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Short communication

Dim light at night increases depressive-like responses in male C3H/HeNHsd mice

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HIGHLIGHTS

- Exposure to light at night is common in modern society.
- Aberrant light exposure may affect mood.
- Mice housed in dim light at night increase depressive-like responses.
- Dim light at night does not affect hippocampus-dependent learning and memory.
- Hippocampal inflammation does not mediate light induced depressive-like responses.

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ABSTRACT

Daily patterns of light exposure have become increasingly variable since the widespread adoption of electrical lighting during the 20th century. Seasonal fluctuations in light exposure, shift-work, and transmeridian travel are all associated with alterations in mood. These studies implicate fluctuations in environmental lighting in the development of depressive disorders. Here we argue that exposure to light at night (LAN) may be causally linked to depression. Male C3H/HeNHsd mice, which produce nocturnal melatonin, were housed in either a standard light/dark (LD) cycle or exposed to nightly dim (5 lux) LAN (dLAN). After four weeks in lighting conditions mice underwent behavioral testing and hippocampal tissue was collected at the termination of the study for qPCR. Here were report that mice exposed to dLAN increase depressive-like responses in both a sucrose anhedonia and forced swim test. In contrast to findings in diurnal grass rats, dLAN mice perform comparably to mice housed under dark nights in a hippocampus-dependent learning and memory task. TNFα and IL1β gene expression do not differ between groups, demonstrating that changes in these pro-inflammatory cytokines do not mediate dLAN induced depressive-like responses in mice. BDNF expression is reduced in the hippocampus of mice exposed to dLAN. These results indicate that low levels of LAN can alter mood in mice. This study along with previous work implicates LAN as a potential factor contributing to depression. Further understanding of the mechanisms through which LAN contributes to changes in mood is important for characterizing and treating depressive disorders.

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Throughout most of evolutionary history, the sun and moon were the dominant light sources to which mammals were exposed. Recently, exposure to light during the night has increased because of widespread adoption of electric lights. Exposure to light at night (LAN) is ubiquitous in modern society and generally considered an innocuous environmental perturbation. However, the use of electric lights occurred prior to a strong understanding of circadian biology and too rapidly for adaptations to evolve to counter any deleterious physiological and behavioral changes that may result from LAN [11].

Chronic exposure to LAN is implicated as a contributing factor in several human diseases, including breast and prostate cancer [18], heart disease [26], obesity, and type II diabetes [27]. Furthermore, chronic exposure to light at night through activities such as shift work is associated with higher prevalence of mood disorders [6]. Epidemiological studies indicate mood disorders have likely increased in recent decades (reviewed in [16]). Changing diagnostic criteria and increased public awareness are thought to contribute to this trend; however, additional nontraditional environmental factors are likely involved [16]. Elevated exposure to light at night correlates with increasing rates of depressive disorders. Here we test the hypothesis that exposure to light at night causes depression, likely through disruption of the circadian system.

The rotation of the earth about its axis produces a highly consistent 24 h pattern of light and dark that varies on a seasonal basis. Many organisms have developed endogenous rhythms, called circadian rhythms that are entrained to this external light/dark cycle.

Abbreviations: LAN, light at night; dLAN, dim LAN.

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The master regulator of circadian rhythms in mammals is located in the suprachiasmatic nuclei (SCN) of the hypothalamus [22]. The SCN is principally entrained by light information that is detected by a special population of non-image-forming cells in the retina called intrinsically photosensitive retinal ganglion cells (ipRGCs) [3]. ipRGCs project to hypothalamic and preoptic areas such as the SCN, subparaventricular nucleus, and ventrolateral preoptic area to regulate circadian rhythms. The SCN forms multiple downstream connections with limbic areas suggesting it may be involved in regulation of mood [21]. Moreover, ipRGCs project directly to specific limbic regions such as the lateral habenula and amygdala [15].

Recent work from our lab and others demonstrates that exposure to aberrant light cycles [8,19] and dim LAN (dLAN) [1,2,10] change affective behaviors in rodents. Female Siberian hamsters (*Phodopus sungorus*) chronically exposed to 5 lux of dLAN increase hippocampal cytokine expression and reduce dendritic spine density in CA1 pyramidal neurons. These changes are accompanied by increases in depressive-like behavior. Inhibition of hippocampal cytokine expression with intracerebroventricular administration of a dominant-negative TNF ameliorates the dLAN induced depression [2]. Diurnal Nile grass rats (*Arvicanthis niloticus*) exposed to chronic dLAN display similar changes in affect. Grass rats housed in dLAN alter hippocampal connectivity and increase depressive-like responses compared to rats exposed to dark nights. Additionally, dLAN grass rats impair spatial learning and memory as evaluated in a Barnes maze task [10].

Together these studies suggest that exposure to LAN affects mood and cognition in rodents, potentially through changes in hippocampal inflammation. One limitation to previous studies is the use of alternative rodent models and the effect of dLAN on cognitive behaviors in nocturnal rodents were not assessed. Thus, we investigated whether dLAN affects learning and memory and depression in a common strain of laboratory mice, C3H/HeNHsd. C3H/HeNHsd mice were selected because they are one of two inbred strains of laboratory mice that have intact melatonin production. Melatonin is considered the hormonal signal of darkness; it is produced during the night in both diurnal and nocturnal species and is suppressed by exposure to light at night [23]. Many of the effects of exposure to LAN and shift work are attributed to disrupted sleep, rather than disrupted circadian rhythms. We used nocturnal mice, which normally sleep during the light of day, in order to separate the effects of light at night on brain and behavior from any indirect effects of sleep disruption.

Male C3H/HeNHsd mice (~8 weeks of age) were obtained from Harlan Laboratories for use in this study. Mice were individually housed in propylene cages (dimensions: 27.8 cm × 7.5 cm × 13 cm) at an ambient temperature of 23 ± 2 °C and provided with Harlan Teklad 8640 food (Madison, WI) and filtered tap water *ad libitum*. All mice were maintained in a standard light dark cycle (16:8 light (~150 lux)/dark (0 lux); LD) for one week following arrival. A 16:8 light dark cycle was used rather than 12:12 to avoid providing a seasonally ambiguous signal. Although C3H mice are an inbred strain they may still retain some responsiveness to photoperiod. Mice might interpret a 12:12 light cycle as either a long or short photoperiod, and thus exhibit higher variability in phenotype. After the 1 week acclimation period mice were randomly assigned to either LD or dLAN (16:8 light (~150 lux)/dark (5 lux)). Dim light was administered with a flexible strip of cool white LEDs wrapped around the rack on which the mouse cages were placed. The lighting intensity was measured inside the home cage and was highly consistent between cages. Five lux of light exposure was chosen because it is greater than moonlight (<0.5 lux), but highly distinguishable from light exposure during the light phase (150 lux). After 4 weeks in lighting conditions mice underwent behavioral testing in the following order: Barnes maze, sucrose anhedonia, and forced swim test. Barnes maze and the forced swim test were conducted during the light phase in order to avoid exposing LD mice to light disruptions during the dark. Because sucrose anhedonia took place in the home cage, this behavior was evaluated during the dark when nocturnal rodents are more active.

Learning and memory was evaluated in a Barnes maze task. The Barnes maze is a brightly lit circular arena with 18 evenly spaced holes on the outside. One hole leads to an escape box under the maze which mice are trained to find by putatively attending to extra maze spatial cues. On day 1 of Barnes maze mice were habituated to the apparatus by gently guiding the mice from the center of the maze to the target hole. After entering the target hole mice were left undisturbed in the escape box for 2 min. The maze was wiped clean between tests with mild soapy water. Mice then underwent 4 days of acquisition training with 3 trials per day. Each trial lasted 2 min or until the mouse found the escape hole, where they were left for 1 min. After each trial the mouse was placed back in its home cage for 10 min before beginning the next trial. During the training trials, latency to find the target hole and number of errors were scored. Twenty-four hours following the last training trial, mice underwent a 90 s probe trial in which the escape box was blocked off and percent of visits to the target hole was quantified.

Depressive-like responses were subsequently evaluated with sucrose preference and forced swim tests. These tests assess anhedonia and learned helplessness respectively, two key components of depression. For the sucrose preference test, consumption of a 2% sucrose solution was recorded. Before presentation of the sucrose solution, mice were administered water in modified bottles for 5 h on 3 consecutive nights to control for the novelty of the bottle. The following night mice were given a choice between water and sucrose solution in the modified bottles. Bottles were counterbalanced to control for possible side preference. To quantify sucrose preference, bottles were weighed before and after the 5 h sessions and sucrose consumption was normalized to water consumption. Following sucrose preference testing mice underwent a forced swim test. Mice were placed in ~17 cm water (22 ± 1 °C) within an opaque, cylindrical tank (diameter = 24 cm, height = 53 cm). Swimming behavior was videotaped for 5 min and scored by a condition-blind observer with the Observer software (Noldus Corp, Leesburg, VA) for latency to float and time spent floating.

At the conclusion of the study, mice were anesthetized with isoflurane vapors and rapidly decapitated. Brains were removed and placed in RNA later for ~48 h and hippocampi were dissected out. Total RNA was extracted from hippocampal tissue using a homogenizer (Ultra-Turrax T8, IKAWorks, Wilmington, NC) and an RNeasy Mini Kit (Qiagen, Austin, TX) according to manufacturer instructions. RNA was reverse transcribed into cDNA with M-MLV Reverse Transcriptase enzyme (Invitrogen, Carlsbad, CA). Gene expression for IL1β, TNFα, and BDNF was determined using inventoried primer and probe assays (Applied Biosystems, Foster City, CA) on an ABI 7500 Fast Real Time PCR System using Taqman® Universal PCR Master Mix. The universal two-step RT-PCR cycling conditions used were: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Relative gene expression of individual samples run in duplicate was calculated by comparison to a relative standard curve and standardized by comparison to 18S rRNA signal.

Gene expression and behavioral results from the forced swim and sucrose anhedonia tests were analyzed using Student's *t*-test. For each Barnes maze session (1–4), latency to enter the target hole was averaged per session for each mouse, and data were analyzed with repeated-measures ANOVA. The above statistical analyses were conducted with StatView software (v.5.0.1; SAS Institute, Cary, NC). In all cases, differences between groups means were considered significantly different is $p \leq 0.05$.

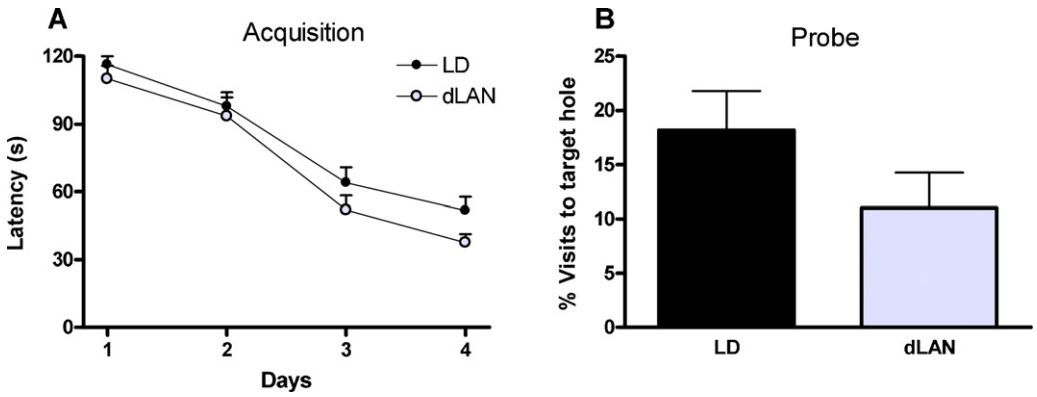

Fig. 1. Mice exposed to dim and dark nights have comparable performance in a Barnes maze task. (A) Latency to reach the target hole over the course of acquisition training and (B) percentage of visits to the target hole in a probe trial.

At the conclusion of the study mice dLAN mice weighed significantly more than mice housed with dark nights ($t_{19} = 2.431$; $p < 0.05$; data not shown). This has been previously shown in Swiss Webster mice and may relate to changes in timing of food intake [12].

Cognitive abilities and memory vary throughout the day [13] and disruption of the circadian system results in memory impairments [14]. We have previously reported that diurnal rodents exposed to dLAN have deficits in learning and memory. Thus, we evaluated the effects of nighttime light exposure on a hippocampus-dependent cognitive task in mice housed in dark or dim nights. Learning and memory did not differ between dLAN and LD mice. Both groups of mice acquired the task with decreasing latencies to reach the target hole over time ($F_{3,57} = 74.863$; $p < 0.0001$). However, there were no differences between groups over the course of the acquisition trials or during the probe trial ($F_{1,57} = 2.727$ and $F_{1,19} = 2.100$, $p > 0.10$; Fig. 1A and B). Multiple factors may account for the disparate results in learning and memory between nocturnal and diurnal rodents exposed to light at night. First, Barnes maze testing occurred during the light phase in both studies. For diurnal grass rats, this means that training occurred during the active phase. Because learning and memory has a circadian component (reviewed in [13]), it is possible that nighttime light exposure dampens cognitive abilities specifically during the active phase when animals are at peak learning capacity. Alternatively, dLAN exposure may be more disruptive to diurnal animals. dLAN may represent a stressor in diurnal but not nocturnal rodents; exposure to dLAN increases corticosterone concentrations in diurnal grass rats [9,10] but does not affect glucocorticoid concentrations in nocturnal hamsters [1] or mice [12]. Elevated circulating glucocorticoid concentrations are predictive of hippocampal atrophy and memory deficits [20]. dLAN may also potentially disrupt sleep in diurnal rodents which can impair memory consolidation [25].

Circadian abnormalities are prominent in depressive disorders. For example, the circadian clock is often phase-delayed in major depressive disorder, with the severity of depression correlating with the extent of the delay [7]. The relationship between

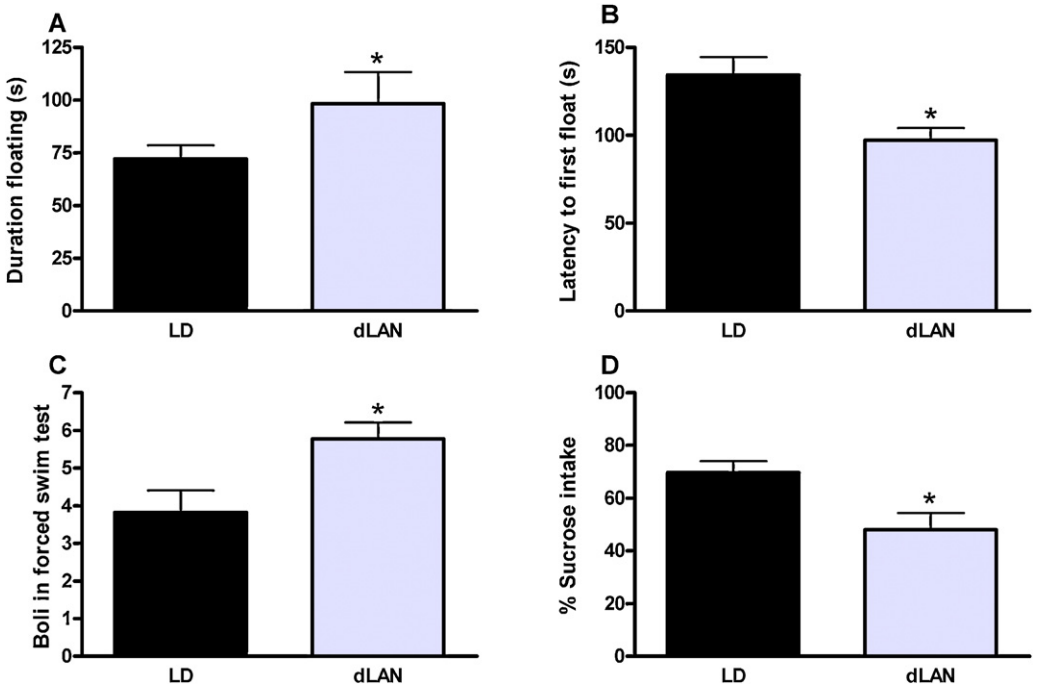

Fig. 2. Exposure to dim LAN increases depressive-like responses in mice. In the forced swim test dLAN mice (A) float for a longer duration, (B) reduce latency to first float, and (C) increase defecation. (D) Sucrose intake is also reduced by exposure to dLAN.

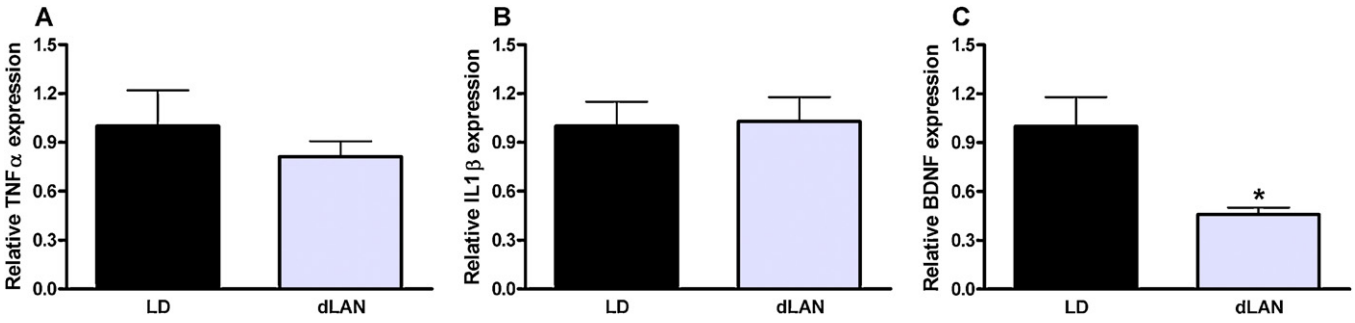

Fig. 3. Pro-inflammatory cytokine expression is unaffected and BDNF expression is reduced by exposure to dLAN. (A) TNFα and (B) IL1β gene expression were comparable between dLAN and LD mice and (C) BDNF gene expression was reduced by dLAN exposure.

depressive-disorders and the circadian system is likely bidirectional as shift workers who are exposed to high levels of LAN in the workplace have elevated risk for developing mood disorders [6]. In agreement with previous findings, exposure to dLAN increased depressive-like responses in mice. In the forced swim test, dLAN mice spent more time immobile and had a shorter latency to first float ($t_{19} = 1.773$ and $t_{19} = 2.749$, $p < 0.05$; Fig. 2A and B). Increases in immobility in the forced swim test are generally interpreted as behavioral despair. dLAN mice also increased defecation in the forced swim test ($t_{19} = 2.585$, $p < 0.05$; Fig. 2C), which is a measure of stress-related autonomic reactivity [17]. In order to confirm changes in affect mice also underwent a sucrose preference test. As predicted, dLAN mice had lower sucrose intake compared to mice housed with dark nights ($t_{19} = 2.862$, $p < 0.05$; Fig. 2D). Together, these results suggest exposure to light at night changes depressive-like behaviors in mice. This adds to the growing body of literature implicating nighttime light exposure as a critical factor regulating mood [1,2,10].

Inflammation is associated with depression [4]; immune function is dysregulated in patients with major depressive disorder and chronic inflammation is associated with depression. Commercially available antidepressant medications typically target monoamine pathways and are an ineffective treatment for >30% of depressed patients [24]. For this reason, it is important to continue to seek alternative causes and treatments for depressive-disorders. Siberian hamsters exposed to dLAN elevate hippocampal TNFα expression and exhibit behavioral changes in the forced swim test that are reversed with ICV administration of a DN-TNF [2]. Therefore, we investigated whether dLAN induces changes in hippocampal inflammation in mice. No differences in hippocampal TNFα and IL1β cytokine expression were apparent between lighting conditions; dLAN and LD mice had equivalent levels of hippocampal TNFα and IL1β mRNA ($p > 0.05$; Fig. 3A and B). LAN does not alter MAC1 or TNFα expression in other murine brain areas such as the hypothalamus (Fonken et al., unpublished observations). Taken together, these studies suggest that mice may not exhibit baseline differences in central inflammation with LAN. Additionally, in the study of hamsters exposed to dLAN, treatment with DN-TNF ameliorated depressive-like responses in the forced swim test, but did not reverse changes in hippocampal structure or behavioral differences in sucrose anhedonia [2]. This indicates that dLAN may work through additional pathways to induce changes in hippocampal structure and behavior. Indeed, hippocampal brain-derived neurotrophic factor (BDNF) gene expression was reduced in mice exposed to dLAN ($t_{17} = 2.510$, $p < 0.05$; Fig. 3C). BDNF gene expression is attenuated by stress-induced depression and rescued by antidepressant drugs. Moreover, BDNF affects dendrite complexity, spine density, and function [5]. These results implicate changes in BDNF and potentially accompanying alterations in hippocampal circuitry in dLAN induced depressive behaviors.

Overall, our findings demonstrate that exposure to low levels of LAN can alter mood in mice. These results along with previous work implicate LAN as a contributing factor to development of depressive disorders. Light at night has grown exponentially in recent decades with little focus on the health implications of its exposure. It is now apparent that use of electrical lighting has significant effects on physiology and behavior [2,18,26,27]. Further understanding of the mechanisms through which LAN contributes to changes in mood is important for characterizing and treating depressive disorders.

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