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Behaviour of laboratory mice is altered by light pollution within the housing environment

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Abstract

*Environmental light-dark cycles play an important role in behavioural and physiological processes. It is essential that laboratory vivaria be designed to properly control the light conditions in which laboratory mice are housed; however, this is not universally the case. Some laboratory vivarium doors are designed with windows, which allow light from the hallways to leak into the housing space during the rodents' dark phase. Personnel entering and exiting the housing space during the dark phase can also create excessive light leak from brightly illuminated hallways. In this study, we investigated the hypothesis that exposure to dim light at night, as commonly experienced in many laboratory rodent housing spaces, alters mouse (*Mus musculus*) behaviour. We specifically analysed patterns of locomotor activity, anxiety- and depressive-like responses. Exposure to dim (5 lux) light at night altered home-cage locomotor activity and increased anxiety and some depressive responses among laboratory mice. These results suggest that light conditions can alter mouse behaviour and potentially influence experimental outcomes. Increased care should be taken to properly control light-dark conditions for laboratory animals.*

Keywords: *animal welfare, anxiety, behaviour, circadian, depression, mouse*

Introduction

Environmental factors can influence experimental results and well-being of laboratory animals. The artificial light-dark cycle is one variable that should be strictly controlled in laboratory experiments. In mammals, light is detected by photoreceptors located in the retina. One population of these cells, called intrinsically photosensitive retinal ganglion cells, contains a photopigment called melanopsin that is most sensitive to blue wavelength light (Panda *et al* 2005; Schmidt *et al* 2011). When activated in the presence of light, melanopsin-expressing cells project directly to the suprachiasmatic nucleus (SCN) of the hypothalamus, the master circadian clock in the brain (Hattar *et al* 2002). In this way, the SCN is entrained to the daily light-dark cycle; disruption of its oscillation by unnatural light exposure can perturb downstream physiological processes. For example, the SCN regulates hormonal output (eg melatonin and cortisol), patterns of gene expression, and behaviour (Mohawk *et al* 2012). Properly controlled environmental light-dark cycles are critical to maintaining appropriate circadian behaviour and physiology. Even relatively small changes in illumination or spectral quality can profoundly disrupt circadian responses (Brainard *et al* 1983; Bedrosian *et al* 2013).

Laboratory animals should be maintained under carefully controlled lighting conditions; however, pollution of the housing environment by light at night (LAN) is a common problem. Laboratory vivarium doors are sometimes designed with windows that allow light to leak into the housing space from the hallways. Personnel entering and exiting housing rooms during the dark phase may also cause LAN pollution. These occurrences may affect experimental outcome by altering animals' physiology and behaviour. The profound effects of LAN on physiology are well established. LAN suppresses secretion of melatonin (Brainard *et al* 2001), provokes metabolic dysfunction (Fonken *et al* 2010), alters clock gene expression (Bedrosian *et al* 2013), and promotes carcinogenesis (Brainard *et al* 2001). Thus, contamination of laboratory animal housing spaces with LAN may interfere with experiments across a wide variety of biological sciences.

In this study, we investigated the effects of exposure to dim LAN in the housing environment on laboratory mouse behavioural responses. We used male C57bl/6 mice, which are commonly used in biological studies across many fields. Levels of LAN were maintained at approximately 5 lux throughout the night. We hypothesised specifically that exposure to LAN alters locomotor activity and increases rodent anxiety and depressive responses. Our results suggest that care should be taken to properly control light-dark cycles for laboratory rodents to prevent unintended effects on behavioural responses.

Materials and methods

Twenty, adult male C57bl/6 mice (*Mus musculus*) (Jackson Labs, Bar Harbor, MA, USA) were maintained upon arrival in our vivarium at The Ohio State University. Mice were individually housed in polypropylene cages (30 × 15 × 14 cm; length × width × height) and allowed one week to acclimate to a 14:10 h light-dark cycle before beginning the experiment. Throughout the study, food (Harlan Teklad 8640, Indianapolis, IN, USA) and water were available *ad libitum*. Housing quarters were maintained at 22 (± 2)°C and ~50% relative humidity. Mice were randomly assigned (n = 10 per group) to experimental light conditions, which consisted of either a 14:10 h light-dark cycle (150/0 lux) or a 14:10 h light-dim light cycle (150/5 lux). Light intensity was measured in the cage using a Traceable Dual-Display light meter (Fisher Scientific, Hampton, NH, USA). Broad-spectrum fluorescent lights in 'cool white' were used to illuminate the housing space. The dim night-light (10059-F8T5/CW) originated from GE Lighting (East Cleveland, OH, USA). Light conditions were carefully controlled throughout the study and all husbandry-activities were restricted to the light phase. The Ohio State University Institutional Animal Care and Use Committee approved all experimental procedures, and animals were maintained in accordance with the recommendations of the *NIH Guide for the Care and Use of Laboratory Animals*.

Home-cage locomotor activity was measured throughout the experiment using an infra-red beam break system (Columbus Instruments, Columbus, OH, USA) and data were analysed using Clocklab (Actimetrics, Wilmette, IL, USA). After four weeks in lighting conditions, mice were tested for anxiety and depressive responses. Anxiety-like responses in an open field were assessed by placing each mouse individually into a 40 × 40 cm (length × width) clear acrylic chamber lined with corncob bedding, inside a ventilated chamber equipped with infra-red beams (Med Associates, St Albans, VT, USA). Movement in the open field was tracked by beam breaks over 6 min and analysed by minute for percent beam breaks in the centre of the open field. Behavioural despair was assessed using the tail-suspension test. Mice were suspended by the tip of the tail using laboratory tape and behaviour was videotaped for 6 min, and then scored for time spent immobile and latency to become immobile. Sucrose preference was assessed by offering mice a choice of drinking tap water or a 1% sucrose solution. Intake was recorded after 24 h by weighing each bottle and analysed as percent preference for sucrose over water.

Open field data were analysed by one-way ANOVA and significant main effects were followed up with Fisher's *post hoc* comparisons. Activity and sucrose preference data were analysed by one-tailed Student's *t*-tests with light condition as the independent variable. Statistics were performed using Statview 5.0.1 for Windows. Mean differences were considered statistically significant when $P < 0.05$.

Results

Total home-cage locomotor activity was equivalent between mice exposed to dark or dim LAN ($P > 0.05$; Figure 1[a]). The activity rhythm remained entrained, but fast Fourier transformation revealed decrements to the strength of the 24-h rhythm after exposure to LAN (Figure 1[b]-[d]).

Exposure to LAN reduced the total percentage of beam breaks in the centre of the open field, as there was a main effect of lighting condition ($F_{1,6} = 9.907$, $P < 0.05$). Analysing the data in 1-min intervals through *post hoc* analysis revealed a reduced percent of centre beam breaks specifically during the first ($P < 0.05$), third ($P < 0.05$), and fourth minute ($P < 0.05$) (Figure 2[a]). Further, LAN exposure reduced preference for a solution of 1% sucrose versus water ($t_{15} = 2.400$, $P < 0.05$) (Figure 2[b]). There were no significant differences in total immobility time or latency to become immobile in the tail suspension test ($P > 0.05$).

Discussion

Light intensities in laboratory vivaria are not always stringently controlled and the design of these spaces is not necessarily conducive to minimising light contamination. Our results indicate that laboratory mice are sensitive, in terms of behaviour, to low-level light at night. For studies investigating circadian responses or affective behaviour, it is important to minimise light pollution within the housing environment. There may be additional physiological effects of LAN that could undermine experimental results across a variety of fields. Even the spectrum of light exposure can significantly influence physiological values. In a recent study, daily plasma levels of melatonin, fatty acids, glucose, leptin, insulin, corticosterone, and other parameters were altered simply depending on whether nude rats were housed in a clear rodent cage or one that was tinted amber or blue (Dauchy *et al* 2013). This finding underscores the importance of controlling lighting environments in biological research.

In our experiment, mice exposed to 5 lux of light showed altered activity and affective responses. Previous research has demonstrated that chronic exposure to 5 lux of light pollution affects hormone secretion and gene expression (Bedrosian *et al* 2013), which could be two mechanisms contributing to changes in behaviour. Altered behaviour may significantly affect experimental results, particularly in those studies investigating mood using animal models, as the open field and sucrose preference tests are typically thought to reflect anxiety- and depressive-like responses. It should be noted, however, that the similarities between these tests and the corresponding clinical conditions in humans are still debatable (Cryan & Slattery 2007). Exposure to low levels of light also influences body mass and metabolic function (Fonken *et al* 2010), immune function (Bedrosian *et al* 2011), tumour growth (Dauchy *et al* 2011), and endocrine physiology (Dauchy *et al* 2010). Furthermore, rodents are sensitive to light intensity as low

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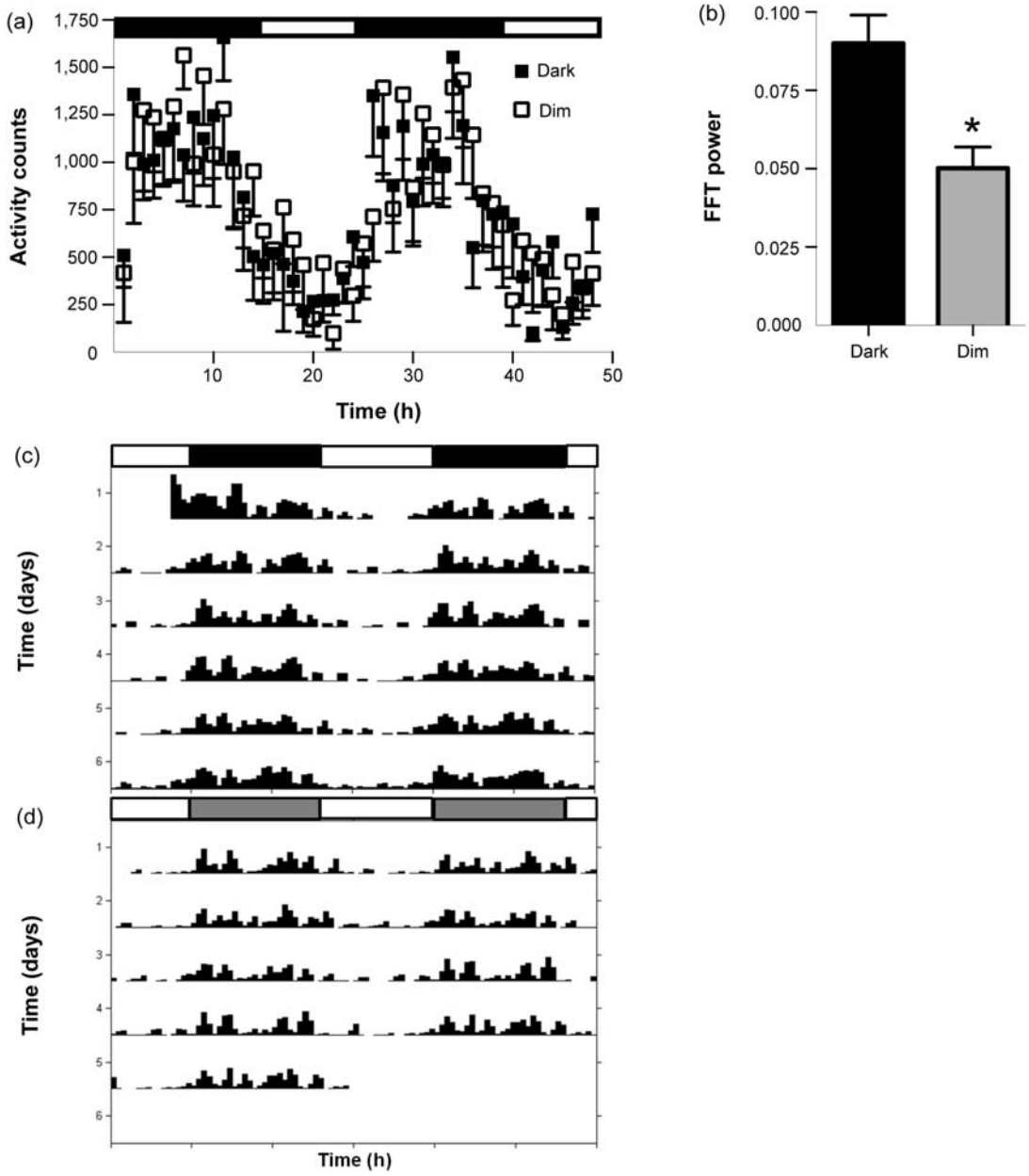
Figure 1

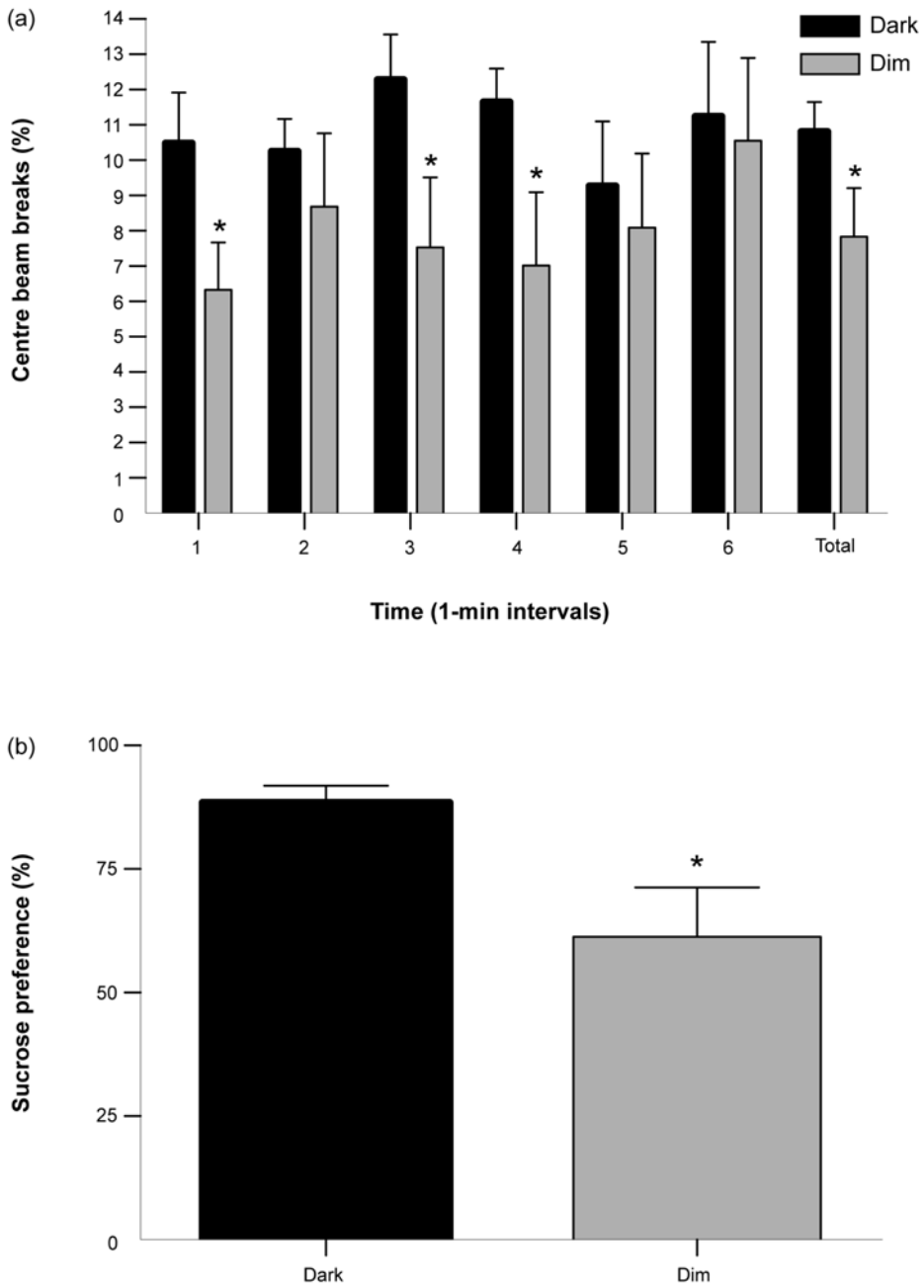


Home-cage locomotor activity showing (a) mean (± SEM) activity counts over 48 h, (b) exposure to dim night-time light reduced fast Fourier transform power of the activity rhythm, (c) double-plotted actigraph depicting activity in dark nights and (d) in dim light at night. * $P < 0.05$.

as 0.2 lux (Minneman *et al* 1974), underscoring the importance of eliminating LAN in the laboratory environment.

There are simple steps that can be taken to minimise exposure to unnecessary LAN among laboratory rodents. First, any windows on vivarium doors should be carefully covered with light-impenetrable material. Hallways should be equipped with red lights than can be used when entering or exiting rodent housing spaces during the dark phase. Door frames must be sealed against light and a double set of doors is recommended to completely prevent light leak from the hallway. Furthermore, any equipment left in the housing space (ie ventilation hoods, power strips, monitoring equipment) should be checked carefully to ensure no light is emitted. Our experiment specifically investigated the effects of constant dim LAN and thus best mimics the conditions experienced by mice with continuous exposure from windows or door frames. The consequences of intermittent exposure due to personnel entering and exiting rooms must be investigated in future studies. Nevertheless, taking steps to

Figure 2



Anxiety and depressive responses showing (a) mean (± SEM) centre beam breaks in an open field test over 6 min (data broken into 1-min intervals and exposure to light at night reduced central tendency) and (b) mean (± SEM) preference for sucrose solution. Preference was reduced after exposure to night-time light. * $P < 0.05$.

minimise light leak will prevent unintended consequences of night-time light pollution on experimental results.

Animal welfare implications

Although manipulation of experimental rodents during the dark phase is sometimes necessary, an effort should be made to prevent exposure to light during these activities. The data presented in this study demonstrate that even dim light contamination of the housing quarters can depress and provoke anxiety within colonies. Consideration must be given to the potential effects on experimental outcome when night-time light contaminates the laboratory vivarium.

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