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Limited evidence for affective and diurnal rhythm responses to dim light-at-night in male and female C57Bl/6 mice

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ABSTRACT

Circadian rhythms are recurring patterns in a range of behavioural, physiological and molecular parameters that display periods of near 24 h, and are underpinned by an endogenous biological timekeeping system. Circadian clocks are increasingly recognised as being key for health. Environmental light is the key stimulus that synchronises the internal circadian system with the external time cues. There are emergent health concerns regarding increasing worldwide prevalence of electric lighting, especially man-made light-at-night, and light's impact on the circadian system may be central to these effects. A number of previous studies have demonstrated increased depression-like behaviour in various rodent experimental models exposed to dim light-at-night. In this study we set out to study the impact of dim light-at-night on circadian and affective behaviours in C57Bl/6 mice. We set out specifically to examine the impact of sex on light at night's effects, as well as the impact of housing conditions. We report minimal impact of light-at-night on circadian and affective behaviours, as measured by the tail suspension test, the forced swim test, the sucrose preference test and the elevated plus maze. Light-at-night was also not associated with an increase in body weight, but was associated with a decrease in the cell proliferation marker Ki-67 in the dentate gyrus. In summary, we conclude that experimental contextual factors, such as model species or strain, may be considerable importance in the investigation of the impact of light at night on mood-related parameters.

1. Introduction

Presently, 99% of individuals living in both the United States and Europe are exposed to light pollution, with a further 80% of the world's remaining population exposed [1]. Notwithstanding the manifold benefits of electric lighting, there are emerging concerns that widespread man-made night-time illumination may result in deleterious impacts on human health [2]. The mechanistic basis for such concerns centre on the impact of light on the circadian system, the endogenous 24-h timekeeping system that imposes a temporal architecture on myriad physiological and behavioural processes [3]. Under the natural solar cycle, the endogenous circadian pacemaker is entrained to the photoperiod via a system involving intrinsically-photosensitive retinal ganglion cells expressing the photopigment melanopsin and projecting to the hypothalamic suprachiasmatic nucleus (SCN), the site of the master circadian clock [4]. Aberrant patterns of light exposure can lead to desynchronisation of the circadian system, which may in turn manifest as deleterious health effects [5].

There has been particular interest recently in the putative impact of light-at-night and other aberrant light exposure patterns on affect and mood regulation, with proposed mechanisms including circadian disruption [6] and non-circadian effects [7]. The link between light-at-night and mood is particularly of interest given an established link between circadian rhythm alterations and affective disorders such as major depression and bipolar disorder [8]. A number of pre-clinical studies have indicated that exposure of nocturnal rodents to dim light (5 lx) during the dark phase (ie. the active phase) induces increases in depressive-like and anxiety-like behaviours [9–13]. These changes were found to be maximally sensitive to blue light (to which the circadian system is most sensitive; [14]), associated with decreases in synaptic spine density [10], increased hippocampal tumour necrosis factor and were reversible following re-establishment of a “dark” night [14]. Importantly, the affective findings were also associated with parallel changes in circadian function, with dim light-at-night exposure leading to decreased circadian rhythm amplitude in locomotion resulting from rhythm fragmentation [10] and decreased rhythmicity in circadian clock molecular components [15]. Dim light-at-night exposure of rats has been described as resulting in decreased rhythms of REM and non-REM sleep, as well as desynchronisation of circadian rhythms in locomotion [16]. Dim light-at-night also disrupts affective processes in

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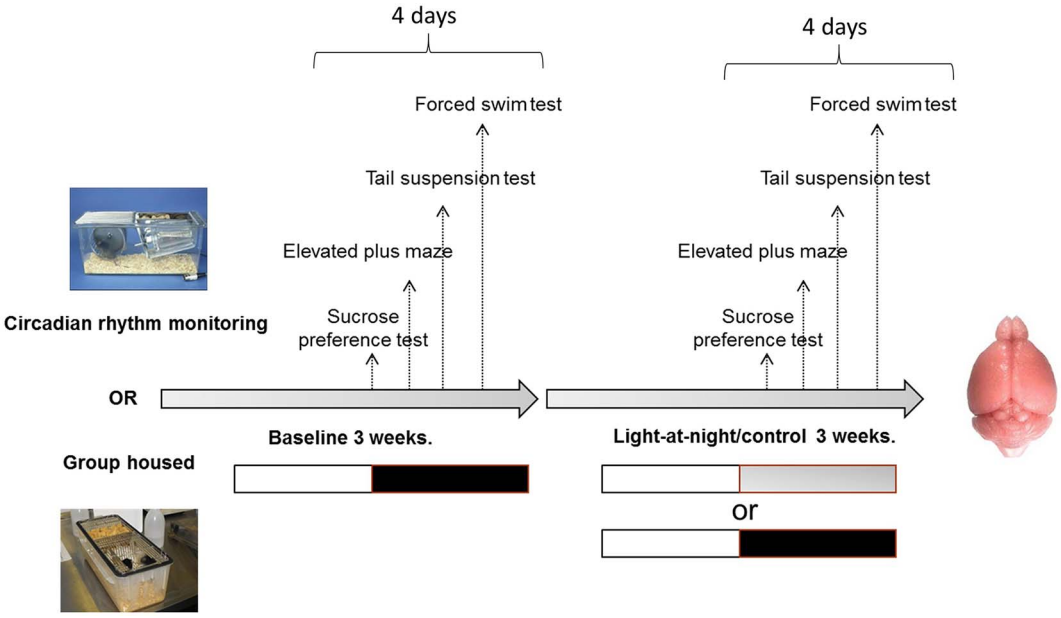

Fig. 1. Cartoon illustrating the experimental design employed in the study.

diurnal rodents [17], indicating that the effects described in rodents may be of importance for human health.

Interestingly, there has not been an explicit examination of potential sex differences in mediating the affective effects of light-at-night in adult animals. Many studies to date have examined either male or female animals separately. Further, a number of studies to date have used either the strongly photoperiodic Siberian hamster (*Phodopus Sungorus*) as a model (eg [10]), or out-bred albino mouse strains such as Swiss-Webster mice [13]. The use of albino strains of mice such as Swiss-Webster may be an important factor as the lack of retinal pigment in such mice renders the animal's retina more sensitive to incident light due to the lack of absorption by pigment [18,19]. That such a consideration may be important is indicated by a recent study in C57Bl/6 mice, which did not report dim light-at-night effects on anhedonia [20]. In order to address these issues, we set out to examine the effects of dim light-at-night on circadian behaviour, a range of affective behaviours and a marker of neurogenesis in the hippocampus in a strain of C57Bl/6 mice commonly used in circadian rhythms research. We hypothesised that: (1) Dim light-at-night would induce significant changes in diurnal rhythms of locomotor behaviour; (2) Dim light-at-night would induce more depression-like and anxiety-like behaviours; and (3) Sex would interact with dim light-at-night in producing these effects.

2. Materials and methods

2.1. Animals

A total of 87 animals of both sexes were used of this study. Animals were fed ad libitum on standard chow, and temperature and humidity were kept constant with an average temperature of 21 ± 1 °C and humidity 50 ± 10%. These animals were from a colony of C57Bl/6 mice which express PER2::luc that was maintained at Maynooth University and derived from mice obtained from the Jackson Laboratory *(B6.129S6-Per2tm1Jt/J;* [21]). This strain has been widely used in circadian neurobiology and the presence of the PER2::luc transgene is not associated with alterations in behavioural parameters compared to wild type C57Bl/6 [21–23]. We chose this strain to allow for future extension of behavioural changes into ex-vivo investigations of clock function, and the widespread use of this strain in chronobiology. All animals were between 10 and 12 weeks old at the onset of the experiments. Sample sizes were calculated with GPower for the appropriate experimental design with a power of 0.9 and an effect size of 0.35. All protocols were approved by the Research Ethics Committee at the National University of Ireland, Maynooth (BSRESC-2013-0018) and approved by the Health Products Regulatory Authority of Ireland. All animals were treated in accordance with the European Union Directive 2010/63/EU (Protection of Animals Used for Scientific Purposes) Regulations 2012 (S.I. NO. 543 of 2012). Every effort was made to minimize the amount of animals used and any suffering and discomfort was kept to a minimum as much as possible.

2.2. Experimental design

Animals were assigned to one of eight different experimental groups, based on the three factors of sex, exposure to light at night or control light:dark, and single housing or group housing. We analysed single or group housing as previous work has indicated that single housing can result in affective changes in the mouse [24–26] and single housing is required for conventional monitoring of circadian rhythms in locomotion. Therefore, housing status could interact with the presence of dim light-at-night in determining behavioural outcomes. The eight groups are 1) Female single-housed control; 2) Female single-housed LAN; 3) Male single-housed control; 4) Male single-housed LAN; 5) Female group housed control; 6) Female group housed LAN; 7) Male group-housed control; and 8) Male group-housed LAN. Single-housed animals were housed in a cage equipped with a running wheel connected to the Chronobiology Kit (Stanford Systems, USA) for monitoring of diurnal rhythms in wheel-running activity. Group housed animals were housed in groups of three with access to running wheels to allow for similar voluntary wheel-running activity, but data on wheel revolutions was not collected.

The basic experimental design is illustrated in Fig. 1. Initially all animals are housed under a 12:12 light;dark cycle in an environmental isolation cabinet for three weeks. At the end of this baseline period animals undergo testing on four behavioural tests, one test per day for four consecutive days in the following order: 1) Sucrose Preference Test; 2) Elevated Plus Maze; 3) Tail Suspension Test; 4) Forced Swim Test. Animals were then exposed to either continuation of the control light:dark cycle for a further three weeks, or exposed to a Light:LAN cycle for three weeks. The control light dark cycle consisted of 12 h white fluorescent light at an average cage-level of 150 lx, and 12 h of complete darkness. Light:LAN cycles consisted of 12 h light (~150 lx)

and 12 h of 5 lx via cool white light-emitting diodes (RS, Ireland). After this three week period, animals were re-assessed on each of the sucrose preference test, the elevated plus maze, the tail suspension test and the forced swim test. Animals were also weighed at the end of each experimental week.

2.3. Behavioural tests

Diurnal rhythms of running wheel activity were recorded as previously described [27]. Briefly, animals were singly housed in polypropylene cages equipped with running wheels (11 cm diameter) housed in an environmental isolation cabinet to allow for full control of the photic environment. Bedding was changed every 14 days. Wheel running was monitored via microswitches attached to the running wheels communicating with the Chronobiology Kit (Stanford Software Systems, CA, USA) to allow for production of actograms of wheel-running behaviour. Period length and rhythm power, were obtained from Poisson Periodograms in the Chronobiology Kit, and phase-angle of entrainment was calculated as the difference in minutes between the onset of the dark phase and the onset of activity (counted as 25 or more wheel revolutions in a 10 min bout).

The sucrose preference tests, elevated plus maze, tail suspension test and forced swim test were carried out accordingly to previously described protocols [28,29]. For the sucrose preference test as a measure of anhedonia, animals were presented simultaneously with two water bottles. One bottle contained ordinary water, the other 1% solution of sucrose. To control for possible side preferences, placement of the bottles in the cage were counterbalanced after 12 h. Bottles were weighed immediately before and after 24-h following placement to ascertain how much sucrose and water were consumed respectively. Sucrose preference was calculated as [sucrose consumed / (water consumed + sucrose consumed)]. The elevated plus maze was used to assess anxiety-like behaviours. The apparatus was plus-shaped in design and was elevated up above the floor, with a centre area of diameter 12.5 cm from which four arms extended of length 34.5 cm, width 5 cm. Two of the arms were open without walls while the other two arms were enclosed by high walls. Each mouse was placed in the centre of the test apparatus facing an open arm. Time spent in open arms, time spent in closed arms and latency to first entry to the open arms were the measures taken during the five-minute period in which the test was being conducted. In order for the test to be novel to animals when they were tested subsequently after experimental condition the layout of the test was altered. Where previously the arms were open the arms were closed and vice versa. Additionally, at the end of each test the whole area of the apparatus was sprayed with 70% ethanol solution to remove any scent of animals and wiped with dry paper towel to remove any mouse dropping and urine. The tail suspension test and the forced swim test were carried out as non-synonymous tests of behavioural despair. For the tail suspension test, rodents were suspended from a burette clamp by adhesive tape attached -1 cm from the tip of the animal's tail. Animals were suspended in the apparatus for a 6 min epoch and the duration of immobility was recorded during this period, with mice considered immobile only when they hung passively and completely motionless. For the forced swim test, animals were placed into an opaque cylinder jar which was filled with room temperature water ($22^{+} - 1$ °C) for a period of 6 min. Dependent measures to assess depressive-like responses were the latency to first immobility and total period of time spent immobile. Following the test each animal was removed from the water, patted dry and placed under a heater to fully dry off. Containers were emptied and cleaned between each test.

2.4. Perfusion and immunohistochemistry for Ki67

We carried out immunohistochemistry for the expression of Ki67 in the subgranular zone of the dentate gyrus. Ki67 is a marker of proliferating cells, and as such can be used a marker for stem cell proliferation without the need for BrdU treatment [30]. Perfusions were carried out in the middle of the light phase. Animals were terminally anaesthetised with an i.p. injection of sodium pentobarbital (Euthathal, Merial Animal Health, UK), the chest cavity opened and animals perfused transcardially with 0.9% saline, and then 4% parapformaldehyde (PFA, Sigma) in 0.1 M Phosphate Buffer (PB) at 4 °C. Brains were then removed, post-fixed overnight in 4% PFA at 4 °C and then placed in 30% sucrose. Brains were mounted on a freezing stage microtome (Leica), using the minimal amount of mounting medium (0.1 M PB). The caudal cut surface was attached to the stage and the ventral aspect faced towards the blade. Brains were quick frozen with dry ice and 30um thick serial coronal sections were cut throughout the rostrocaudal extent of the hippocampus. Sections were then stored in 1 M PB at a pH of 7.4 (Sigma) with 0.1% sodium azide at 4°.

For immunohistochemistry, free floating brain sections were washed twice at room temperature for a period of 10 min. They then underwent antigen retrieval in 0.01 M sodium citrate (pH 6) at 95–100 °C for 20 min. They were then processed via a standard ABC-Nickel DAB protocol (eg. [31]). For the primary antibody step, sections were incubated with a polyclonal antibody against Ki67 (Abcam, UK; ab16667) raised in rabbit diluted 1:500 in 0.1 M PBX and 2% NGS. For the secondary antibody and ABC steps, sections were incubated with 1:400 biotinylated goat anti rabbit (Jackson Immune research Labs) in 2% NGS at room temperature for a 70 min, and then treated with the Vectastain Elite Universal Kit (Vector Laboratories, Peterborough, Cambridgeshire) for 90 mines at room temperature. Immunoreactivity was visualized with nickel-enhanced diaminobenzidine (3, 3′-diaminobenzidene with ammonium nickel chloride, NiDAB, pH 6) and 60 µl glucose oxidase (5 mg/ml). In order to control for inter-run reliability each of the sections when being ran for a specific antibody were reacted at the same time with each section developing for the same period of time. After mounting, clearing and coverslipping, photomircogrpahs of the sections were taken under constant light intensity using a digital camera which was attached to an Olympus BX-51 light microscope at either 40× or 100× magnification. Stem cell proliferating cells with Ki67 were counted by eye under 400× magnification.

2.5. Statistical analysis

Data was analysed via factorial ANOVAs, either between groups or mixed between-groups/within-groups as appropriate to the dependent variable and the experimental design. An alpha level of 0.05 was set for statistical significance. The statistical analysis was hypothesis testing, rather than exploratory [32]. Given that part of the data was analysed via 4-way factorial ANOVAs (with time point, LAN, sex and housing conditions as the factors), and as such analysis generates a large number of F values, and the experimental design illustrated in Fig. 1, we focus on interaction terms incorporating time point and LAN terms to be most illustrative in testing our hypotheses, and our reporting of results reflects this emphasis. When factorial ANOVAs revealed significant interaction terms incorporating a LAN and time point term, these were probed further with more discrete ANOVAs or corrected pairwise comparisons. Effect sizes in factorial ANOVAs are reported as partial ETA squared values, and are interpreted as per Cohen [33].

3. Results

Exposure of both male and female mice to the LAN regime resulted in limited impact on rhythms in wheel running activity. As illustrated in Fig. 2, the initial 4–5 cycles of the exposure to LAN are characterised by a delay in the onset of activity and/or fragmentation of activity within the LAN period. However, when the 21 days under LAN are analysed, there are not significant effect of LAN on the phase-angle of entrainment (time point x LAN interaction $F_{1,37} = 1$, $P = 0.32$), rhythm power ($F_{1,37} = 0.84$, $P = 0.38$) and a borderline effect on period length (time point × LAN interaction $F_{1,37} = 4.4$, $P = 0.043$, partial ETA

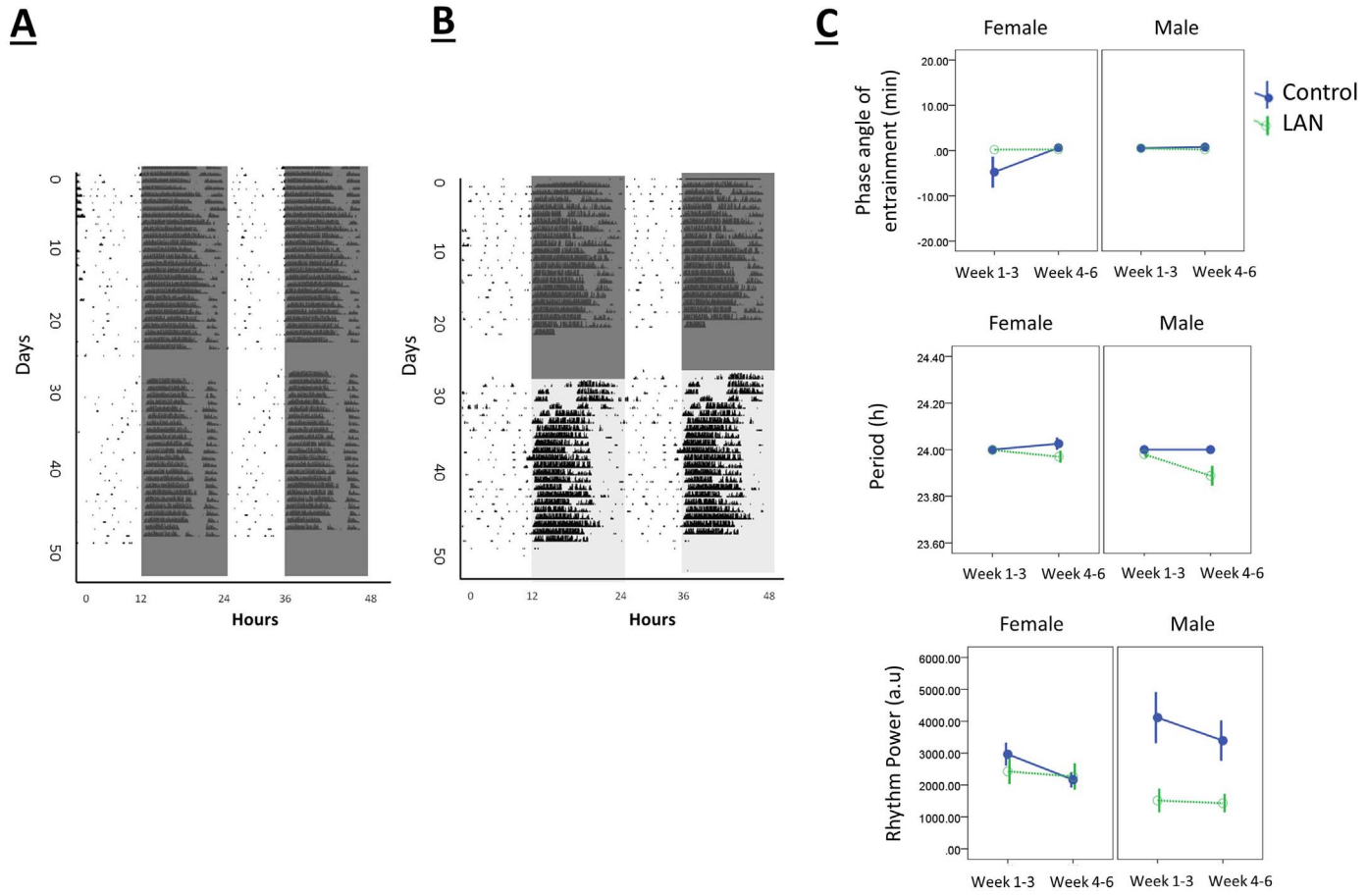

Fig. 2. Effects of LAN on wheel-running diurnal rhythms. Actograms showing representative daily patterns of running wheel activity under (A) control 12 h light:12 h dark conditions, and (B) under 12 h light:12 h LAN for 3 weeks following a 3 week period of 12 h light:12 h dark. The gaps in the activity records are the periods the animals were undergoing EPM, TST and FST. Note the delayed activity onset for the first 6–7 cycles in (B) following initiation of LAN exposure. (C) Impact of LAN on phase-angle of entrainment, rhythm period and rhythm power under standard LD (weeks 1–3 and 4–6 for control animals, week 1–3 for LAN animals) and LAN (weeks 4–6 for LAN animals). The group sizes, with complete data sets for analysis, were for LAN group, male = 9, female = 10; control group, male = 6, female = 16. Control represented by the filled circles and whole line, LAN represented by the open circles and dashed line.

squared = 0.11, indicating a moderate effect size) in both male or female animals when compared to the baseline period via mixed between-within ANOVAs. There were no statistically significant interactions involving sex as a term on any diurnal measure, and only singly-housed animals could be examined on these measures.

For the tail suspension test, there was no statistically significant effects of any LAN × time point interaction term (LAN × time point = 0.06, LAN × time point x housing, $P = 0.26$, LAN × time point × sex, $P = 0.43$; LAN × time point × housing × sex, $P = 0.06$; Fig. 3A). There was a statistically significant main effect of time point on time spent immobile ($F_{1,72} = 9.2$, $P = 0.003$, partial ETA squared = 0.112, a moderate effect size) reflecting a general trend towards more immobility on re-test. When data from the forced swim test was analysed there were main effects of time point ($F_{1,72} = 50.3$, $P < 0.001$, partial ETA squared = 0.36, a large effect) reflecting a general trend towards more immobility on re-test. There was a LAN × time × sex interaction on time spent immobile in the FST ($F_{1,72} = 7$, $P = 0.01$, partial ETA squared = 0.085, a moderate effect size; Fig. 3B). When probed further, female single housed animals did not differ in time spent immobile in the forced swim test compared to female single housed control animals ($P = 0.74$), nor did group housed female animals differ to LAN exposed animals ($P = 0.19$). Male singly housed animals did not differ after LAN compared to controls ($P = 0.55$), but male group housed animals showed significantly more immobility following LAN exposure than controls ($P = 0.013$ for time point x LAN interaction in this group, ETA squared = 0.35, a large effect). This result might be explained by the unexpectedly low amount of immobility observed in the male, group-housed control animals at baseline, and that this group did not show the increase in immobility at re-rest that was observed in the other three control groups in the FST (Fig. 3B). Testing this concern with a factorial ANCOVA with immobility on the FST at week 6 as the dependent variable, and immobility at week 3 as the covariate, for male group housed animals there was a significant effect of LAN ($F_{1,\ 15} = 29.4$, $P < 0.001$). For anhedonia, data from the sucrose preference test showed that there was a significant main effect of time point on sucrose preference ($F_{1,72} = 12.1$, $P = 0.001$, with an increase in sucrose preference at week 6 over week 3; Fig. 4B). However, there were no significant interaction terms including LAN and time point (LAN × time point, $P = 0.43$, LAN × time point × housing, $P = 0.88$; LAN × time point × sex, $P = 0.73$; LAN × time point × housing × sex, $P = 0.09$).

In order to examine the impact of LAN on anxiety-like behaviours, performance in the elevated plus maze was examined. There was a main effect of time on time spent in the open arms ($F_{1,72} = 14.7$, $P < 0.001$, with time in open arms being lower on retest at week 6) and a statistically significant time point × LAN × sex × housing interaction ($F_{1,72} = 4.6$, $P = 0.035$). When the nature of this interaction was probed, it was revealed that group-housed control animals spend *less* time in open arms at week 6 than LAN group housed animals; Fig. 4B. There were no further significant main effects or interaction terms involving LAN on time spent in open arms (LAN × time point = 0.49, LAN × time point × housing, $P = 0.97$, LAN × time point × sex,

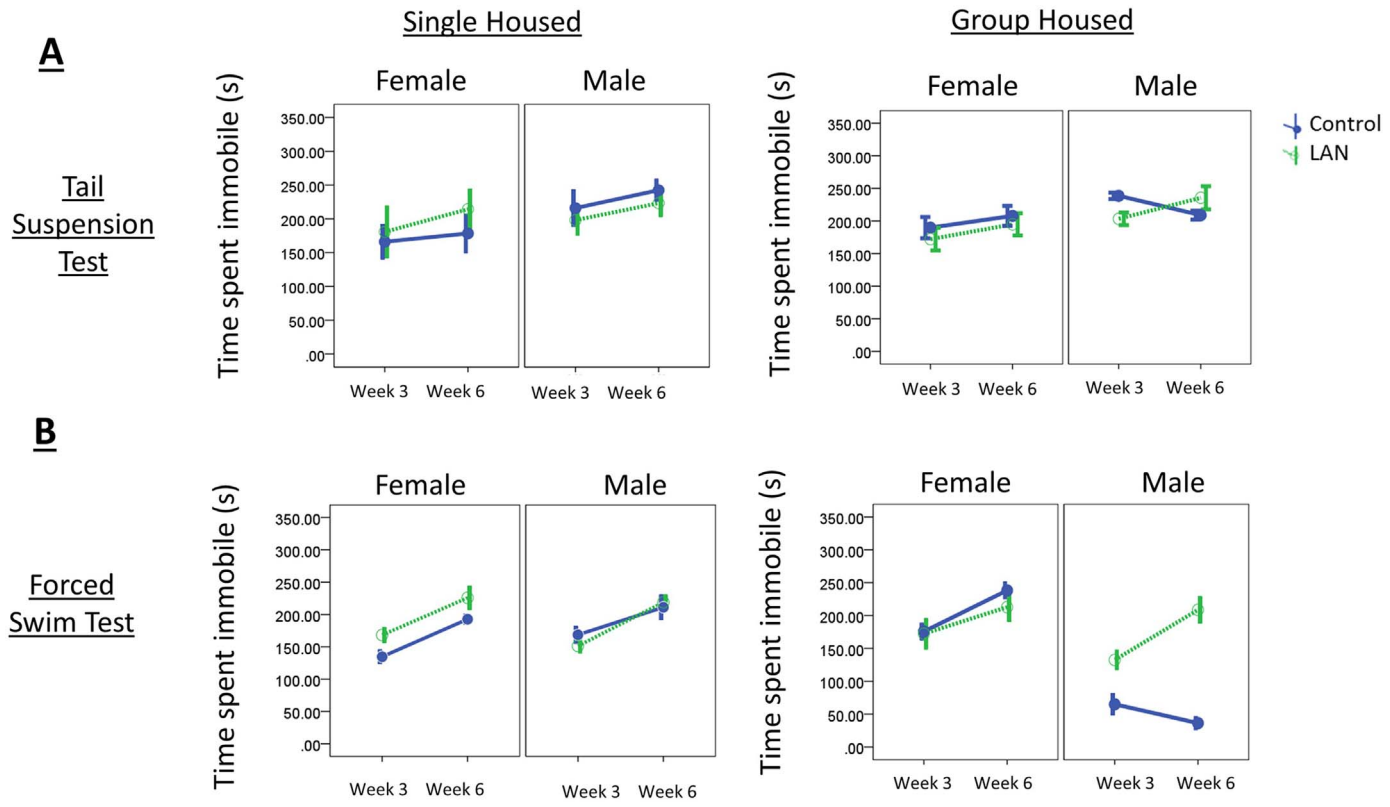

Fig. 3. Effect of LAN exposure on time spent immobile on the tails suspension test (A) or the forced swim test (B). Control represented by the filled circles and whole line, LAN represented by the open circles and dashed line. Week 3 is the time point for the control LD period for both control and LAN groups, week 6 is the testing point after further LD for the control group or after LAN. The only significant increase in immobility noted was for male group housed animals following LAN on the forced swim test. The group sizes were: female single housed controls = 14; female single house LAN = 11; male single housed control = 7; male single housed LAN = 19; female group housed controls = 7; female group housed LAN = 8; male group housed control = 7, male group housed LAN = 8.

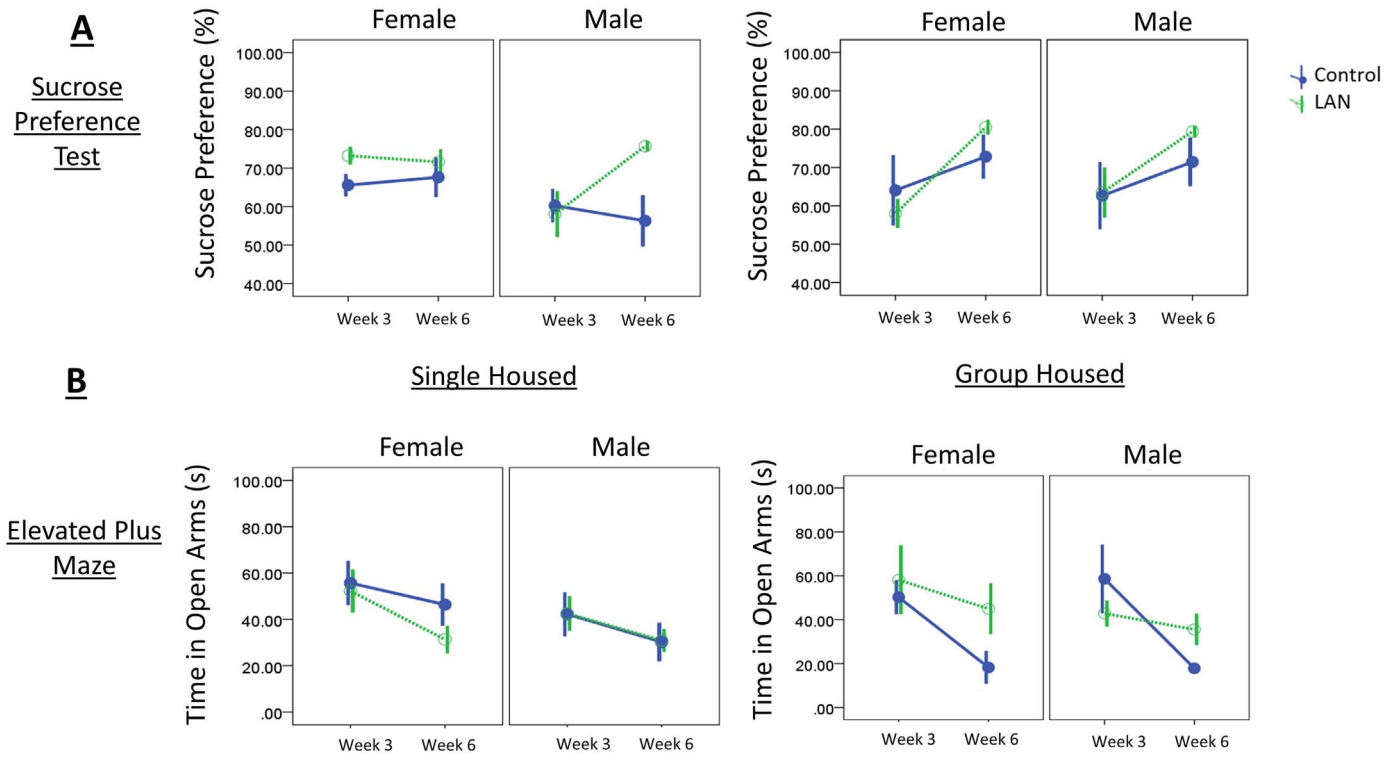

Fig. 4. Effect of LAN exposure on sucrose preference (A) or time spent in open arms in the elevated plus maze (B). Control represented by the filled circles and whole line, LAN represented by the open circles and dashed line. Week 3 is the time point for the control LD period for both control and LAN groups, week 6 is the testing point after further LD for the control group or after LAN. The only significant increase in immobility noted was for male group housed animals following LAN on the forced swim test. The group sizes were as described for Fig. 3.

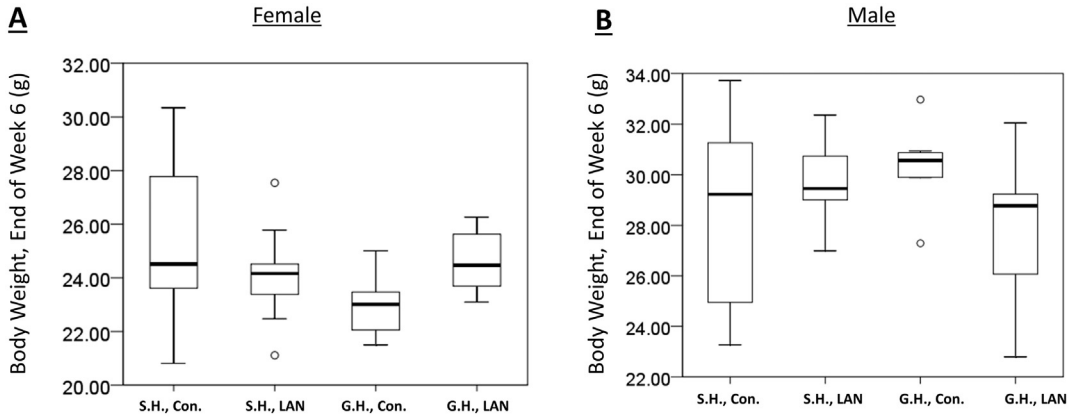

Fig. 5. Impact of LAN on body weight. Boxplots representing body weight on the end of the experiment for each of the 8 groups are presented. S.H. = single housed; G.H. = group housed; Con. = control, LAN = light at night. Group sizes are as described in the legend for Fig. 3. The open circles indicate moderate outliers.

$P = 0.38$).

Exposure to LAN was found to have no statistically significant effect on body weight at week 6 of the experiment (Fig. 5; $P = 0.08$ for single housed females between control and LAN groups, $P = 0.115$ for group house females; $P = 0.19$ for single housed males between control and LAN groups, $P = 0.06$ for group housed males). When the effect of LAN was examined on Ki-67, a marker for cell proliferation, in the dentate gyrus there was an effect of LAN ($F_{1,\ 35} = 5.4$, $P = 0.027$), but not a sex × LAN interaction ($P = 0.84$) or a main effect of sex ($P = 0.58$; only singly housed animals were examined for Ki-67; Fig. 6).

4. Discussion

At the outset of our study, we aimed to test the hypothesis that the impact of LAN on circadian and affective behaviours in C57Bl/6 mice would be moderated by sex. As circadian rhythm recording of locomotor activity usually required single housing of animals, and single housing can be a stressor for rodents [34], we also set out to examine if single or group housing would moderate the impact of LAN on affective behaviours. Given a number of previous reports of effect of LAN on depression- or anxiety-like behaviours in mice [6], we were somewhat surprised to find that in our hands, dim LAN produced only restricted effects on the behavioural repertoire that we examined.

In terms of LAN-induced changes in diurnal rhythms of locomotor activity, in contrast to the effects of dim white or blue LAN resulting in markedly decreased rhythm power in Siberian hamsters [14] and Swiss-Webster mice [35], we find only a modest change in rhythm period under LAN, and no changes in rhythm power and phase-angle of entrainment. Martynhak et al. [20] report that in wild type and PER3 knockout C57Bl/6 mice, dim LAN results in delays of the timing of the peak of nocturnal activity. This is similar to the effect we observe in the first number of cycles under dim LAN, but we find that this delay attenuates after approximately 7 cycles. In Wistar rats, dim LAN exerted a relatively subtle effect on diurnal activity rhythms, introducing a free-running activity component with a period of ~25 h that interferes with the entrained 24 h rhythm [16]. A study of LAN in the diurnal rodent *Arvicanthis niloticus* (Nile grass rat) showed no impact of LAN on diurnal activity rhythms [17]. Therefore, there appears to be a range of circadian changes associated with dim LAN, and these differences may be, in part, a result of species and strain differences. It is interesting to note a previous report that very low levels of LAN significantly alters subsequent responsivity of the circadian system to photoperiodic changes [36], although in the current study we did not examine whether LAN altered subsequent plasticity of the circadian system and so cannot rule out that such changes might have taken place. Overall, the impact of dim LAN on diurnal activity rhythms appears to be context dependent.

In our experiments, we found limited impact of LAN on any affective behaviours examined. We set out to examine how sex may moderate any impact of LAN, as sex influences characteristics of animal models of affective disorders (eg [37]) and the prevalence of depressive and anxiety disorders in humans [38]. We also included housing conditions as a factor, as protocols involving monitoring of circadian activity patterns in rodents usually entail single housing of these animals, which itself may be a stressor [34] (although access to running wheels, as in our experiments, may buffer the effects of isolation [39]). Somewhat to our surprise, we found limited affective responses to LAN during our experiments. The only observed increase in behavioural despair was for group housed male animals in the forced swim test, and this finding may be confounded by the low levels of immobility observed in control animals in this experiment. Increases in immobility in

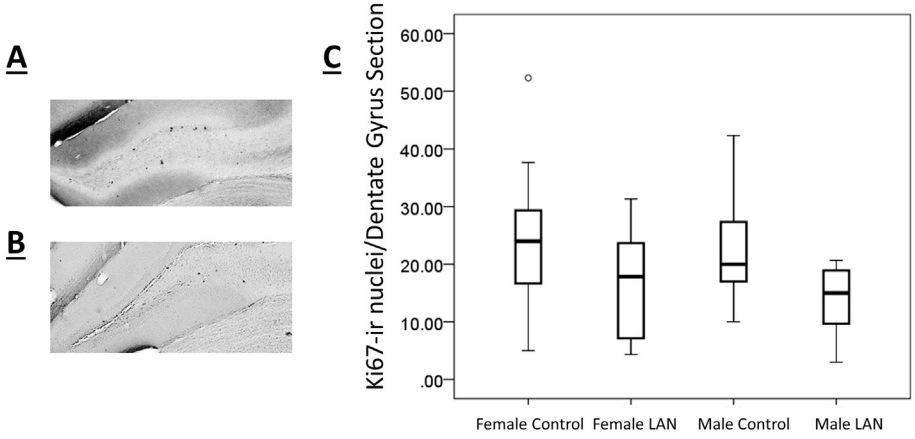

Fig. 6. Impact of LAN on Ki-67 expression in the dentate gyrus. Representative photomicrographs of Ki-67 expression at week 6 in (A) control and (B) LAN animals. (C) Boxplots showing the impact of LAN on Ki-67 expression in the dentate gyrus. Group sizes: Female control = 12, male control = 7, female LAN = 8, male LAN = 8. The open circles indicate moderate outliers.

the forced swim test following LAN exposure has been reported previously in female Siberian hamsters [9,10], in male Nile grass rats [17], in melatonin secreting male C3H mice [12]. We observe no impact of LAN on immobility in the tail suspension test, and are not aware of other reports of TST performance after exposure dim LAN (this may be in part due to the lack of suitability of this test for some animal models used in LAN research, such as the Siberian hamster). There have been a number of reports of an increase in anhedonia, as measured via the sucrose preference test, which accompanies increased behavioural despair following dim LAN exposure in female Siberian hamsters (eg [9,10].), in male Nile grass rats [17], and in melatonin secreting male C3H mice [12]. However, Martynhak et al. [20] report no decrease in sucrose preference in wild type male C57Bl/6 mice after 4 week of dim LAN, although PER3 knockout mice did show a transient increase in anhedonia. When we examined performance on the elevated plus maze as a measure of anxiety-like behaviour, we found a decrease in anxiety-like behaviours in group housed animals following LAN, and no change in single housed animals. Bedrosian et al [9] also report a decrease in anxiety-like behaviour in the elevated plus maze in female Siberian hamsters, although early-life exposure to LAN in mice increases anxiety-like behaviours in adult animals [13].

We examined Ki-67 expression in the dentate gyrus, as previous studies have shown LAN-induced changes in neuroplasticity, including changes in dendritic spine density in the hippocampus [9], decreased hippocampal BDNF and increased TNF-α [10], and that constant light induces a decrease in hippocampal neurogenesis [40]. We did find a significant reduction in Ki-67 following LAN, which suggests that LAN may impair hippocampal neurogenesis. It is of interest to note the seeming mismatch between changes in Ki-67 expression and the relatively sparse behavioural phenotypes observed in the same animals; it may be that the experimental time course employed was not sufficiently extended to allow for neuroplastic change to manifest behaviourally.

A parsimonious explanation for the relative lack of impact observed in response to LAN in the current study is that the LAN paradigm in C57Bl/6 mice produces an insufficiently large circadian desynchrony to manifest as substantial affective changes. Desynchronisation of the circadian system under different paradigms has been shown to induce a range of affective changes [41] as well as other health consequences [5]. That this may be the case is spoken to by our current finding that LAN-exposed animals did not gain body weight in comparison to the control group, a finding that is different from studies in models in which LAN leads to both significant changes in the diurnal or circadian activity rhythm, and metabolic and endocrine changes manifesting ultimately as weight gain [35]. Here it is interesting to note that the study of Stenvers et al. [16] which noted a subtle impact of LAN on circadian behaviour, did not report changes in body weight or glucose tolerance.

There are a number of potential sources of variability that may moderate the impact of LAN in rodents, including retinal pigmentation, photoperiodism of the species and strain used and melatonin secretion competence. C57Bl/6 mice, as used in the current study, have retinal pigment, which renders them less photosensitive than albino strains [19] and therefore possibly less reactive to similar cage-levels of LAN. C57Bl/6 mice, like most commonly used laboratory strains, are melatonin deficient, and clearly LAN-induced suppression of nocturnal melatonin is a putative mechanism through which effects may arise (although it is important to consider whether LAN levels could be sufficient to induce melatonin suppression in any given model (eg [42])). The effect of LAN may be moderated via 12 h light:12 h dim light cycles being interpreted as either short or long days by the exposed animals [43], and as such LAN imposed on less potentially ambiguous photoperiods (8:16, or 16:8) may yield insight into this, as may the systematic study of highly photoperiodic vs. weakly photoperiodic models. Other experimental factors, such duration of LAN exposure, exact spectral composition of LAN, ontogeny and prior behavioural history may all shape the response to LAN. Further, estrous cycle stage may impact on behavioural results in female mice, or on Ki67 expression; however, by the nature of the experimental paradigm run across a number of weeks, it is inevitable that estrous cycle differences will occur in relation to behavioural testing and/or LAN exposure. Further, behavioural outcomes in C57Bl/6 mice has previously been shown to be relatively insensitive to estrous cycle stage [44]. LAN may itself impact on estrous cycling in female mice, and future work should address this as a mechanism through which LAN may induce behavioural change. Although sleep architecture was not examined in the current study, it is interesting to note that one study in mice reported no impact on sleep timing or quality [13], while a study in rats did report non-REM sleep disruption in response to LAN [16], further illustrating the differential responses to similar LAN procedures in different models.

In conclusion, our current study indicates that careful consideration should be given to model selection for studies of LAN on circadian and other functions. Such factors include species, strain, photoperiod, sex, housing condition, melatonin secretion, and LAN protocol and behavioural assessment protocols. Such considerations will enhance the validity and reliability of future animal model-based enquiry into the important issue of man-made artificial night-time light, and the health consequences thereof.

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