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Neither artificial light at night, anthropogenic noise nor distance from roads are associated with oxidative status of nestlings in an urban population of songbirds

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ABSTRACT

Increasing urbanization is responsible for road-related pollutants and causes an unprecedented increase in light and noise pollution, with potential detrimental effects for individual animals, communities and ecosystems. These stressors rarely act in isolation but studies dissecting the effects of these multiple stressors are lacking. Moreover, studies on urban stressors have mainly focused on adults, while exposure in early-life may be detrimental but is largely ignored. To fill this important knowledge gap, we studied if artificial light at night, anthropogenic noise and road-related pollution (using distance from roads as a proxy) explain variation in oxidative status in great tit nestlings (*Parus major*) in an urban population. Artificial light at night, anthropogenic noise and distance from roads were not associated with variation of the nine studied metrics of oxidative status (superoxide dismutase-SOD-, glutathione peroxidase-GPX, catalase-CAT-, non-enzymatic total antioxidant capacity-TAC-, reduced glutathione-GSH-, oxidized glutathione-GSSG-, ratio GSH/GSSG, protein carbonyls and thiobarbituric acid reactive substances-TBARS). Interestingly, for all oxidative status metrics, we found that there was more variation in oxidative status among individuals of the same nest compared to between different nests. We also showed an increase in protein carbonyls and a decrease of the ratio GSH/GSSG as the day advanced, and an increase of GPX when weather conditions deteriorated. Our study suggests that anthropogenic noise, artificial light at night and road-related pollution are not the most important sources of variation in oxidative status in great tit nestlings. It also highlights the importance of considering bleeding time and weather conditions in studies with free-living animals.

1. Introduction

Our world is urbanizing rapidly, with more than half of the global population already living in cities, and this proportion is still growing (UN-Habitat, 2012). The creation and growth of cities and towns impact heavily on ecological processes and have been identified as major threats to global biodiversity (McDonald et al., 2008; McDonnell and Hahs, 2015). Urbanization is responsible for modification, fragmentation and loss of habitat, with a consequent decline of many animal species (Seto et al., 2012; McDonnell and Hahs, 2015), and furthermore for chemical pollution (Grimm et al., 2008). Recently, there has been a growing interest in two major threats that are closely linked to urbanization, artificial light at night (ALAN), also known as light pollution (Hölker et al., 2010), and noise pollution (Barber et al., 2010). Light pollution is mainly caused by street lights and noise pollution is mainly caused by traffic noise (Seress and Liker, 2015).

Recent research has shown that road-related pollutants such as air pollution and both light and noise pollution may influence a large variety of physiological and behavioural aspects (Pauley, 2004; Isaksson et al., 2005; Blask, 2009; Isaksson, 2015; Swaddle et al., 2015). Chemical pollution has been suggested to affect physiology in wild animals (reviewed in Isaksson, 2015). For example, oxidative

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status appeared to be affected in urban wild great tits, *Parus major* (Isaksson et al., 2005; but see also Isaksson et al., 2009). ALAN may have direct effects on animal physiology, e.g. it suppresses melatonin (Bedrosian et al., 2016) and increases stress hormones (corticosterone) in songbirds (e.g. Russ et al., 2015; Ouyang et al., 2016). Indirect effects on physiology may also occur through altered animal behaviour, e.g. ALAN affects daily rhythms (e.g. Dominoni et al., 2014), disrupts sleep (e.g. Raap et al., 2015), affects foraging (e.g. Russ et al., 2015) and nestling provisioning in songbirds (Stracey et al., 2014). Likewise, noise pollution may have direct effects on bird physiology as it increased stress hormones (Crino et al., 2013) and decreased telomere length (Meillère et al., 2015). Noise may also indirectly affect animal physiology, e.g. through impairment of parent-offspring communication which is likely to affect offspring health (Lucass et al., 2016).

An important aspect of the physiological system that might be affected by urbanization through road-related pollutants, such as chemical, light and noise pollution, is the oxidative balance (Isaksson, 2015). Recent work has shown that oxidative stress may be a mediator of some life-history trade-offs (Costantini, 2014). This is because increased molecular damage and depletion of antioxidants may influence growth, reproductive strategies and survival. In the urban environment, stressors such as light, noise and chemical pollution may influence the oxidative balance through their effect on hormones like melatonin and corticosterone (Isaksson, 2015). In fact previous studies have shown that melatonin, which is also an antioxidant, and corticosterone, which has pro-oxidant effects, can affect the oxidative status (Yadav and Haldar, 2014; Costantini et al., 2011) together with chemical pollutants (Isaksson, 2010). Furthermore, in an urban environment, stressors rarely act in isolation. Studies that dissect the effects of these multiple stressors (e.g. light and noise pollution) are lacking and therefore they are urgently needed to comprehend the impact of urbanization on wild animals and for effective management of protected areas (Barber et al., 2010; Isaksson, 2015; Swaddle et al., 2015).

To fill this important knowledge gap, we examined if road-related pollutants can explain variation in oxidative status in an urban population of the great tit, an important model species. Because conditions experienced during early-life may produce effects that can persist throughout life, we used developing nestlings (Lindstrom, 1999; Metcalfe and Monaghan, 2001; Fonken and Nelson, 2016). We tested whether the variation in oxidative status was better explained by distance of the nest box from the road/highway (which is used as a proxy that integrates information on environmental stress at different levels), noise or light pollution in isolation or by a combination of noise and light pollution. Given the complexity of the oxidative balance (Dotan et al., 2004; Cohen and McGraw, 2009; Costantini et al., 2013), we examined multiple metrics of antioxidant protection and oxidative damage.

2. Material and methods

2.1. Study area

The study was carried out in the surroundings of Wilrijk (Antwerp), Belgium (51°9′44″N, 4°24′15″E) in a free-living urban population of great tits breeding in nest boxes. Great tits are cavity breeders that readily accept nest boxes for breeding in spring and roosting during winter. Nest boxes are made out of plywood (120 mm wide × 155 mm deep × 250 mm high) with a metal ceiling and an opening of 30 mm ø. They were installed in an urbanized area in patches of deciduous woodlands crossed by small roads illuminated during the night by street lights and close to a highway which represents the main source of background noise (Lefebvre et al., 2011). Therefore, nest boxes are exposed to different levels of artificial light and noise depending on their location (in proximity of roads or far away). Since their installation in 1997, the nest boxes have been monitored all year round (see e.g. Rivera-Gutierrez et al., 2010, 2012; Van Duyse et al., 2005; Vermeulen et al., 2016). To allow individual identification, all individuals are metal-ringed as nestlings or when first caught.

2.2. Data sampling

During the breeding season of 2015, the nest boxes were regularly checked as part of our long-term study to obtain information about the start of nest-building, egg-laying, clutch size, hatching date and fledging success. All nestlings were ringed with a numbered metal ring when they were between 10 and 13 days old (hatch day = 1). On day 15 post-hatch, between 7:45 and 15:45 (between 4th of May and 29th of June), a blood sample (≤ 150 μl) was taken from the brachial vein of the nestlings with a Microvette CB 300 lithium-heparin tube (Sarstedt, Numbrecht, Germany). The time of blood collection of each nest was recorded. Directly after blood collection, the body mass of the nestlings (± 0.1 g) was measured using a digital balance (Kern TCB 200-1). The blood samples were kept cool and centrifuged (10,000 rpm for 5 min; within an average of 2 h after sampling) to separate plasma from red blood cells. After centrifugation, the resulting plasma and red blood cells of each sample were divided into different tubes to avoid repeated thawing and freezing of the same aliquots and then stored at − 80 °C. In total, a sample of blood from 561 nestlings (85 nests) was collected. Due to blood volume limitations, sample sizes vary between oxidative status metrics (*N* = 543–561).

2.3. Light and noise measurements

Ambient light intensity and noise were measured at each nest box after sunset (May 26–28 2015, between 22:00 and 01:00). Additional noise measurements were taken during the day between 09:30 and 12:30 (June 3–5 2015). Maximum light intensity (lux) was measured with an ILM 1335 light meter (ISO-TECH, Northamptonshire, UK) by placing the photo detector vertically at the nest box opening. Noise amplitude (dB SPL re 20 μPa) was measured with a DVM 401 environmental meter (Velleman Inc., Fort Worth, TX, USA) by placing the microphone at the level of the nest box opening and registering the highest value of background noise amplitude. Both light intensity and noise amplitude were measured at the nest box opening in order to minimize disturbing the nestlings and/or parents. These measurements were considered as a proxy of the levels of light and noise pollution that the nestlings were exposed to inside the nest box. The reliability of our noise measurements was confirmed by the high correlation of noise measurements over years (see Section 2.8, Statistical analyses).

2.4. Distance to the road and the highway

We determined the distance between each nest box and the road and highway in metres using Google Earth. For 13 nest boxes the closest road was the highway and the same value is used for distance to either.

2.5. Collection of data on weather condition

Weather may affect oxidative status, especially low temperatures (Costantini, 2014). Therefore, when the nestlings were sampled, we collected data on weather conditions (rain in mm, average wind speed in km/h and temperature in °C) from a local meteorological station for the area of the city of Antwerp.

2.6. Sex determination

DNA was extracted from 1 μl of red blood cells (RBCs) using Chelex 100 resin (Bio-Rad Laboratories; Walsh et al., 1991). CHD-W and CHD-Z genes were amplified from sex chromosomes using polymerase chain reaction with primers P2 and P8 (Griffiths et al., 1998). PCR products were separated by electrophoresis on agarose gel stained with ethidium bromide and visualized by UV transillumination. Birds were sexed