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Exposure to dim light at night during early development increases adult anxiety-like responses

Jeremy C. Borniger *, Zachary D. McHenry, Bachir A. Abi Salloum, Randy J. Nelson

Department of Neuroscience, The Ohio State University Wexner Medical Center, Columbus, OH 43210, USA

HIGHLIGHTS

- Early life dim white light exposure reduced post-weaning body weight gain that normalized during adolescence.
- Early life dim light exposure increased anxiety-like responses in adulthood on the elevated plus and passive avoidance tests. No differences were observed on the open field, forced swim, novel object, or sucrose anhedonia tests.
- No differences were observed in *bdnf* expression in the hippocampus or pre-frontal cortex in adulthood.
- No differences in corticosterone were observed at 3 weeks of age.

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ABSTRACT

Early experiences produce effects that may persist throughout life. Therefore, to understand adult phenotype, it is important to investigate the role of early environmental stimuli in adult behavior and health. Artificial light at night (LAN) is an increasingly common phenomenon throughout the world. However, animals, including humans, evolved under dark night conditions. Many studies have revealed affective, immune, and metabolic alterations provoked by aberrant light exposure and subsequent circadian disruption. Pups are receptive to entraining cues from the mother and then light early during development, raising the possibility that the early life light environment may influence subsequent behavior. Thus, to investigate potential influences of early life exposure to LAN on adult phenotype, we exposed mice to dim (~5 lux; full spectrum white light) or dark (~0 lux) nights pre- and/or postnatally. After weaning at 3 weeks of age, all mice were maintained in dark nights until adulthood (9 weeks of age) when behavior was assessed. Mice exposed to dim light in early life increased anxiety-like behavior and fearful responses on the elevated plus maze and passive avoidance tests. These mice also displayed reduced growth rates, which ultimately normalized during adolescence. mRNA expression of brain derived neurotrophic factor (BDNF), a neurotrophin previously linked to early life environment and adult phenotype, was not altered in the prefrontal cortex or hippocampus by early life LAN exposure. Serum corticosterone concentrations were similar between groups at weaning, suggesting that early life LAN does not elicit a long-term physiologic stress response. Dim light exposure did not influence behavior on the open field, novel object, sucrose anhedonia, or forced swim tests. Our data highlight the potential deleterious consequences of low levels of light during early life to development and subsequent behavior. Whether these changes are due to altered maternal behavior or persistent circadian abnormalities incurred by LAN remains to be determined.

1. Introduction

Early life experiences can promote changes in brain structure and function that persist into adulthood, ultimately influencing adult behavior, health, and disease [34,35]. Extensive research has focused on early life environment in relation to altered temporal patterns of gene expression, HPA axis sensitivity, and neurogenesis implicated in psychiatric disease and neurodevelopmental disorders [4,7,54]. Although most early environmental studies have focused on spatial environmental perturbations, temporal alterations may also influence adult phenotype as well. The circadian system is uniquely situated to transduce photic input to influence neuronal function. Not only do intrinsically photosensitive retinal ganglion cells (ipRGCs) innervate the suprachiasmatic nucleus (SCN), they make direct connections to brain areas involved in the regulation of behavior [1,48,71,76].

Within the past century, increased levels of light at night have become ubiquitous throughout the developed world; this light affects both domesticated and wild animals [57,63]. Also, 99% of the human

* Corresponding author at: 636 Biomedical Research Tower, 460 W. 12th Avenue, Columbus, OH, 43210, USA.
E-mail address: Jeremy.Borniger@osumc.edu (J.C. Borniger).

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population in the United States and Europe are exposed to regular 'light pollution' [31,57]. Furthermore, ~14% of newborn infants of low weight worldwide are kept in neonatal intensive care units (NICUs) under abnormal light environments [23,65]. Neonates have increased susceptibility to retinal light exposure, due to increased pupillary size and decreased reactivity of the pupil in response to light [17,66]. Studies of nurseries have demonstrated wide variations in light levels [19,47] and continuous dim light exposure is common and has been related to abnormal weight gain and retinopathy of prematurity [22]. Although there has been extensive investigation of the use of "night-lights" in relation to the development of myopia [36,37,62,70], and preliminary research on bright and dim 'tablet' use in adolescence [39], little is known about how differences in ambient lighting may influence behavioral development.

The light environment may influence offspring development directly via mistimed signaling from ipRGCs in the retina or indirectly through modulation of maternal behavior. The extent and quality of mother-pup interaction is a powerful determinant of offspring behavior later in life (e.g., [25,33,43,78]). Because the maternal circadian system acts as the primary entraining cue to offspring during embryonic and early neonatal life [56], it has been investigated in relation to maternal behavior and mother-pup interactions. Altered adult behavioral and gene expression phenotypes have been observed in offspring reared by mothers kept in short photoperiods [74] or with mutations in the core clock gene *Clock* [40,45]. Artificial lighting has been linked to altered avian reproductive physiology [29], and the physiological and behavioral implications of dim nighttime light (~5 lux) exposure have been investigated in several species of rodents in adulthood (e.g., [5,10,18,30,41]). The enduring effects of dim light exposure during early development on adult phenotype remain largely unknown.

We hypothesized that dim light exposure during development alters behavioral responses in adulthood. We predicted that mice maintained under dim light at night conditions would display altered depressive- and anxiety-like responses and learning and memory deficits in adulthood compared to mice kept in dark night conditions. Specifically, we predicted that early post-natal LAN exposure would elicit the strongest behavioral changes in adulthood, as this is when light (and not maternal cues) becomes the primary zeitgeber for entrainment [23]. Changes in adult behavior following modulation of the early life environment have also been linked with altered neurotrophin expression [16,20,21,68]. Specifically, early life stress and maternal separation decreases *bdnf* expression in the prefrontal cortex (e.g., [68]) and hippocampus (e.g., [67]), which are crucial areas in the regulation of mood and cognition. We therefore predicted that dim light at night would alter central *bdnf* expression in adulthood, potentially contributing to altered behavioral phenotypes.

2. Methods

2.1. Animals and lighting conditions

Thirteen male and female adult (>9 weeks old) Swiss-Webster (Charles River Laboratories, International, Inc.) mice were group housed (4-5 per cage) and allowed 1 week to acclimate to our laboratory and a 14:10 light/dark schedule upon arrival. Over the course of the following two weeks, male and females were paired to allow mating for a two hour period during the early dark phase. Once pairs were observed mating (i.e., lordosis followed by intromission), females were assumed pregnant and were moved with the stud males into one of two experimental conditions: (1) either a 14:10 light (~150 lx)/dark (0 lx) cycle or (2) a 14:10 light (~150 lx)/dim light (~5 lx) cycle (Lights on: 0200 h, Lights off: 1600 h Eastern Standard Time (EST)). Cool white light emitting diodes (LEDs) were used to ensure even dim light distribution at cage level as previously described [11]. This study was conducted under approval of The Ohio State University Institutional Animal Care and Use Committee and procedures followed the National Institutes of Health *Guide for the Use and Care of Laboratory Animals* and international ethical standards [61].

Males were removed from the cage 10 days after mating to ensure that pregnancy was achieved over multiple mating bouts and prevent the possibility of a subsequent pregnancy after primary parturition. Upon birth (~21 days), offspring were counted and cages were kept in their pre-natal light conditions, or moved to the opposite light condition to examine post-natal influences of the light environment. This resulted in 4 groups of a full-factorial design with either pre-natal (DLAN-LD; $n = 16$), post-natal (LD-DLAN; $n = 34$), or pre- and post-natal (DLAN-DLAN; $n = 39$) dim light, or no dim light (LD-LD; $n = 22$) exposure (see Fig. 1 for experimental design overview). At 3 weeks of age, all mice were briefly anesthetized under 1.5% isoflurane and ear-punches were made to aid in identification. Mice were sexed and split by sex at this time as well. Regardless of experimental group, mice were placed into dark night conditions at weaning (21 days of age) until the conclusion of the study, effectively restricting dim light exposure to discrete developmental stages. To limit variations in litter size from contributing to body mass or other variables, at post-natal day 10, the sexes of all pups were determined (via anogenital distance inspection) and litters were culled down to a maximum of 10 pups per litter (5/13 litters were culled). Care was taken to cull an equal amount of male and female pups when possible.

2.2. Behavior

At nine weeks of age, mice underwent a battery of behavioral tests consisting of the following assays in order: the open field, elevated plus maze, passive avoidance test, forced swim test, novel object recognition, and sucrose anhedonia. These tests were chosen as adult exposure to dim light at night or early life experiences have been demonstrated to affect performance on these tasks (e.g., [8,9,11,45,74]). The open field and passive avoidance tests were conducted during the light phase and all other tests were conducted under dim red light during the dark phase. Mice were allowed to acclimate to the testing room for at least 30 min prior to all tests. The novel object, forced swim, and elevated plus maze tests were scored via video tape by a single observer unaware of experimental conditions using Observer XT 8.0 software (Noldus Information Technology, Leesburg, VA, USA). The open field and passive avoidance tests were collected and scored automatically using computerized software (described below).

Day 1: The open field chamber consisted of a 40 × 40 cm transparent acrylic box flanked by two stacked grids of intersecting infra-red beam emitter/detectors. Mice were placed into the apparatus with fresh cage nesting material loosely covering the ground and left to acclimate for 5 min before starting the test. All open field testing was completed between 900–1200 h. Total locomotor activity and central tendency was measured over a single 10 minute interval automatically using Photobeam Activity System (PAS) software (San Diego Instruments, San Diego, CA). Between each session, chambers were wiped clean with soap and water to eliminate olfactory cue carryover without introducing a potential aversive stimulus.

Anxiety-like behavior was assessed on the elevated-plus maze over a single period during the early dark phase (0–2 h after lights off). Mice were placed in the center of the platform facing a closed arm, and the following variables were scored for the subsequent 5 minutes: time in center (time to enter either arm), time spent in open arms, time spent in closed arms, number of closed arm entries, and number of open arm entries. Upon entering a closed or open arm, mice were scored as remaining on that arm until they entered a different (open or closed) arm of the maze.

Day 2: Mice were tested in the passive avoidance apparatus (Gemini Avoidance System; San Diego Instruments, San Diego, CA) over the course of two days. On the first day, individual mice were placed into the right chamber of the apparatus and allowed 30 sec to acclimate to their surroundings. After acclimation, a light would turn on (CS) and a

Fig. 1. Experimental Design. Pregnant mothers were maintained in either dark (groups 1 and 2) or dim (groups 3 and 4) night conditions during gestation and then switched to dark (group 3) or dim (group 2) nights on the date of birth (DOB; P0). After weaning at P21, all pups were separated by sex and maintained in dark night conditions until the conclusion of the study.

gate separating the two chambers would open, allowing free passage into the neighboring dark chamber. The apparatus would measure latency to enter the dark chamber (LAT1). As soon as the mouse entered the dark chamber, the gate would close, and the mouse would receive a 1.5 mA shock (US) for 2 sec. Following the shock, mice would be immediately returned to their home cage. The inside of each chamber was thoroughly wiped with 70% ethanol followed by distilled water (dH_2O) after each test.

Day 3: Approximately 24 h following the shock, mice were again tested for latency to cross into the dark chamber (LAT2). Mice were placed into the right chamber and allowed 30 sec to acclimate. The light (CS) would turn on, and the gate would open, allowing free movement between the right and left chamber. The latency to enter the dark chamber was measured, with a maximum test time of 5 min. Longer latencies to enter the chamber where the animal previously experienced a shock are interpreted as evidence of fear memory retention [2,58], and have been linked to anxiety-like behavior and fearfulness [15].

Day 4: Mice were tested for depressive-like behavior on a 6 minute forced swim test 0-2 h after lights off. Briefly, mice were placed into clear 3000 mL containers filled with ~2000 mL room temperature water (~22 °C). They were videotaped for the following 6 min and then removed, patted dry, and placed into a new cage to be singly housed for the remaining tests. Containers were emptied and cleaned between each test. Upon scoring, the first 2 min were omitted due to very little floating activity usually observed in this timeframe [24]. The final four minutes were analyzed for time spent swimming (i.e., vigorous swimming or scratching directed at the wall of the container) and time spent immobile (i.e., minimal movement necessary to keep head elevated above water surface). Increased immobility time is interpreted as a 'learned-helplessness' behavior indicative of a depressive-like response [8]. Following the forced swim test, mice were separated and individually housed (to allow sucrose preference to be tested) for the rest of the study. To allow acclimation to a new 2-bottle water system, and test for side preference, mice were supplied 2 bottles of water daily for 3 days prior to a sucrose solution being added to one bottle (see day 7).

Day 5: During the early dark phase (0-2 h after lights off), mice were placed into an empty 45 × 24 × 22 cm black chamber and allowed to freely explore the enclosure for 10 minutes. This acclimation day was to control for the novelty of the chamber itself in the day 6 novel object test. The chamber was cleaned with 70% EtOH between animals.

Day 6: Approximately 24 h later, mice were tested in the novel object paradigm in the same black containers they had explored the previous day. As before, mice were allowed 10 min to explore the chamber, this time with two identical objects (small (<10 cm diameter) convex aluminum half-circles) placed ~20 cm equidistant on the right and left from the center of the chamber. Mice were scored for amount of time exploring the cage, or amount of time investigating the right or left object to determine if a side preference existed. Investigation was defined as facing the object and actively sniffing or touching the object. Both the familiar and novel objects were selected to be hard for the animals to climb on and any climbing behavior was not scored as 'investigation'. After 10 min, mice were removed from the chamber and returned to their home cage for a 2 h period. Then, mice were placed into the chamber with one familiar object (aluminum half-circle), and one novel object (a plastic inverted funnel) randomly placed on either the left or the right side for 5 min. Mice were scored on time investigating either the familiar or novel object for the first two minutes of the test. Data are represented as a discrimination index (DI), where DI = (Total Time with Novel Object − Total Time with Familiar Object)/(Total Time with Novel Object + Total Time with Familiar Object). Positive values represent a preference for the novel object over the familiar object, zero values indicate no preference, and negative values indicate preference for the familiar object.

Day 7–9: On days 7–9, mice were tested for anhedonia-like behavior on the sucrose preference test. Two water bottles were presented as before (starting on day 4), except one contained a 1% sucrose solution. Bottles were weighed immediately before and 24 h following placement into the cage for 2 consecutive days. Values from the second day were used during analysis to further reduce novelty effects of the sucrose solution. Reduced sucrose preference in rodents is interpreted as an anhedonic response and is indicative of a depressive-like state [9,11,69,80].

2.3. Gene expression

Following behavioral testing (~10 weeks of age), mice were deeply anesthetized under isoflurane vapors and then rapidly decapitated in the light phase during a three hour window from 900-1200 h. Trunk blood was collected and brains were dissected out. Hemispheres were immediately separated and placed on ice in RNAlater reagent (Qiagen). Hemispheres remained in RNAlater for ~1 week at 4 °C, and then hippocampi and a section of the pre-frontal cortex were dissected out and maintained at -80 °C until RNA extraction.

RNA extraction was completed using TriZol reagent (Life Technologies, Thermo Fisher Scientific Inc.) according to the manufacturer's instructions. RNA pellets were resuspended in 30 μL sterile RNase-free water and quality was determined using a spectrophotometer (NanoDrop, Thermo Fischer Scientific Inc.). If RNA yield was low (<200 ng/μL) or quality was compromised (260/280 or 260/230 ratio < 1.8), RNA was re-extracted from the contralateral hemisphere. cDNA was synthesized using M-MLV reverse transcription and diluted 1:15 for subsequent PCR. Inventoried primer/probe pairs from Applied Biosystems (Life Technologies) for mouse brain derived neurotrophic factor (*bdnf*) (Assay ID: Mm01334047_m1; catalog #: 4453320) were used. A probe that spans multiple exons was used to ensure amplification of mature mRNA and not residual genomic DNA. The universal 2-step RT-PCR cycling conditions used were as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. BDNF expression was normalized to human rRNA 18 s signal and quantified using a relative standard curve as previously described [81]. All samples were run in duplicate single-plex reactions.

2.4. Corticosterone quantification

To determine whether early life dim light at night exposure elicits a physiologic stress response (i.e., elevated glucocorticoids), we assayed corticosterone levels in a separate cohort of pups in each lighting condition at 3 weeks of age (n = 7–13 pups/group). At ZT 7 at post-natal day 21, individual pups were removed from the litter and brought to an adjacent procedure room. Quickly, a small (<40–50 μL) blood sample was taken via submandibular bleed. The pup was then immediately returned to its mother (<1.5 min per pup). ZT 15 samples were taken the following day to allow corticosterone elevations from the stress of the initial measurement to return to baseline. The ZT 15 measure was taken under dim red light conditions as this time point corresponds to 1 h after dark phase onset. Blood from all pups at each time point was collected as quickly as possible (<7 min from start to finish all mice), and gloves were changed between each successive blood draw to minimize olfactory carryover between subjects. Serum was stored at −80 °C until an EIA was performed. There was no correlation between order of sampling and subsequent corticosterone levels ($R = -.053$, $p > .05$). Initially, serum was diluted in assay buffer and dissociation reagent 1:100 and corticosterone was determined via Enzyme Immunoassay (K014-H1; Arbor Assays, Ann Arbor, Michigan, USA) on a single plate following manufacturer instructions. The maximum intra-assay co-efficient of variation (CV) was 12.29% with an average inter-well CV of 4.32%.

2.5. Statistical analysis

Outliers were identified for each variable within each lighting group using the box plot method [79]. Initially, repeated measures ANOVAs were used to examine potential differences between groups in body mass over time with litter as a covariate (with post-birth week as the within groups variable and light condition as the between groups variable). General linear model (GLM) univariate ANOVAs were used to examine differences between sexes and then lighting groups in other analyses. If significant *F* values between the sexes were detected, groups were analyzed within each sex separately. ANOVAs between light conditions were conducted followed by post-hoc Tukey HSD tests if initial *F* values were significant. Mean differences were considered statistically significant when $p \leq 0.05$. Statistics were completed using SPSS Statistics version 21 (IBM, Armonk, NY, USA) and visualized using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Body mass

Because a significant degree of sexual dimorphism in body size exists in Swiss Webster mice, body mass gain was analyzed within each sex separately. Differential exposure to dim light at night influenced body mass trajectories in both males and females. Males that were exposed to dim LAN weighed significantly less at week 5 ($F(3,50) = 3.148$, $p < 0.05$) and then more at week 10 ($F(3,46) = 3.643$, $p < 0.05$) compared to males exposed to dark nights. Females exposed to dim LAN weighed significantly less at week 5 ($F(3,44) = 3.947$, $p < 0.05$), 6 ($F(3,44) = 5.063$, $p < 0.05$), and at week 7 ($F(3,41) = 2.352$, $p < 0.05$). Relationships among lighting groups in body mass gain between each sex are presented in Fig. 2.

3.2. BDNF expression

Mean BDNF did not differ between the sexes within the prefrontal cortex ($F(1,94) = .612$, $p > 0.05$) or hippocampus ($F(1,99) = .002$, $p > 0.05$). Therefore, sexes were combined for subsequent analyses. Pre-and post-natal light conditions did not influence adult BDNF expression in the PFC ($F(3, 92) = 1.892$, $p > 0.05$) or hippocampus ($F(3,97) = 1.343$, $p > 0.05$) (Fig. 3).

3.3. Corticosterone

Total corticosterone did not differ among light condition groups at 3 weeks of age ($F(3,34) = .284$, $p > 0.05$) (Fig. 4). Corticosterone concentrations did not differ between 800 (ZT 7) or 1600 (ZT 15; one hour after lights off) ($F(1, 36) = .185$, $p > 0.05$). Corticosterone did not differ among light condition groups at ZT 7 ($F(3,12) = 1.696$, $p > 0.05$) or ZT 15 ($F(3, 18) = .148$, $p > 0.05$). These data indicate that at 21 days of age, dim light at night exposure does not elicit an enduring physiologic stress response.

3.4. Elevated plus maze

Adult anxiety as assessed on the elevated plus maze increased after early life dim light exposure. Males and females did not differ in time spent in the open arms ($F(1,100) = .176$, $p > 0.05$) or in latency to enter either arm ($F(1,100) = .003$, $p > 0.05$). Therefore, sexes were combined for these analyses. Light treatment groups differed in time spent on the open arms ($F(3,98) = 3.701$, $p < 0.05$) and in latency to enter either arm ($F(3,98) = 5.226$, $p < 0.01$). Specifically, DLAN-LD mice showed the greatest anxiety-like response in time in the open arms (vs. LD-LD $p < 0.01$) and DLAN-DLAN showed the greatest response in latency to enter either arm (vs. LD-LD $p < 0.05$). Group differences are shown in Fig. 5.

3.5. Open field

Within the open field, males showed greater central tendency than females ($F(1,100) = 5.270$, $p < 0.05$), and no differences in rearing behavior ($F(1,100) = .268$, $p > 0.05$) or total locomotor activity

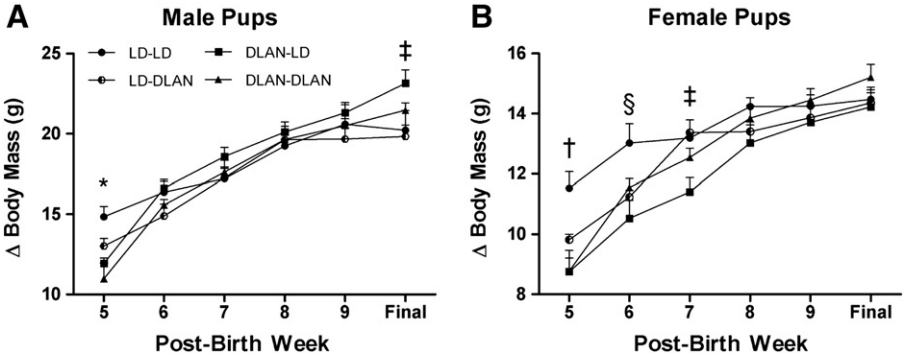

Fig. 2. Early life dim light at night alters early post-weaning body mass trajectory. (A) Post-weaning body mass gain in male and (B) female pups. (LD-LD N = 9 male, 6 female; LD-DLAN N = 14 male, 20 female; DLAN-LD N = 10 male, 6 female; DLAN-DLAN N = 22 male, 16 female). Error bars represent SEM. (Significance markers: P < 0.05 * = LD-LD vs DLAN-DLAN; † = LD-LD vs DLAN-LD, DLAN-DLAN; ‡ = LD-DLAN vs DLAN-LD; § = LD-LD vs LD-DLAN, DLAN-LD).

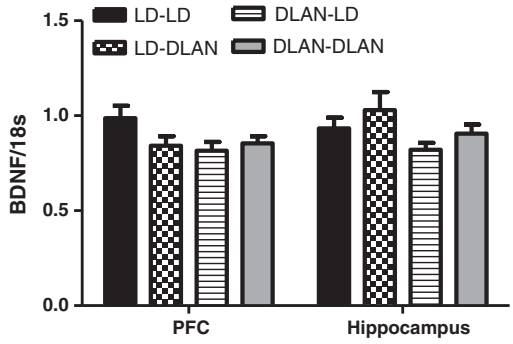

Fig. 3. Early life dim light does not significantly alter BDNF expression in the pre-frontal cortex or hippocampus in adulthood. BDNF expression is normalized to 18 s rRNA signal. Error bars represent SEM. LD-LD N = 17; LD-DLAN N = 24; DLAN-LD N = 16; DLAN-DLAN N = 39.

($F(1,100) = 1.846$, $p > 0.05$). Males of each light condition group did not differ in rearing behavior ($F(3,49) = 1.89$, $p > 0.05$), central tendency ($F(3,49) = 1.17$, $p > 0.05$) or in total locomotor activity ($F(3,49) = 1.647$, $p > 0.05$). Similarly, females of different lighting groups did not differ in rearing behavior ($F(3,45) = 1.81$, $p > 0.05$), central tendency ($F(3,45) = 1.024$, $p > 0.05$) or total locomotor activity ($F(3,45) = 1.522$, $p > 0.05$). These data indicate that early life dim LAN does not influence adult behavior on the 10 minute open field test.

3.6. Passive avoidance test

Early life dim light exposure increased latency to cross into the dark chamber in the passive avoidance task 24 h following shock. Males and females did not differ in time to cross into the dark on the acquisition day ($F(1,101) = 2.003$, $p > 0.05$), but differed in test latency to cross into the dark ($F(1,101) = 7.303$, $p < .01$), indicating a sex difference in performance on this task. Therefore, subsequent analysis of the passive avoidance tests assessed males and females separately. Acquisition latency to cross into the dark chamber was not different between lighting groups within males ($F(3,49) = 1.081$, $p > 0.05$) or females ($F(3,46) = 2.357$, $p > 0.05$). However, significant differences among light condition groups within males ($F(3,49) = 6.668$, $p < 0.01$) and females ($F(3,46) = 6.074$, $p < 0.01$) were detected in latency to enter the dark chamber 24 h post-shock, with animals exposed to dim LAN displaying increased latency to cross. LD-LD females had reduced latencies on the test day compared to LD-DLAN ($p < 0.05$), DLAN-LD ($p < 0.01$), and DLAN-DLAN ($p = <0.01$) females. Similarly, LD-LD males had reduced test day latencies compared to LD-DLAN ($p = 0.01$), DLAN-LD ($p < 0.01$), and DLAN-DLAN ($p < 0.01$) males. These data indicate that mice exposed to dim LAN during development exhibited more fearful behavior 24 h post-shock in the passive avoidance test. Differences between sexes and among light condition groups are shown in detail in Fig. 6.

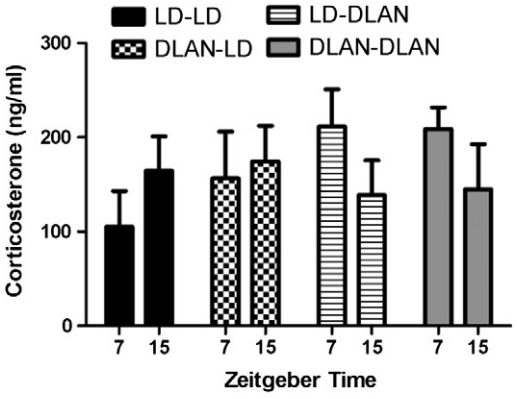

Fig. 4. Early life dim light at night does not elicit a stress response in pups at 3 weeks of age. Corticosterone was sampled at ZT 7 (800 h) and 15 (1600 h). Error bars represent SEM. (LD-LD N = 13; DLAN-LD N = 9; LD-DLAN N = 9; DLAN-DLAN N = 7).

3.7. Forced swim

Males and females differed in % time immobile during the forced swim test ($F(1,101) = 3.934$, $p < 0.05$). Therefore, light treatment groups were further analyzed separately for each sex. Neither males ($F(3,49) = .661$, $p > 0.05$) nor females ($F(3,46) = 3.53$, $p > 0.05$) in each light treatment group differed in % time immobile, suggesting no effect of early life dim light at night exposure on this measure of depressive-like behavior.

3.8. Sucrose anhedonia

No difference in sucrose preference was observed between males and females ($F(1,101) = .857$, $p > 0.05$), or among light condition groups ($F(3,99) = 1.312$, $p > 0.05$).

3.9. Novel object recognition

Mice did not display a preference for either the left or the right object during the 10 minute acquisition trial (mean time with left object = 44.2 sec (Standard Deviation (SD) = 15.6), right object = 37.58 sec (SD = 14.67)) suggesting that no side bias was apparent. Males and females did not differ in amount of time spent with the left ($F(1,100) = 2.285$, $p > 0.05$) or right ($F(1,100) = .119$, $p > 0.05$) object during the 10 min acquisition.

After a 2 h inter-trial interval, males and females did not differ in preference for a novel object (discrimination index) ($F(1,100) = .158$, $p > 0.05$). Therefore, sexes were combined for analysis of novel object recognition between treatment groups, which did not differ in preference for a novel object ($F(3,98) = 1.066$, $p > 0.05$). Dim LAN during development does not seem to influence adult novel object recognition performance.

4. Discussion

Our data indicate that dim light exposure during early life can have transient effects on weight gain and long lasting effects on anxiety-like behavior persisting into adulthood. Pups exposed to dim LAN pre- and post-natally reduced post-weaning growth rates (Fig. 2), decreased time spent in the open arms of the elevated plus maze, and increased latency to cross into the dark chamber 24 h post-shock in the passive avoidance test (Figs. 3, 4). The anxiety-like phenotype on the elevated plus maze was strongest in mice exposed to dim LAN prenatally (DLAN-LD) and both pre- and post-natally (DLAN-DLAN), suggesting a critical period for the effect of light on adult phenotype may exist during gestation.

LAN could elicit an anxiety-like phenotype in adulthood by altering maternal circadian output, mother-pup interaction, or by influencing developing photic entrainment functions within the pups directly. Although maternal circadian function or behavior was not assayed in the present study, its deregulation by light at night likely contributed to behavioral differences observed in pups. This is reasonable because mice exposed to LAN prenatally, but not postnatally (DLAN-LD) displayed similar anxiety-like responses to mice receiving dim light pre- and post-natally (DLAN-DLAN) (Figs. 3, 4). Because these pups were not exposed to aberrant lighting after birth, their unique behavioral phenotype was likely influenced by disruption of a maternal entrainment signal via LAN. Additionally, when pups received LAN during the time when they characteristically switch from maternal to photic entrainment cues (LD-DLAN), they did not display increased anxiety-like responses on the elevated plus maze (Fig. 3), further suggesting a strong role of maternal circadian function in the development of subsequent

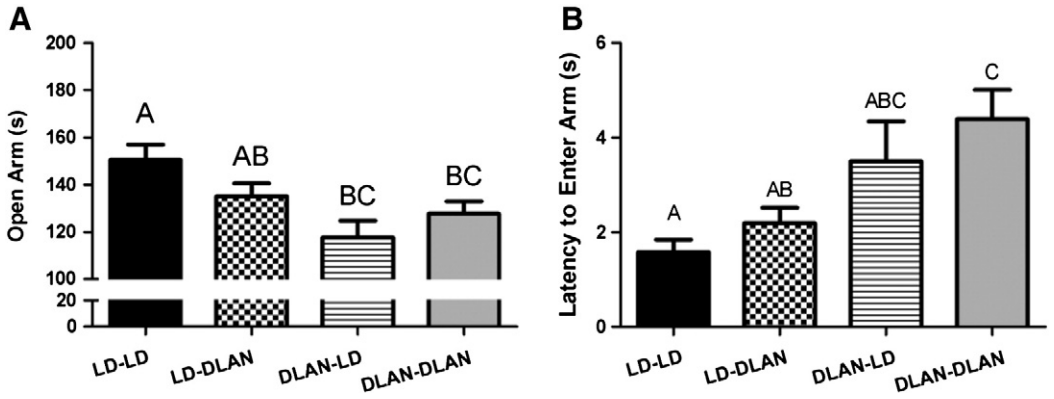

Fig. 5. Early life dim light at night alters anxiety-like responses in adulthood. (A) Time spent on the open arms of the elevated plus maze during a 5 min test and (B) latency to enter either arm after being placed in the center facing the closed arm. Error bars represent SEM. (LD-LD N = 17; LD-DLAN N = 31; DLAN-LD N = 16; DLAN-DLAN N = 38).

behavioral phenotypes. In the passive avoidance paradigm, the increased latency to cross into the dark chamber 24 h post-shock (LAT2) in LAN exposed mice is suggestive of an anxiety-like response. Anxiety traits and performance on this test are intimately linked because anxiety appears to enhance performance in emotional memory tests [15,50]. The avoidance of potential harm (i.e., increased LAT2) is a key component of anxiety and anxiety responses in rodents and humans, and an exaggerated fear response is characteristic of pathological anxiety [27,46]. Whether increased LAT2 in LAN exposed mice demonstrates 'normal' anxiety or a 'pathological' response requires further investigation [14]. The lack of an anxiety-like response in the open field may be due to circadian variation in behavioral responses [77], as the open field test was conducted during the light (inactive) and the elevated plus maze test was conducted during the dark (active) phase. Furthermore, the open field test lasted 10 minutes, while the elevated plus task consisted a single 5 minute session. 10 minutes may be too long to resolve differences in anxiety-like behavior between groups in the open field in the present study.

To assess whether early life dim light exposure elicits a stress response, corticosterone was assayed in a separate cohort of mice at 3 weeks of age. We determined that early-life dim LAN exposure does not elicit chronic glucocorticoid elevation in pups at weaning (Fig. 6), further suggesting that the phenotype we observed were not a result of environmental stress. Maternal behavior is modulated by environmental stress and variations in maternal care strongly influence the development of stress reactivity and behavior in pups [32,49,55]. This raises the possibility that the mother, but not the pups, interpreted LAN as a stressor. This is unlikely, as we have previously demonstrated that chronic dim LAN does not increase baseline corticosterone in adulthood [8,30]. Dim LAN, however, does influence the circadian rhythm of glucocorticoid secretion in Siberian hamsters (*Phodopus sungorus*) [13], and it seems reasonable that a similar phenomenon occurs in mice. It is possible that corticosterone phase, but not amplitude, may be modulated by dim LAN. Whether this subsequently alters maternal behavior to influence offspring phenotype remains to be determined. It should be noted, however, that we did not assay other stress-related systems (e.g., sympathoadrenal actions or endogenous opioids) in the present study, and these could additionally contribute to the behavioral phenotypes we observed in adulthood.

Physiologic stress reactivity (i.e., glucocorticoid response to an acute stressor) was not assessed in adulthood and the question of whether early life dim light exposure exacerbates stress responses remains to be determined. We previously described altered delayed-type hypersensitivity (DTH) responses to acute stress in Siberian hamsters (*Phodopus sungorus*) exposed to chronic dim light at night [12], suggesting that potential interactions may exist between the light environment and stress reactivity even when baseline glucocorticoids are unchanged.

During late embryonic and early post-natal life, the circadian system is plastic and responsive to entrainment factors (via maternal, then light cues) [23,26,28,56,64]. The SCN of rodents fully develop and produce synchronous, rhythmic output at ~10 days of age [72], and this development is susceptible to changes in the environment [42]. Circadian abnormalities may play a role in the observed phenotype indirectly as temporal organization of maternal behavior is important for normal pup development and adult behavior [59]. Mice homozygous for a mutation in the core clock gene, *Clock*, display disrupted nursing behavior and cross-fostered wild-type offspring have reduced growth rates [40]. Furthermore, these offspring display increased anxiety-like (but

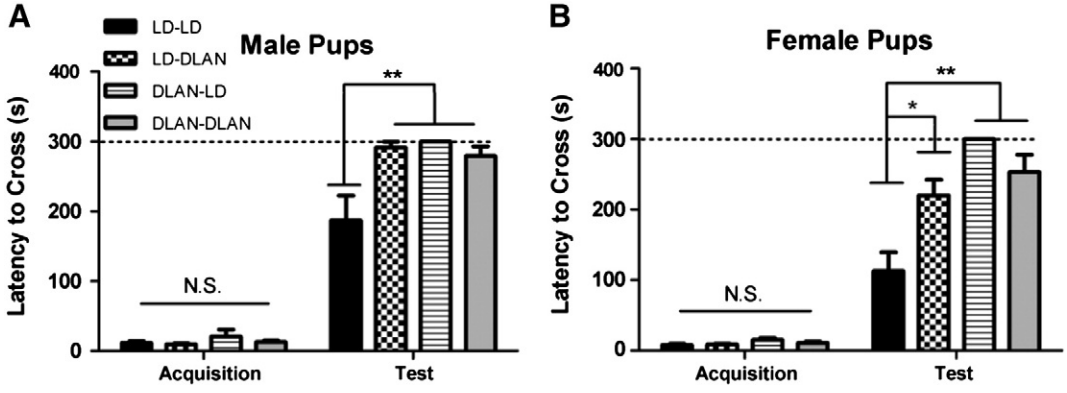

Fig. 6. Early life dim light at night alters passive avoidance responses 24 h post-1.5 mA shock. (A) Male and (B) female pup latencies to cross into the light chamber following 30 sec acclimation prior to shock (acquisition) and 1 day post-shock (test). Error bars represent SEM. NS = non-significant *p < 0.05; **p < 0.01. LD-LD N = 9 M, 8 F; LD-DLAN N = 11 M, 20 F; DLAN-LD N = 10 M, 6 F; DLAN-DLAN N = 23 M, 16 F). Dotted line represents 5-min testing time limit.

not depressive-like) behavior in adulthood and reduced brain serotonin levels at P14 [45]. The growth and behavioral phenotypes of pups exposed to dim LAN (pre- and/or post-natal) in the present study are similar to those of pups reared by *Clock* mutant mice, however it is unknown whether this is a result of altered maternal behavior prior to weaning induced by dim LAN or persistent circadian system dysfunction extending into adulthood. Environmental lighting can influence maternal behavior and fetal growth; rat dams maintained in short photoperiod (8:16) conditions display impaired pup licking and grooming, and their pups exhibit increased anxiety-like behavior and altered hippocampal glucocorticoid receptor mRNA expression in adulthood [74]. Rats exposed to constant light during pregnancy have reduced fetal weight [52], and mice subjected to repeated shifting of the light/dark cycle during pregnancy have significant trouble carrying pregnancies to term [73].

Other studies examining early life environment have largely focused on stressful (e.g., [51,54,75]) or enriching (e.g. [6,44]) stimuli and subsequent behavioral alterations in adulthood. The role of the neurotrophin BDNF in the development of altered behavioral phenotypes following early life experiences has been intensely investigated (e.g. [16,21]) with changes in the early life environment eliciting lasting epigenetic changes on the BDNF gene [68]. We were unable to detect significant differences in *bdnf* expression in the hippocampus or prefrontal cortex of LAN exposed mice, suggesting that (in adulthood) this neurotrophin is not implicated in the anxiety-like phenotype we observed. Alternatively, because behavioral and cognitive testing itself alters *bdnf* expression [38], assaying gene expression following behavioral testing may have masked actual group differences that would have remained evident in the absence of behavioral testing.

5. Conclusions

The developing nervous system is exquisitely sensitive to changes in environmental stimuli. Dim LAN exposure limited to discrete developmental stages (i.e., gestation, pre-weaning) has long lasting effects on the development of anxiety-like behavior similar to phenotypes observed in pups reared by *Clock* mutant dams. Because the circadian system is largely entrained by maternal cues during embryonic and early post-natal life, we suggest that dim LAN likely disrupts the maternal circadian system to alter mother-pup interaction. This, in turn, would impair the development of offspring behavior. Dim light induced anxiety-like behavior in mice is likely not due to a stress response or altered *bdnf* expression in adulthood. Further research is required to investigate potential disruptions of mother-pup interactions via dim LAN. In relation to human development, only a few studies have examined the effects of the neonatal intensive care environment (including light exposure) on growth and future neurobehavioral deficits (e.g., [53,60]). The exact role aberrant light exposure plays in shaping infant development remains unclear, and potential interventions have remained ineffective [3]. The present study provides preliminary evidence of a causal link between the perinatal light environment on adult behavior and growth that may be of relevance to children or newborns exposed to relatively low levels of artificial light at night.

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