportunities for refinement

Challenges

Challenges

- Too many changes in photoperiod may lead to mice becoming stressed
- Lux as a unit of measurement is based on human experiences of light intensity, so is not very relevant
- Wavelengths measurements not in Code of Practice
- Spectrophotometers are very expensive and impractical
- Limiting exposure to Light at Night (LAN)
- The effect of lighting on experimental results is not fully understood
- Information may be hard to read
- Risk of eye strain
- Maintaining acclimatisation periods can delay experiments
- Gaps in awareness and legislation
- Lack of awareness on the effect of LAN and inadequate acclimatisation periods

Refinement

Refinement

- Opaque bags used for transport of cages to better block out light
- Reduced brightness of red lighting and red-light torches
- Dusking and dawning function allows for gentle transitions between light and dark, reducing stress in the animals
- Increased acclimatisation periods up to 2 weeks
- Increased use of reverse lighting in research leading to a better understanding of light on results
- Limiting working time under red light
- Eliminating light pollution at night e.g. turning off switches, printers and covering lights with card
- Better reporting on the spectral distribution in labs
- Increased wider recognition of the impact of LAN
- Wider recognition of the impact of wavelength range on reproducibility

Summary

Red light is the most effective approach to studying rodent innate nocturnal behaviour, given their inherent insensitivity to long wavelength light. However it is important to carefully regulate the dimness of the lighting to ensure the mice cannot perceive the red lights.

Prior to commencing any procedural work an acclimatisation period for the mice becomes necessary due to the alteration in photo periods.

Additionally, disruptions in darker conditions such as LAN pose significant welfare concerns, potentially modifying rodent natural nocturnal behaviours.

It is crucial to acknowledge the potential impacts on employees as prolonged exposure to red light may interrupt the human circadian rhythm and give rise to various health issues.

Wider industry awareness on the significance of LAN exposure, with a view to prioritising both employee wellbeing and Animal Welfare.

Better reporting on the spectral distribution in labs could improve scientific outcomes and reproducibility.

Acknowledgements

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