The use of sodium lamps to brightly illuminate mouse houses during their dark phases

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Summary
The human and murine diurnal rhythms are out of phase. Consequently in conventionally-lit mouse houses the mice's deep sleep is often disrupted, the daily welfare monitoring of the mice is limited by their inactivity, and scientific data obtained from the mice model the sleeping rather than awake human. Sodium light is bichromatic, with both wavelengths being in the human visual field but at the margin of murine vision. We report here that sodium lamps can be used to light mouse houses to a level that is comfortable for humans, but still sufficiently dull to permit nocturnal behaviour in mice. The response of mice to sodium light was initially monitored by recording the locomotor activity of BALB/c mice. The movement of mice in their cages greatly increased at the start of the nocturnal phase. Alterations in the white light cycle caused an acute change in the onset of nocturnal behaviour. In contrast, sodium light did not suppress the onset of nocturnal locomotor behaviour, even though the lighting was sufficiently bright for humans to read without light adaptation. The sodium lighting was then used to observe the nocturnal behaviour of over 150 mice of various strains, for over 1.5 years. Mice were invariably awake and alert during the nocturnal/sodium light phase. All exhibited high locomotory activity, except for nursing mothers. Some tasks, such as cage cleaning and minor surgery, were more easily done under white than sodium lighting. We therefore adjusted the timing of the light cycles to provide white light in the morning and sodium light [nocturnal phase] in the afternoon. This provided for easy operation of the mouse house, while yielding both animal welfare and scientific advantages.

Keywords  Nocturnal; circadian; behaviour; welfare monitoring

The use of mice in biomedical research is increasing due to the mouse genome project and the increasing ease with which genetic modification of mice can be undertaken. Genetic modification carries the risk of adversely affecting the welfare of mice, increasing the importance of daily monitoring. However, the nocturnal behaviour of mice makes accurate monitoring difficult during the day, as the mice tend to be asleep in their nests. For this reason, a Working Party of the Royal Society for the Prevention of Cruelty to Animals recommended that mice should be monitored during the dark, using red light to observe them [Jennings et al. 1998].

There are also scientific advantages to experimenting on mice during the dark phase. Many metabolic, endocrine, immunological and behavioural parameters have profound circadian rhythms [Kolaczkowska et al. 2001, Buijs et al. 2003, Fu & Lee 2003].
Although knowledge of the entire pattern is important, most biomedical studies aim to model the daytime characteristics of humans. Whenever this is a tacit aim of a study, the nocturnal mouse should be the preferred model, as its biological rhythms will more closely correspond to the daytime human than will that of a mouse in its daytime sleeping phase.

The use of red light to detect mice makes use of the differences between human and rodent eyes. The photoreceptors of mammalian retina can be divided into rods and cones. The rods are responsible for low light vision, whereas the cones are important in colour vision and for acuity (Barr & Kiernan 1988). Circadian rhythms are dominated by cones, but normal rhythms require both rods and cones (Lupi et al. 1999). Murine eyes are sensitive to green, blue and near ultraviolet light but have very limited ability to detect light in the red portions of the spectra (Fig 1) [Sun et al. 1997, Lyubarsky et al. 1999]. In contrast, humans have greater red vision than most other mammals. This is because we, and some other primates, have a third cone in our retina, with two of our cones being sensitive to red [Fig 1] (Dartnall et al. 1983).

The traditional light source for red-light observation of mice has been incandescent darkroom red light bulbs. However, the light from red light bulbs only minimally activates human cones and rods [Fig 1], making it difficult to see and precluding tasks that require visual acuity. Nocturnal monitoring of mice is unlikely to become routine until a more adequate nocturnal lighting is achieved. Alternative incandescent bulbs, such as darkroom yellow bulbs, provide a better environment for human sight but are unsuitable for animal houses as their spectrum is too broad, resulting in stimulation of murine eyes. A better option may be to use light sources with narrow spectra.

The light emitted from sodium lamps is dominated by the bright sodium D-lines at 589 and 589.6 nm [variably described as orange or yellow]. These wavelengths provide near maximal activation of two of the human cones. Consequently, humans perceive sodium light to be brighter than it actually is and have good visual acuity in sodium light. Mice, on the other hand, have very limited capacity to see 589 nm light as it falls on the very margins of their visual sensitivity [Fig 1]. We report here that sodium lighting can be used to create a mouse house that facilitates both experimental objectives and the welfare monitoring of mice.

Material and methods

Animals

This study reports observations made in an animal room that was being used to breed mice for an experimental programme.
involving the post-mortem analysis of animal tissues. Consequently, the number and age of each strain of mice in the room varied during the study period.

At any one time, the room held a minimum of 150 mice of various strains, of which at least 50 were wild-type mice. The mice were bred in the facility except for the C57BL/6 mice, which were obtained from the central facility of the University of Otago. For each colony, the approximate number of cages allocated to the colony, the total number of mice held during the study period, the duration the colony was held, and the origin of the mice used to establish the colonies are indicated in parenthesis. The main colonies were: BALB/c (2–6 cages; 89 mice, 10 months; Australian National University); heterozygous transforming growth factor-beta2 null mutant (TGF-ß2+/−) mice (Sanford et al. 1997) with a mixed C57BL/6 × 129 background and maintained by breeding TGF-ß2+/− studs with congenic wild-type dams (4–12 cages; 250 mice, 16 months; Jackson Laboratories, Stock #003102); mutant human G93A superoxide dysmutase 1 (SOD1) transgenic mice (Gurney et al. 1994) with a B6SJL background and maintained by breeding SOD1 studs with congenic dams (2–6 cages; 110 mice, 15 months; Jackson Laboratories, Stock #002726). The colonies of TGF-ß2+/− and SOD1 mice contained approximately equal numbers of wild-type and genetically-modified (GM) mice. The facility also housed 24 lines of founder transgenic mice with either BALB/c (Australian National University), or FVB [AgResearch, Hamilton, New Zealand] backgrounds that were being tested and bred with C57BL/6 mice to change their genetic background (10–25 cages; 1200 mice). These transgenic mice were designed to express non-functional proteins in a neuron- or muscle fibre-specific manner and were phenotypically normal. The ages of the mice varied from neonate to one year old, with most mice being young (3–16 weeks old). Each colony contained similar numbers of males and females.

The unit was operated as a specified pathogen free (SPF) unit. Some of the new transgenic mouse lines were made in a facility with isolated instances of mouse hepatitis virus infections and were held in quarantine during the study period. No pathogens were detected during the study period.

The University of Otago Animal Ethics Committee had statutory oversight of the operation of the animal facility and approved the trial of the sodium lighting.

Feeding

Mice had ad libitum access to water and Diet 86 pellets (Sharpes Grain and Seeds Ltd, New Zealand) that had been sterilized by gamma radiation.

Husbandry during the experiment

The room was newly constructed and located on the Dunedin campus of the University of Otago. The initial 5 months of operation of the room were used as a trial period, to optimize the lighting (see below). During this period, the room was only used to breed mice. The environment in the room was then standardized and the facility was operated for over 1.5 years.

The mice were housed in a vented M.I.C.E. caging system [Animal Care Systems, Inc, Littleton, CO, USA. www.animalcaresystems.com]. The system contained 42 cages in a single rack, with the back of the rack adjacent to a wall. The cages were made of clear polycarbonate and were fully transparent. The inner dimensions of the cages were 34.3 cm long, 17.8 cm wide, 16.5 cm high at the front and 21.6 cm high at the rear. The bedding was wood shavings that had been kiln-dried and autoclaved at the University of Otago.

The cages had a V-shaped stainless-steel feeding bin in their centre, which served as a vertical climbing area for the mice. Nylon-enrichment tunnels were installed beneath the feeding bin. The tunnels were opaque, 16.5 cm long, 8 cm wide and 3 cm high, with fifteen 29 mm² ventilation/observation holes equally spaced along each side of the tunnel. The single-sex stock cages contained between one and six mice, with more than 80% of the cages having four or five mice.
Breeding cages typically contained a stud and two dams.

The air entering and leaving the cages was filtered, with the exhaust air from the cages being ducted to the outside of the building. This maintained sterility and eliminated pheromonal influences between cages, thus diminishing fighting and enabling stock mice and various colonies to be housed in a single room. The room temperature was 21 ± 2°C. Excessive humidity was prevented using a dehumidifier (Mitsubishi MJ-E2OPX) set to operate at 45% humidity.

**Lighting**

The room was 2.4 × 3.4 m, with a height of 3.0 m. White light was produced by incandescent bulbs in the ceiling and was under the control of a dimmer switch and a timer. The intensity of the light was adjusted to the minimum level that was comfortable for staff. The light intensity was measured with a standard light meter (Hagner Digital Lux Meter, Model EC1, accuracy better than 1%) and was 200 lux at 2 m high and 67 lux at floor level. The day length was experimentally varied between 12/12 and 14/10 and then fixed at 14/10, with the night time beginning at 13:00 h.

The sodium light source was an 18 W low-pressure lamp (SOX18E, Philips Lighting Co.), which has an average lumen output of 1650 to 1800. The lamp was mounted on the ceiling, one metre in front of the cages. The brightness of the light in the room was determined using a Laser Checker (Coherent, Auburn, CA, accuracy better than 5%) set to measure light intensity at 589 nm. The brightness at 2 m was 80 lux, attenuating to 43 lux at floor level. The intensity of the light within the cages varied from the top (9.6 lux) to the bottom rack (1.2 lux).

The timing of the sodium light was initially experimentally varied and then fixed to warm up during the last 30 min of the white light cycle. It remained on for the first 4 h of the dark cycle.

**Locomotor activity monitoring**

Graphic illustration of the locomotory activity of mice was obtained using an activity monitor designed by Prof. I. A. Hendry (Leck 2003). Briefly, two red light emitting diodes were attached to the side of a standard M.I.C.E. cage with corresponding red light detectors on the other side of the cage. When a mouse walked through the red light beam a pulse was sent to a computer, which recorded the movement and the time of day. One of the emitter/detector pairs was located just in front of the feeding trough and recorded each time the mouse walked to the front of the cage. The other detector was just behind the feeding trough and recorded movement towards the rear of the cage (Fig 2). The red light emitted was not visible to humans and would thus have been invisible to the mice.

![Diagram of the cages used to detect locomotory activity](https://via.placeholder.com/150)

**Fig 2** Design of the cages used to detect locomotory activity. (A) View from above. The locations of the red-light emitters and detectors are indicated by black squares and circles, respectively. The light path is indicated by dotted lines. The location of the water tube is indicated by a line and a ‘w’. (B) View of the left-handing side, indicating the position of the red-light emitters.
Four of these activity cages were located on a shelf on the wall opposite the M.I.C.E. rack, at a height of 0.4 m. These cages were operated as standard non-vented mouse cages. Each cage housed one 10-week-old male BALB/c mouse. The mice were given one week to adapt to being singly housed after which their locomotory activity was recorded for 4 months.

**Statistical analysis of activity monitoring**

The hourly activity values for each mouse were averaged from observations made on 4 consecutive days and are illustrated in Figs 3 and 4. The difference in the time the mice were active under the different light regimes was compared using a repeated measure two-way ANOVA with both time and light as within-subject factors. The activity in the 4 h before and the 4 h after the white light was turned off were also calculated for each mouse. The difference between these two 4 h periods was analysed using two-way Student's $t$-test.

**Welfare monitoring and qualitative observations**

The physical characteristics of the room, and the standard operating procedures were designed to optimize the care of mice at risk of developing neurological disorders. However, in the current study period the only at-risk colony was the SOD1 mice, which develop age-related motor neurone disease (Gurney et al. 1994). The SOD1 colony was exclusively used to obtain tissues from young mice that were pre-symptomatic. No SOD1 mice developed symptoms.

Each mouse was observed at least once per day to determine its behaviour and its well-being. The welfare aspect of this monitoring involved checking for abnormal movement or other signs of ill health, and was routinely done at a random time during the sodium light/night phase. The welfare monitoring could be done with the cages in situ, except for nursing mothers. Sliding the cage forward caused the dam to stand up, enabling both her and her pups to be inspected. Mice were also closely examined during routine cage changing. The behaviour of mice during the light phase was observed whenever the room was used, which was typically 2–3 times per week.

The enrichment tunnels were removed from the SOD1 colony as they limited observation of mice with low motor activity. We were also concerned that mice with precious neurological symptoms could become trapped in the tunnels.

![Fig 3](image-url) **Locomotor activity of mice with a 12/12 h light–dark cycle beginning at either 07:00 h (A) or 10:00 h (B).** The locomotor activity of the mice was totalled for each hour and expressed as the percentage of activity for the day. The data in the figures are the mean ± the standard error of the mean of the four mice in the activity cages. The time of light onset had a significant effect on when mice were active (ANOVA, $P < 0.01$). Note that in both figures, the mice were significantly more active in the 4 h immediately after the lights went out, than at the end of the dark phase or the start of the light period (Student's $t$-test, $P < 0.01$)
Results

Traditional light cycles

When a standard dark–white light regime was used, the mice slept during the day, with only occasional short periods of activity. The daylight episodes of movement were more common as the dark period approached (Fig 3). During the daytime, mice often huddled together, making observation of their individual well-being difficult. Young newly weaned mice tended to cluster together in the enrichment tunnels, completely preventing observation of them unless the cage was opened.

Shortly after the lights were turned off, the behaviour of the mice switched to their nocturnal state of continuous activity interspersed with short periods of rest. This active period typically lasted 5–6 h (Fig 3). It was followed by a period of sleep during which the mice rarely moved around the cage. This sleep period began before the lights were turned on and typically lasted for at least 6 h (Fig 3).

The total number of times a mouse passed a detector (total activity) varied from mouse to mouse, and from day to day for a given mouse. However, the time of day that the mice were active varied little, as evidenced by the small standard errors in Figs 3 and 4.

The length and/or the time of onset of the light cycle were changed six times. As expected, the timing of the onset of the nocturnal locomotory behaviour changed within 24 h to correspond to the new light regime. The circadian rhythms in Figs 3a and 3b are not significantly different, other than that the cycle is displaced by 3 h in Fig 3b relative to Fig 3a.

Sodium lamps

In marked contrast to the effect of white light, having the sodium lamp lit for the first 4 h of darkness did not prevent the mice in the locomotory cages initiating their nocturnal behaviour (Fig 4). The activity of the mice in the first 4 h of the dark phase was not significantly different (Student's t-test) for any of the lighting conditions, including when the sodium light was on. For instance, in the illustrated examples, 45.5 ± 3.2% (sodium light on, Fig 4), 43.8 ± 0.1% (sodium light off, Fig 3a) and 41.1 ± 2.7% (sodium light off, Fig 3b) of the mice's daily activity occurred in the first 4 h after the white lights were turned off.

The sodium light permitted the nocturnal behaviour of the mice to be observed, which was done for over 1.5 years, with a 42-cage system that typically held in excess of 150 mice. The difference between the mice in their diurnal (white light) and nocturnal (sodium light/dark) states was overt. During their nocturnal state, all mice were awake and were noticeably more alert and more active. They frequently climbed the spout of their water bottle and the food trough. This facilitated the accurate assessment of their well-being. Mice that were not moving during the inspection period responded to minor movement of their cages, which during the diurnal phase would often have been insufficient to elicit a response.

The routine observation of numerous strains of mice indicated that the mice in the activity cages were representative of all the mice in the room. The onset of nocturnal behaviour at the beginning of darkness was an invariant characteristic that occurred every day in all mice, irrespective of their
age, sex and strain, and whether they were housed individually, as single-sex groups or as breeding pairs. As with the quantitative studies, the absolute activities of individual mice varied greatly. In particular, older mice were noticeably less active than younger mice.

The various mouse colonies have bred well during the period that the sodium light has been operating. For instance, C57BL/6 × 129 dams crossed with congenic TGF-β2+/- studs have raised an average of 7.8 ± 0.5 pups to weaning per litter (34 litters). Nursing mothers are relatively inactive during the nocturnal phase, although they are clearly awake and alert.

The intensity of the sodium light was sufficient for researchers to clearly observe the mice and to read cage labels and laboratory notebooks without either additional light sources or a period of light adaptation. However, some of the limitations that were present during white light observation of the mice remained. The presence and well-being of pups could not be determined without moving the cage. This was because nests were commonly in a rear corner of a cage, making them difficult to observe, and because the pups could only be observed if the mother was persuaded to move off the nest.

The enrichment tunnels occasionally limited observation of some mice, although this was less of an issue than during white light observation, as the mice were not asleep in the tunnels and as an observer could detect movement of the mice in the tunnels. In the absence of enrichment tunnels, the sodium light regime was suitable for observing mice at risk of developing motor neurone disease.

Wetness within cages created by leakage of water bottles was more difficult to detect under the sodium light than under white light, as was the cleanliness of spare cages and the floor of the animal house. Enclosed spaces, such as the inside of a class II biohazard hood, had impractical levels of lighting. The level of lighting was, for instance, sufficient for behavioural testing of the mice but was insufficient for collecting ear biopsies for genotyping of the mice.

Discussion

The diurnal and nocturnal characteristics of animals are instinctive behaviours. When nocturnal and diurnal behaviour occurs, light cues but does not create these behaviours. Nocturnal behaviour is triggered by melatonin (Sharma & Chidamabaram 2003) and is acutely terminated by light (this study and previous studies, e.g. Edelstein & Mrosovsky 2001). Even very low levels of white light are sufficient to suppress nocturnal melatonin (Brainard et al. 1982). Consequently, the observations that all mice in the sodium light/dark phase of our study were awake and exhibiting nocturnal locomotory behaviour indicates that the sodium lamps used in this study did not suppress nocturnal behaviour in the way that very weak white light does. This is initial proof that sodium light that is bright to the human eye can be used to observe mice without affecting the main characteristics of the mice’s circadian cycle.

Circadian rhythms are only altered if the intensity of night-time light exceeds a threshold (Brainard et al. 1982, Meijer et al. 1986). The light thresholds to extinguish the various aspects of nocturnal physiology may vary (Hashimoto et al. 1996, Edelstein & Mrosovsky 2001). Consequently, further proof is needed to exclude the possibility that the sodium lights subtly alter some nocturnal characteristics of mice. This notwithstanding, the fact that the awakeness, alertness, locomotor activity and reproductive capacity of mice is insensitive to sodium light makes sodium lamps suitable for most studies, and for the routine care, of mice.

It is well documented that the absolute locomotor activity levels of mice are sensitive to multiple variables, including housing, ambient temperature, sex, strain, light intensity, etc. (Wax & Goodrick 1975, Kopp et al. 2000). The variability in the diurnal and nocturnal activities of mice observed in this study is thus not surprising. The complexity of absolute locomotor activity makes it an unsuitable parameter in which to detect subtle effects of lighting on behaviour. In contrast, the abrupt change
in locomotor activity that occurs at the beginning of the nocturnal phase is a highly reproducible behaviour that is light-sensitive and independent of the multiple other parameters that influence absolute activity levels. Consequently, the measure of whether, or not, mice undergo a change in behaviour at the start of night is a particularly sensitive measure of whether the nocturnal light levels are appropriate. It is for this reason that our study is based on the observation of nocturnal behaviour rather than on absolute locomotor activity levels.

The timing of the light cycle was initially varied in the room in order to establish an optimal working environment. For our purposes, it was most convenient to operate the animal house under white light conditions in the morning with sodium light/dark conditions operating from 13:00 to 17:00 h. This enabled the benefits of both white and sodium light to be harnessed. The relative inactivity of the mice in their diurnal phase facilitated cage changing and tasks such as routine biopsies for genotyping. These activities were done inside a biohazard hood located within the room. The hood’s supplementary lighting was useful for various tasks but transmitted too much light to the animal cages to be used during the nocturnal phase.

The sodium light/dark phase was ideal for the daily observation of the mice. Individual mice could be clearly viewed through the cage walls, as they were active rather than huddled together with their sleeping cage-mates, as often occurred during the light phase. The alertness of mice in their nocturnal state is also a clue to their well-being and should aid the early detection of health problems. The decreased need to open cages to inspect mice should assist in preventing infectious disease and should limit stress on the mice. Despite the usefulness of sodium light observation, we still consider it essential that every mouse is given a careful examination during cage changes.

Mice sleep deeply at the beginning of the light phase, which in conventionally-lit mouse houses often corresponds to the period when their cages are changed or when they are experimented on. Consequently, the deep sleep of mice is often disturbed in animal houses. The use of sodium lights in the afternoon enabled the white light cycle to be set from 24:00 h to 13:00 h. This in turn moved the period of deep sleep of the mice to the early hours of the morning, when humans were not usually present in the mouse house. Judging from human experience, the lack of disturbance of the mice’s deep sleep is possibly a non-trivial improvement to the quality of the mice’s lives.

As a consequence of sleeping deeply during night time, the mice were in their late diurnal phase during the beginning of the human working day (08:00 h to 12:00 h). While in this phase, the mice were only lightly asleep, resting or in a low activity state. Observation of the mice at this phase of the diurnal rhythm is more difficult than in a sodium light/dark phase, due to the lower locomotory activity/alertness of the mice. Nevertheless, observation of mice during the late diurnal phase is clearly easier and superior to observation when they were deeply asleep. This provides flexibility to the management of the animal house, as it enables the daily monitoring of the mice to be done at a time that least interferes with experimental programmes.

The current lighting regime provides an investigator with the opportunity to study both the late-diurnal and nocturnal states of mice, simply by varying whether the experiments are done in the morning or afternoon. Nocturnal mice have rarely been studied in the past due to the difficulty of using photographic red lights. However, the few studies that have used nocturnal mice have revealed phenomena that were not apparent with diurnal mice (Jansen van’t Land & Hendriksen 1995, Kriegsfeld et al. 1999), pointing to the potential value of the nocturnal mice for scientific study.

In conclusion, this study demonstrates that sodium lights can be used in mouse houses, with benefits for both animal welfare and science.

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