Environmental Pollution xxx (2016) 1–6

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Short communication

Early life exposure to artificial light at night affects the physiological condition: An experimental study on the ecophysiology of free-living nestling songbirds☆

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ARTICLE INFO

Article history:
Received 23 June 2016
Received in revised form
4 August 2016
Accepted 6 August 2016
Available online xxx

Keywords:
Artificial light at night
Light pollution
Physiology
Development
Early-life

ABSTRACT

Light pollution or artificial light at night (ALAN) is increasingly recognised to be an important anthropogenic environmental pressure on wildlife, affecting animal behaviour and physiology. Early life experiences are extremely important for the development, physiological status and health of organisms, and as such, early exposure to artificial light may have detrimental consequences for organism fitness. We experimentally manipulated the light environment of free-living great tit nestlings (*Parus major*), an important model species in evolutionary and environmental research. Haptoglobin (Hp) and nitric oxide (NOx), as important indicators of immunity, health, and physiological condition, were quantified in nestlings at baseline (13 days after hatching) and after a two night exposure to ALAN. We found that ALAN increased Hp and decreased NOx. ALAN may increase stress and oxidative stress and reduce melatonin which could subsequently lead to increased Hp and decreased NOx. Haptoglobin is part of the immune response and mounting an immune response is costly in energy and resources and, trade-offs are likely to occur with other energetically demanding tasks, such as survival or reproduction. Acute inhibition of NOx may have a cascading effect as it also affects other physiological aspects and may negatively affect immunocompetence. The consequences of the observed effects on Hp and NOx remain to be examined. Our study provides experimental field evidence that ALAN affects nestlings' physiology during development and early life exposure to ALAN could therefore have long lasting effects throughout adulthood.

1. Introduction

Over the last 100 years, the night-time environment in much of the world has greatly been disrupted through the introduction of artificial light at night (ALAN), also known as light pollution. It is increasingly being recognised as a widespread and important anthropogenic environmental pressure on wildlife (Hölker et al., 2010). Recent studies (reviewed in Swaddle et al., 2015) have documented effects of ALAN on a wide variety of behavioural traits, such as reproduction, foraging, and migration. Several physiological effects have also been reported, including alterations in immune response, melatonin, and testosterone levels (reviewed in Swaddle et al., 2015).

The immature circadian system may be particularly sensitive to circadian disruption through artificial light as experiences during early life profoundly affect the developing brain, influence adult behaviour, physiology, health, and disease (Fonken and Nelson, 2016). ALAN can influence foraging behaviour of adult songbirds (Stracey et al., 2014) as well as begging behaviour of nestlings (Raap et al., 2016). These behavioural effects may have physiological consequences. Moreover, laboratory studies showed that ALAN can cause direct changes in physiology, including a decreased immune response to challenges, an increase in stress hormones, and a decrease in melatonin levels (reviewed in Fonken and Nelson, 2016; Swaddle et al., 2015). Effects of ALAN on individual physiological condition and health state of developing birds in the wild are therefore likely (Fonken and Nelson, 2016; Isaksson, 2015; Salmon et al., 2016) but are unknown at present.

Studies to examine the effects of ALAN in the wild are important

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.
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http://dx.doi.org/10.1016/j.envpol.2016.08.024
0269-7491/

but rare. Nonetheless, experiments using laboratory animals and wild derived animals in captivity have provided useful insights into how artificial light may affect animal physiology (see Table 1 for some particularly relevant studies on wild derived animals). Even though studying ecophysiology in the wild remains challenging, examining the effects of ALAN on developing animals in ecologically realistic situations is urgently needed because the laboratory is a simplified environment that fails to capture the complexity of natural conditions. To the best of our knowledge, experimental studies on effects of ALAN on the physiology of developing animals in the wild are completely lacking. Altered physiology together with demands of limited resources and harsh environmental conditions may however seriously impact survival outside of the laboratory. Studies in the wild have often compared urban versus non-urban populations and focused on adult individuals (see Table 2). In these types of studies effects of light pollution may be confounded by other major urban stressors such as noise (Swaddle et al., 2015) and chemical pollution (Isaksson, 2015). Experimental studies in the wild that manipulate light conditions but keep all other influencing factors as stable as possible (e.g. Ouyang et al., 2015) are therefore necessary to fully comprehend the effects of ALAN on developing animals.

ALAN may directly affect an individual's physiological condition, e.g. increased oxidative stress (Navara and Nelson, 2007), and indirectly as it decreases melatonin (Swaddle et al., 2015) which may lead to a cascade of other physiological effects (Tan et al., 2010). Melatonin has multiple functions and is involved in regulation of oxidative stress and immunological modulation (Tan et al., 2010). We therefore hypothesized that artificial light at night would affect the physiological condition of developing animals.

In this study, we experimentally investigated whether artificial light at night affected haptoglobin (Hp) and nitric oxide (NOx) in free-living developing great tits (*Parus major*). Haptoglobin is an acute phase protein that plays an important role in inflammation, infection and trauma. It is part of the non-specific immune response but also acts as an antioxidant (reviewed in Matson et al., 2012). Plasma NOx is an easily measurable multifunctional signalling molecule involved in inflammatory processes but uncontrolled production may lead to cell damage and death (reviewed in Sild and Horak, 2009). Changes in haptoglobin and nitric oxide are especially interesting as they provide useful information on changes in physiological condition, health state and innate immunity (Matson et al., 2012; Sild and Horak, 2009).

In the present study, we experimentally exposed wild great tit nestlings to two nights of artificial light (3.0 lux) and compared these to nestlings which were not exposed to ALAN. We then assessed individual changes in Hp and NOx to determine the effects of ALAN on the physiological condition of developing animals.

2. Method

2.1. Study area and general procedures

Our study was performed during the 2015 breeding season (between 8 and 25 May) in a resident suburban nest box population of great tits in the surroundings of Wilrijk, Belgium (51°9′44″N, 4°24′15″E). Nest boxes were put up in 1997 and ever since this free-living population is continuously monitored (see e.g. Rivera-Gutierrez et al., 2010; Rivera-Gutierrez et al. 2012; Van Duyse et al., 2005; Vermeulen et al., 2016b). During winter and breeding seasons, great tits are caught inside the nest boxes after which they are ringed. This study was approved by the ethical committee of the University of Antwerp (ID number 2014-45) and performed in accordance with Belgian and Flemish laws.

2.2. Experimental design

While field studies on physiology often rely on single point measurements and experiments on free-living animals are often unfeasible (van de Crommenacker et al., 2010), we used an experimental field study with repeated measurements. We looked at individual changes in physiology of wild animals caused by ALAN as this takes into account that physiological condition likely differs between individual nestlings (e.g. Vermeulen et al., 2015). We randomly assigned 32 nests to one of the two treatment groups: a control (dark) and a light treated group, which was exposed to two consecutive nights of light (day 13 and 14). We obtained a blood sample (≤150 μL) from all nestlings of a nest when they were 13 days old, to determine their baseline Hp and NOx levels and subsequently weighed them (digital balance; Kern TCB 200-1). To quantify changes in physiological condition, this procedure was repeated after two nights when the nestlings were 15 days old. Taking repeated measurements is crucial for understanding physiological responses (van de Crommenacker et al., 2010) and

Table 1
Examples of particularly relevant experiments on the physiological effects of artificial light at night on wild derived animals in captivity. All studies used adult animals.

Species	Physiological measurement	Light intensity used (lux)	Main results	References
Siberian hamsters *Phodopus sungorus*	Delayed-type hypersensitivity Blood plasma bactericidal capacity	5	*ALAN supressed immune responses* • Delayed-type hypersensitivity response was reduced • Blood plasma bactericidal capacity was reduced	Bedrosian et al. (2011)
Indian weaver birds *Ploceus philippinus*	Melatonin	Different intensities between 0.1 and 100	*ALAN supressed melatonin levels*	Singh et al. (2012)
Western scrub-jay *Aphelocoma californica*	Luteinizing hormone Testosterone Estradiol Melatonin	3.2	*ALAN did not stimulate the reproductive axis* • Luteinizing hormone was reduced in males, but not in females • Testosterone was reduced in females but not in males • ALAN increased melatonin • ALAN disrupted the correlation between testosterone and estradiol	Schoech et al. (2013)
Blackbird *Turdus merula*	Testosterone	0.3	*ALAN advanced reproductive physiology* • Earlier increase in testosterone secretion	Dominoni et al. (2013a)
Blackbird *Turdus merula*	Melatonin	0.3	*ALAN decreased melatonin secretion*	Dominoni et al. (2013c)
Blackbird *Turdus merula*	Testosterone	0.3	*Long term exposure to ALAN affects the reproductive system* • Long term exposure to ALAN caused testosterone to remain at baseline (non-reproductive state)	Dominoni et al. (2013b)

Please cite this article in press as: Raap, T., et al., Early life exposure to artificial light at night affects the physiological condition: An experimental study on the ecophysiology of free-living nestling songbirds, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.08.024