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Research report

Influence of light at night on murine anxiety- and depressive-like responses

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ABSTRACT

Individuals are increasingly exposed to light at night. Exposure to constant light (LL) disrupts circadian rhythms of locomotor activity, body temperature, hormones, and the sleep-wake cycle in animals. Other behavioural responses to LL have been reported, but are inconsistent. The present experiment sought to determine whether LL produces changes in affective responses and whether behavioural changes are mediated by alterations in glucocorticoid concentrations. Relative to conspecifics maintained in a light/dark cycle (LD, 16:8 light/dark), male Swiss-Webster mice exposed to LL for three weeks increased depressive-like behavioural responses as evaluated by the forced swim test and sucrose anhedonia. Furthermore, providing a light escape tube reversed the effects of LL in the forced swim test. LL mice displayed reduced anxiety as evaluated by the open field and elevated-plus maze. Glucocorticoid concentrations were reduced in the LL group suggesting that the affective behavioural responses to LL are not the result of elevated corticosterone. Additionally, mice housed in LD with a clear tube displayed increased paired testes mass as compared to LL mice. Taken together, these data provide evidence that exposure to unnatural lighting can induce significant changes in affect, increasing depressive-like and decreasing anxiety-like responses.

1. Introduction

With the advent of electrical lighting at the turn of the 20th century, individuals of many species, including humans, became exposed to bright and unnatural light at night. Urban development has further exacerbated the issue of light at night as lighting from infrastructure strays into the atmosphere. This "light pollution" is now affecting 99% of the population in the US and Europe and 62% of the world population [26]. Electric lights have not only created light pollution, but have permitted shift work at night, generally perturbing the sleep-wake patterns of humans [33]. Individuals exposed to light at night are at increased risk for heart disease [17], cancer [10,35], sleep disturbances [12,20], circadian rhythm dysfunctions [3], disrupted rhythmicity of neuroendocrine function (such as corticotrophin releasing hormone, glucocorticoids, and prolactin) [7,30], mood disorders [13], and reproductive dysfunction [14,36].

Housing animals in constant light (LL) conditions is useful for studying the effects of light at night in animal models. The majority of studies indicate that maintaining animals in LL conditions is deleterious, but the mechanisms underlying these harmful effects remain unspecified [26]. Continuous exposure to light strongly suppresses circadian rhythms of locomotion, body temperature, and the sleep-wake cycle of rodents [18], as well as generally elevating corticosterone concentrations [1,38]. It is possible that exposure to light at night produces harmful effects on animals directly via disruption of biological clock function [28]. Another possibility, albeit not mutually exclusive, is that light exposure at night represents a chronic stressor [22] which can indirectly affect physiological and behavioural processes [21].

Seasonal lighting, abnormalities in circadian clock [2], and sleep disorders are associated with depression in some subpopulations [5]. Although depression is traditionally considered maladaptive in humans, depressive-like behavioural responses persist in other species and may be advantageous under certain conditions. For example, symptoms of human seasonal affective disorder (SAD), such as lethargy, anxiety, altered food intake, and loss of sexual behaviour may be adaptive and conserve energy during the reduced day lengths of winter for individuals of some rodent populations [32]. This study is designed to address whether another form of circadian disruption, light at night, also negatively impacts affective behaviour. Depressive behaviours in humans may have evolved under a similar seasonal context as that of rodents and remain

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susceptible to changes in environmental lighting. The unnatural light cycles to which humans are now exposed, and the irregular sleep patterns evoked by light at night, may interfere with typical responses to the annual cycle of changing day lengths.

Reports on the interaction of LL with depressive- and anxiety-like responses have been inconsistent. Although previous studies have reported altered brain morphology due to LL [21] and other forms of circadian disruption such as sleep deprivation [43], previously reported behavioural effects of LL are inconsistent. For example, LL has been reported to both influence memory [22] and have no effect on memory [6]. Additionally, although circadian disruption has been reported to lessen anxiety [34], the effect of LL on anxiety has not been well established [6,22].

In the present experiment, we examined behavioural and glucocorticoid responses to LL exposure, focusing on the possible link between altered lighting and affective responses. Male Swiss-Webster mice were housed in either LL or a light/dark cycle. We attempted to ameliorate the stress-evoking effects of constant light by providing half the mice with an opaque tube to serve as a light escape. As a control for the environmental-enriching effects of the tube, half of the mice were provided with a clear tube. We hypothesized that LL would increase corticosterone concentrations and elevate depressive-like behavioural responses and that providing light escape would partially reverse these effects.

2. Experimental procedures

2.1. Animals

Twenty-four male Swiss-Webster mice (~8 weeks of age) were obtained from Charles River Labs (Kingston, NY) for use in this study. The mice were individually housed in propylene cages (30 cm × 15 cm × 14 cm) at an ambient temperature of 22 ± 2 °C and provided with Harlan Teklad 8640 food (Madison, WI) and filtered tap water *ad libitum*. Upon arrival all mice were maintained under a 16:8 light/dark (lights on at 23:00 Eastern Standard Time [EST]) cycle for one week to allow them to entrain to local conditions and recover from the effects of shipping. Following the recovery period, mice were randomly assigned to either a control or experimental treatment group. Mice assigned to the control group ($n = 12$) were maintained under a 16:8 light/dark (LD) cycle (lights on at 23:00 EST), whereas the experimental group was maintained in constant light (LL; $n = 12$) for the remainder of the study. The mice were housed in separate rooms with fluorescent ceiling lights controlling the light condition to which the mice were exposed. Each cage was provided with a PVC tube (length = 13.0 cm; inner diameter = 5.2 cm; outer diameter = 6.0 cm) that was either opaque providing light escape (LE; $n = 12$), or clear (C; $n = 12$). All experimental procedures were approved by The Ohio State University Institutional Animal Care and Use Committee, and animals were maintained in accordance with the recommendations of the National Institutes of Health and the *Guide for the Care and Use of Laboratory Animals*.

2.2. Experimental design

After three weeks in lighting conditions, the mice underwent a battery of behavioural tests to measure anxiety- and depressive-like responses. All testing occurred during the light phase between 8:00 and 13:00 EST with the exception of the sucrose anhedonia test (see below). Testing occurred in the following order to minimize stress effects in the most sensitive tests [8]: open field, elevated-plus maze, sucrose anhedonia, Porsolt forced swim test. Following testing, the mice were killed and their adrenals, spleens, testes, and fat pads were collected and weighed. Blood samples were collected immediately prior to the start of the experiment, after two weeks of experimental light condition, and at death.

2.3. Behavioural tests

To assess locomotor behaviour and anxiety-like responses, mice were placed in a 40 cm × 40 cm clear acrylic chamber lined with corncob bedding, inside a ventilated cabinet (Med Associates, St. Albans, VT). Mice were allowed to acclimate to the testing room for 30 min before testing began. The test chambers were rinsed with 70% ethanol and the bedding was changed between each test. The center of the open field was defined as the central 30 cm × 30 cm. A frame at the base of the chamber consisting of 32 photobeams in a 16 × 16 arrangement, in addition to a row of beams above, detected the location of horizontal movements and rearing, respectively (Open Field Photobeam Activity System, San Diego Instruments Inc., San Diego, CA). Total movement was tracked for 30 min and analysed for: (1) the percentage of beam breaks in the center of the open field, (2) number of rears, and (3) total locomotor behaviour. Increases in central tendency and rearing are generally interpreted as low anxiety-like responses [9].

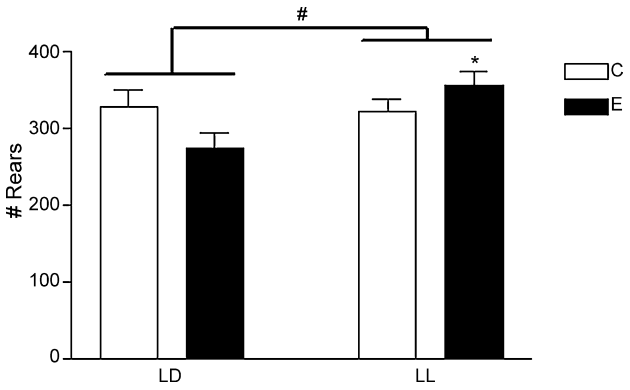

Fig. 1. Mean (±Standard Error of the Mean (SEM)) number of rears in the open field test for anxiety-like behaviours. Total duration of test was 30 min. #$p < 0.05$ between LL and LD groups; *$p < 0.05$ across light and tube type.

To further assess anxiety-like responses, mice were placed in a maze elevated 1 m above the floor made of dark-tinted acrylic and consisting of two open arms bisected by two arms enclosed by walls (the top of the entire maze was open) [16]. Prior to testing, mice were allowed to acclimate to the room for 30 min. Mice were placed in the central maze area facing a closed arm and recorded on video for 5 min. The maze was wiped with a mild soapy water solution between tests. An open arm entry was scored when the two forepaws and half of the body entered an open arm. A condition-blind observer using Observer software (Noldus Corp., Leesburg, VA, USA) scored tapes for: (1) latency to enter an open arm, (2) total time spent in the open arms, and (3) number of open arm entries.

Consumption of a 3% sucrose solution over 5 h during the active phase, 15:00–20:00 EST, was recorded in all mice to measure sucrose anhedonia [40]. Prior to the presentation of the sucrose solution, mice were administered tap water in modified water bottles for three consecutive nights, to control for novelty of the modified water bottles. The modified water bottles were weighed before and after the 5 h sample time to quantify the liquid volume consumed. After the three nights of tap water measurements, a 3% sucrose solution was provided for two nights. Sucrose consumption during both nights was normalized to the average pre-testing water consumption.

To assess depressive-like responses, mice were placed in room-temperature (22 ± 1 °C) water ~17 cm deep, within an opaque, cylindrical tank (diameter = 24 cm, height = 53 cm). Swimming behaviour was recorded on video for 5 min and scored by a condition-blind observer with the Observer software (Noldus Corp.). Latency to float, total number of floating bouts, and total time spent floating served as dependent measures. High percent time floating is interpreted as an increased depressive-like response [31].

2.4. Radioimmunoassay

Blood samples (~0.20 ml) were collected for radioimmunoassay (RIA) of corticosterone from the retro-orbital sinus of mice prior to entering the experimental treatment condition, after two weeks of experimental light condition, and at death. Blood samples were allowed to clot, the clot was removed, and the samples were centrifuged at 4 °C for 30 min at 6000 rpm. Serum aliquots were then aspirated and stored in sealable polypropylene microcentrifuge tubes at −80 °C until assayed. Total serum corticosterone concentrations for mice were determined in duplicate using an ICN Diagnostics ^{125}I double antibody kit (Costa Mesa, CA, USA). The high and low limits of detectability of the assay were 1200 and 3 ng/ml, respectively. All procedures were followed as described by the manufacturer guidelines.

2.5. Statistical analyses

Main effects of light condition (LD, LL) and tube condition (LE, C), and interactions thereof, on behavioural measures were assessed using one-way analysis of variance (ANOVA). *Post hoc* statistical analyses were performed using unpaired *t*-tests because pair-wise comparisons were limited. Mean differences were considered statistically significant when $p \leq 0.05$.

3. Results

3.1. Open field

LL and LE affected rearing in the open field ($F_{1,20} = 5.488$; $p < 0.05$; Fig. 1). Among LL mice, presence of an LE tube in the home cage sig-