Exposure to artificial light at night increases innate immune activity during development in a precocial bird


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ABSTRACT

Humans have greatly altered Earth's night-time photic environment via the production of artificial light at night (ALAN; e.g., street lights, car traffic, billboards, lit buildings). ALAN is a problem of growing importance because it may significantly disrupt the seasonal and daily physiological rhythms and behaviors of animals. There has been considerable interest in the impacts of ALAN on health of humans and other animals, but most of this work has centered on adults and we know comparatively little about effects on young animals. We exposed 3-week-old king quail (Excalfactoria chinensis) to a constant overnight blue-light regime for 6 weeks and assessed weekly bactericidal activity of plasma against Escherichia coli—a commonly employed metric of innate immunity in animals. We found that chronic ALAN exposure significantly increased bactericidal activity and that this elevation in immune performance manifested at different developmental time points in males and females. Whether this short-term increase in immune activity can be extended to wild animals, and whether ALAN-mediated increases in immune activity have positive or negative fitness effects, are unknown and will provide interesting avenues for future studies.

1. Introduction

Humans have greatly altered the night-time photic environment from local to global scales via the production of artificial light at night (ALAN; e.g., street lights, lit buildings) (Falchi et al., 2016). ALAN is problematic because it can significantly disrupt the seasonal/daily physiological rhythms (e.g., immune and reproductive physiology; Swaddle et al., 2015) and behaviors (e.g., predator avoidance, foraging; Kempenaers et al., 2016; Swaddle et al., 2015) of animals, including humans (Cho et al., 2015). Some animals avoid ALAN-rich areas or suffer significant fitness consequences from ALAN exposure, thereby rapidly selecting against some species and ultimately depleting biodiversity (Swaddle et al., 2015).

Among the diverse potential effects of ALAN on organisms, much attention has been paid to its health consequences (Navara and Nelson, 2007). Alterations to light-entrained biorhythms can impact disease exposure and susceptibility, as well as the activity of the immune system (Navara and Nelson, 2007) in a range of taxa (Bedrosian et al., 2011). In 2012, the American Medical Association House of Delegates went as far as adopting a policy statement on nighttime lighting, summarizing the deleterious effects of ALAN on a host of circadian-disruption- and melatonin-suppression-related health outcomes in humans (e.g., cancer, obesity, diabetes, depression; Blask et al., 2012).

Surprisingly, despite the wealth of work in and attention to this area, the majority of studies on ALAN and health have centered on adult animals (Moore and Siopes, 2000; Phillips et al., 2015). Growing young animals might be especially susceptible to ALAN-driven perturbations to metabolic, immunological, or tissue growth/repair systems (Fonken and Nelson, 2016). In human children, ALAN increases body mass (Pattinson et al., 2016) and suppresses melatonin secretion (Higuchi et al., 2014), which regulates both sleeping behavior and immunity. Similarly, ALAN was found to suppress immune activity in young Japanese quail (Coturnix japonica) (Moore and Siopes, 2000) and chickens (Gallus domesticus) (Kirby and Froman, 1991). However, a recent study found that ALAN significantly elevated levels of haptoglobin (an acute phase protein) in wild great tit (Parus major) nestlings, suggesting that further work is needed to refine our understanding of how ALAN affects immunological state in developing animals (Raap et al., 2016).
Here we experimentally tested the effect of ALAN exposure on the bacterial-killing response of developing king quail (Excalfactoria chinensis). We exposed 3-week-old quail to a constant overnight blue-light regime for 6 weeks and drew blood weekly to assess the ability of the plasma to kill microbes (e.g. *Escherichia coli*) ex vivo (i.e. bacterial-killing assay, which assesses basal innate immunocompetency; French and Neuman-Lee, 2012). Note that, while bactericidal activity is an integrated measure of basal innate immunity, other components not measured in this study may differ in their response to ALAN. We hypothesized that chronic ALAN exposure during development would weaken innate immunity compared to animals exposed to a regular daily light/dark cycle. Also, although prior work in very young nestling great tits found no sex differences in the immune effects of ALAN (Raap et al., 2016), we tested effects over a longer range of development (including sexual maturation) and thus hypothesized that males may be more immunocompromised by ALAN exposure than females due to photostimulation of immunosuppressive testosterone secretion (Koutsos and Klaing, 2014).

2. Methods

2.1. Study species

King quail are an ideal study species because they develop to adulthood quickly (in about 50–60 days; Pis and Lusnia, 2005), are amenable to captivity (Adkins-Regan, 2016), and are preocial (i.e., removing complex indirect, parental effects on chicks). This non-migratory species inhabits Old World (e.g. southeast Asia, Australia) coastal tropical and temperate grasslands that are dense with ground vegetation.

2.2. Pre-experiment

We artificially-incubated and hatched 41 king quail eggs. During egg incubation, humidity was kept at 45–65% during the first 13 days and then maintained above 65% during the following 11 days. We stopped automatic egg rotation on day 15, and placed each egg into an individual custom cardboard cubby (3.81 cm W × 3.81 cm L × 5.08 cm H) inside the incubator, which allowed us to track which individual hatched from which egg. On day 16, we began checking twice daily for hatches. We allowed hatchlings to dry in the incubator for 12 h after discovery before placement into one of two small plastic brooding chambers. Each brooding chamber had a ceramic heat lamp set atop the wire-mesh chamber lids to maintain a constant temperature of 37.8°C for the first week of development, which we decreased by ~5°C weekly until reaching a final ambient temperature of 23.9°C by week 3. The ceramic heat lamps produce no visible light, so no bird in our study had ever been exposed to overnight artificial light prior to experimental treatment. We had quail of 5 different plumage types (i.e. appearance was wild-type, silver, white, black, or fawn).

Two weeks after all individuals hatched, quail were split into two experimental groups (*n* = 20–21 per group; overnight-light-treated vs. overnight-dark; see more below), and each group was housed in separate tightly regulated climate- and photoperiod-controlled chambers (Conviron, Winnipeg, Canada). Within each group, quail were housed in random-sex pairs in large cages (dimensions: 38 cm L × 46 cm W × 46 cm H) with opaque barriers between cages to visually separate pairs and limit between-pair social contact. Quail were split between treatments groups evenly from the two brooders and alternating based on hatch order, to mitigate any possible confounds of group size, brooder, or hatch order. Pairs were randomly assigned. Cage floors were lined with rubber matting to prevent the quail from slipping and developing splayed legs. We allowed quail to acclimate to paired housing for 1 week before beginning the experiment. Food (Gamebird Starter Crumble, Purina, St. Louis, Missouri) and tap water were provided ad libitum and fresh daily in petri dishes. All husbandry procedures were performed by trained researchers to ensure that animal husbandry was performed similarly between groups. Husbandry procedures generally followed the guidelines for king quail (Landry, 2015) and were approved by the Institutional Animal Care and Use Committee at Arizona State University.

2.3. Room lighting

During the pre-experimental portion of the study (i.e. the first 3 weeks post-hatch), birds received no night-lighting. Our night-light manipulation began 19–23 days (depending on individual hatch date) after hatch for the full group of birds. The experimental group experienced weak blue light (ca. 0.3 lx; irradiance spectra in Fig. S1) throughout the entire subjective night (18 h light:6 h dark; chosen for optimal growth and survival of hatchlings; Landry, 2015); the blue light was shone directionally at the cages (distance approximately 0.3 m), though reflection off walls and other surfaces provided diffuse, omnidirectional exposure. This weak intensity night-lighting was chosen based on individual-level estimates of ALAN exposure in wild and free-flying European blackbirds (*Turdus merula*) (Dominoni et al., 2013a). We chose to use blue light because of its dominance in natural moon-light (Gaston et al., 2013), its increasing incorporation into artificial nightlight sources (Gaston et al., 2013), and its specific neurophysiological effects (i.e. absorption by non-visual opsins in retina and brain; Ouyang et al., 2018).

The control group had the same night-lighting structure set up in their housing room, but the night-lights were not turned on. Day lighting was solely supplied in the form of bright overhead white fluorescent bulbs (GE Landing, Ashland, Ohio). Housing rooms were window-less, so there was never any solar illumination. The experimental phase of the study lasted for 6 weeks, which was sufficient for all quail to complete skeletal growth and reach sexual maturity. At the end of the study, all birds were euthanized to confirm sex based on gonads.

2.4. Bacterial-killing assay (BKA)

Starting at 3 weeks of age (the morning before the treatment began) and continuing weekly through the end of the study, we collected blood from each quail via the alar vein (< 2% of total body mass). We used an ex vivo bacterial-killing assay to determine plasma immunocompetency (French and Neuman-Lee, 2012; Gao et al., 2016). This method measures humoral components (e.g. natural antibodies, cytokines, acute phase proteins), which are the first line of defense against invading pathogens (Tielemans et al., 2005).

Blood samples were centrifuged to extract the plasma, and then frozen at −80°C until analysis. The bacterial-killing assay does not require any specific antibodies, allowing it to be adaptable to a number of species and serving as an integrative measure of immunity. Our method generally follows the procedure outlined in French and Neuman-Lee (2012). A stock solution of 10^7 colony-forming units (CFU) was created using pre-warmed phosphate buffered saline (PBS; at 37°C) and *E. coli* (*E. coli*, ATCC no. 8739) in the form of a pellet (Epower Assayed Microorganism Preparation; Microbiologics Inc., Saint Cloud, MN). A working solution of 10^5 CFU was created from the stock solution for each BKA analysis. Every plate included 8 negative (24 μL of PBS) and 16 positive controls (18 μL of PBS and 6 μL of E. coli 10^5 CFU). Each sample well had 7 μL of plasma, 11 μL of PBS, and 6 μL of bacteria (10^5 CFU). We then added 125 μL of tryptic soy broth (6 g/200 mL nanopure water; #T8907 Sigma-Aldrich, St. Louis, MO, USA) to each well. Each plasma sample was run in duplicate in neighboring wells.

The plates were initially placed in a microplate reader (Multiskan GO, Thermo Scientific, Waltham, MA, USA) to acquire a background reading at 300 nm. The plates were then incubated at 37°C for 12 h and absorbance was again measured at 300 nm (Gao et al., 2016). We subtracted the 12-h spectral reading from the background reading, and then averaged the two values for duplicates of each sample. We
calculated percentage of *E. coli* killed by each sample as 1 minus the sample average, divided by the average of positive controls, and multiplied by 100. The negative controls were used in this assay to ensure that there was no contamination. Inter-assay (of positive and negative controls) coefficient of variation ranged from 1.26% to 4.22% and inter-assay (of samples in neighboring wells) coefficient of variation ranged from 0.75% to 2.29%.

2.5. Statistical methods

All statistical analyses were performed in the R computing environment (Vers. 3.3.2) with $\alpha = 0.05$ (R Core Team, 2014). We used a generalized linear mixed model with a beta error term. Our main model included sex, time (week), and light treatment, and their interactions as predictors, with percentage of bacteria killed as the response variable. We included both morphotype and individual identity as random effects. We compared differences within sex at each time point using beta regression, but were unable to adjust for multiple comparisons for two main reasons: first, we are unaware of any Tukey-like post-hoc multiple comparisons for mixed beta regression models; and second, other methods for correcting p-values are often considered too conservative, especially in the context of post-hoc pairwise comparisons (Nakagawa, 2004). Therefore, we report uncorrected pair-wise comparisons (Table S1).

3. Results

Prior to the night-light manipulation, there were no differences in plasma bactericidal activity among any of the groups (Fig. 1, Table S1). We found that experimental ALAN exposure significantly increased the bactericidal capacity of plasma for both males and females compared to controls, but at different time points for each sex (Table 1). After 4 or 6 weeks of treatment, for females and males respectively, the plasma of ALAN-exposed birds had greater bactericidal activity than that of control birds (Fig. 1, Table S1). Individual-level repeatability in bactericidal activity was quite low across the weeks of the experiment (bootstrapped $R = 0.014$, 95% CI: 0–0.155, $p = .378$).

4. Discussion

Counter to our predictions, we found no evidence that ALAN decreased immune activity during development in king quail. Instead, our findings likely indicate an ephemeral increase in immunocompetence during chronic experimental exposure to ALAN over development. Prior research suggests that extended photoperiod can compromise immune performance in both adult (Moore and Siopes, 2000) and juvenile birds. However, we found no evidence that ALAN-driven immunocompetition or ALAN-unexposed developing king quail. Instead, our findings likely indicate an ephemeral increase in immunocompetence during chronic experimental exposure to ALAN over development. Prior research suggests that extended photoperiod can compromise immune performance in both adult (Moore and Siopes, 2000) and juvenile birds.

### Table 1

<table>
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<th>Effect</th>
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Significant factors are shown in bold.

(Kirby and Froman, 1991), but we found that the plasma of ALAN-exposed quail showed a superior ability to kill *E. coli* than that of control birds. Our laboratory study complements a recent study on the impact of ALAN on immune activity in wild birds that showed light pollution caused an increase in an acute phase protein (haptoglobin) in great tit nestlings (Raap et al., 2016). Haptoglobin is an important, singular component of the avian immune system, but our study extends these findings using a broad metric of innate immune activity. Additionally, because king quail are precocial (unlike great tits), we could exclude in our study the potentially indirect effects of parental behavior on hatching immunity.

ALAN may have increased bacterial-killing ability in young quail through its sleep-restrictive effect. It has been shown in mice that chronic sleep restriction, especially of rapid-eye movement (REM) sleep, increases levels of cytokines (Gómez-González et al., 2012) central to immune-cell signaling. Since developing birds have proportionally higher amounts of REM sleep relative to adults (Scriba et al., 2013), this may explain why the immune-enhancing effect of sleep restriction has been observed in developing, but not adult, birds. Future studies should manipulate ALAN-exposure in adult and young birds to understand the potential physiological mechanisms underlying ALAN-driven immunomodulation.

We also found that ALAN-induced immunoenhancement manifested at different developmental time points in male and female king quail. One possible explanation for these results comes from evidence that ALAN can increase both circulating corticosteroids and sex steroids (Dominoni et al., 2013b; Ouyang et al., 2015). In male zebra finches (*Taeniopygia guttata*) there is evidence that testosterone is immunosuppressive when corticosteroids are low, but simultaneous treatment with these hormones increases immune activity (Roberts et al., 2007). We may have found sex differences in the timing of this effect due to differences in rates of sexual maturation. In Japanese quail (a species from the same phasianid subfamily – Perdicinae – as king

Fig. 1. Bactericidal activity of plasma for ALAN-exposed and ALAN-unexposed developing king quail. Week 1 sampling occurred immediately prior to the onset of the ALAN treatment. The red asterisk denotes a statistically significant difference among treatment groups within females, whereas the blue asterisk denotes a statistically significantly different among treatment groups within males (see text and Table S1 for results). Points and bars represent sample means ± SEM, respectively.
quail), females sexually mature and produce sex steroids earlier than males, and at similar developmental time periods to when we found immunoenhancing effects of ALAN in our study (Brain et al., 1988; Ottinger and Brinkley, 1979). Sex steroids may have opposing effects on the activity of various immune components, which may explain why the increase in light-treated females was short-term (Bereshchenko et al., 2018).

Though we found that ALAN increased bactericidal activity in our study, the effect was transient for females (and possibly for males, since the length of our study precludes interpretation of any lasting effects of the week 6 immune increase). To what extent might short-term enhanced bactericidal activity early in life be expected to impact fitness? Others have shown that short-term prioritized investment in immune performance comes at a cost and is traded-off with other developmental processes (Hanssen et al., 2005), limiting later-life investment into maintenance, growth, and/or reproduction (Pihlaja et al., 2006). Alternatively, early investment into immunity may train the innate immune system (Netea et al., 2011), leading to more effective innate immune responses in later life. We were able to detect short-term increases in bactericidal activity because our experimental design included both a large portion of the developmental period and regular resampling of individuals. We suggest future studies on the developmental impacts of ALAN include similar study design so that they may reveal potentially critical short-term effects. Additionally, future studies should evaluate short-term (week-to-week physiological trade-offs) and long-term (lifespan and reproductive output) effects of ALAN on immune activity and other limiting resource investments (Hanssen et al., 2005) to better understand the spectrum of costs and benefits incurred by the observed short-term, early-life boosts in immune performance. Taken together with other recent literature, our findings here highlight the sex-, life-history-, and life-stage-specific effects that ALAN exposure can have on animal health.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2019.04.002.

**References**


