Differences in the reproductive hormone rhythm of tree sparrows (*Passer montanus*) from urban and rural sites in Beijing: The effect of anthropogenic light sources

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**Abstract**

The pervasiveness of anthropogenic light in urban environments has increased the exposure to light of many animals. Since photoperiod is a regulator of the timing of reproduction in most temperate region birds, such light sources could potentially change the timing of reproduction. We compared the luteinizing hormone (LH), testosterone (T), and estradiol (E2) levels of tree sparrow (*Passer montanus*) populations sampled at two urban and two rural sites in China, and also performed a controlled photoperiod experiment to determine the influence of artificial light on the endocrine rhythm of these populations. LH levels of urban tree sparrows increased earlier than those of rural ones, but rural populations had higher LH peaks. A linear mixed model (LMM) indicates that increased exposure to light at night (LAN) significantly influenced the LH, T and E2 concentrations of free-living tree sparrows in urban environments compared to their rural counterparts. The results of the controlled photoperiod experiment showed that tree sparrows that were exposed to 6 lux of light during the dark phase of the artificial photoperiod began to secrete LH earlier, and had lower peak LH levels, than control birds. A LMM indicates that LAN had a significant effect on LH levels in this experiment. Although urban tree sparrows began to secrete LH earlier than their rural counterparts, we found no corresponding advance in T or E2 secretion. On the contrary, peak T and E2 levels of urban birds were lower than those of rural birds. These results suggest that although anthropogenic light sources appear to advance the onset of LH secretion in urban tree sparrow populations, they also lower peak LH, and consequently levels of T and E2. A possible explanation for these observations is that greater exposure to anthropogenic light in urban environments stimulates LH secretion and may influence photosensitivity, but further experimental work is required to test this hypothesis.

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1. Introduction

Anthropogenic light pollution is a consequence of global urbanization (Shochat et al., 2006) that is becoming an increasingly important problem for urban animal populations (Longcore and Rich, 2004). Light pollution threatens biodiversity by changing the normal day length (photoperiod) of animal species. Negative impacts upon animals due to LAN exposure include interference with behaviors such as migration, hibernation, and reproduction, as well as increased predation pressures (Longcore and Rich, 2004; Miller, 2006; Rodriguez and Rodriguez, 2009; Kempenaers et al., 2010).

Reproduction in birds is strongly influenced by photoperiod in mid- to high-latitude areas (Dawson et al., 2001). In natural environments, birds use reliable environmental cues to anticipate the onset of predictable environmental changes (Dawson et al., 2001). Because change in photoperiod is entirely predictable at any particular latitude, both within and among years, it is used as an important cue to time the physiological preparations required for reproduction (Wingfield, 1993; Gwinner, 2003; Dawson, 2007). Variation in photoperiod controls the initiation of reproduction through the hypothalamus–pituitary–gonad (HPG) axis. In spring, an increase in photoperiod leads to elevated secretion of GnRH-I from the median eminence at the base of the hypothalamus (Yamamura et al., 2006). This stimulates the synthesis and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the pituitary (Pawson and McNeilly, 2005). These two hormones are secreted into the blood and induce gonadal maturation. The maturing gonads secrete T and E2 which are critical for both gamete maturation and the initiation and maintenance of reproductive behaviors (Dawson, 2008).
Disturbance of light cycles by anthropogenic light may influence the HPG axis to the extent that the timing of reproduction, and ultimately breeding productivity, is affected. Partecke et al. (2005) found that urban male European blackbirds (Turdus merula) had lower plasma LH and T levels during the testicular growth period than those in forest habitats. Similarly, Schoech et al. (2013) found that experimental exposure to low levels of LAN tended to inhibit reproductive hormone secretion in the western scrub-jay (Aphelocoma californica), however, this paper did not compare the plasma LH rhythm of free-living birds with and without exposure to anthropogenic light. More recently, Dominoni et al. (2013a,c) demonstrated that exposure to light during the normal hours of darkness affected the reproductive physiology of captive European blackbirds. These authors not only found that exposure to LAN advanced both the development of the reproductive system and moult, but that urban blackbirds responded differently to this stimulus compared to rural birds.

Although the above authors all found evidence that exposure to anthropogenic light in urban environments can influence the production of hormones in urban birds by disrupting natural photoperiod cues, no one has yet tested this hypothesis by combining studies of free-living birds with controlled laboratory experiments.

In this paper, we present the results of an investigation of the relationship between increased exposure to anthropogenic light and hormone secretion in the tree sparrow (Passer montanus), a small and relatively sedentary passerine bird (Ruan and Zheng, 1991; Pan and Zheng, 2003), in Beijing, China, and at nearby rural sites. We compared plasma LH, T and E2 concentrations between free-living urban and rural tree sparrows. We also performed a controlled photoperiod experiment on birds from both habitats.

2. Materials and methods

2.1. Study area

Beijing is situated on the North China Plain in the north temperate zone. The climate is hot and humid in summer and cold, windy and dry in winter. The approximate average maximum daytime temperature in January is 1.6 °C and in July is 30.8 °C. Two urban and two rural sites within the greater Beijing municipality were selected as sampling sites. The urban sites were Zhongshan Park (39°54′34.1″N, 116°23′18.9″E; a public park in the central urban area of Beijing) and the Minzu University campus (39°56′53.8″N, 116°18′50.8″E; a university campus in the Beijing urban area). The rural sites were two small rural villages; Beilangzhong (40°12′25.9″N, 116°33′36.8″E) and Jishanying (40°11′40.4″N, 116°32′37.4″E). The night-time light intensity and air temperature at these four sample sites on each sampling day are shown in Figs 1 and 2. We measured night-time light intensity at ten randomly selected locations within each sample site at 9:00 p.m., 11:00 p.m. and 1:00 a.m. using a DT-1300 photometer. The mean light intensity at each group of ten locations was taken as the light intensity of each sample site. The maximum and minimum temperature data for each site were obtained from the Beijing Meteorological Bureau.

2.2. Sample design

Adult tree sparrows were captured with mist-nets at each sample site from March to September 2011. Birds were individually marked with leg-bands at first capture, thereby ensuring the avoidance of repeated sampling of individuals. To assess early spring patterns of LH secretion across sample sites, birds were captured on 10, 21, and 30 March and 6, 12, and 22 April in 2011. After egg-laying had commenced, birds were captured once a month until July, then captured in September to evaluate autumn LH levels. At least 25 birds were captured at each site between 6:00 a.m. and 10:00 a.m. on each sampling day. Within 1–3 min of being caught in the net, about 100 μL of whole blood was collected by puncturing a brachial wing vein with a disinfected 23 G needle and collecting the blood that exuded from the puncture site into heparin-soaked microcapillary tubes. The skin around the puncture site was disinfected with medical alcohol before and after puncturing. Pressure was applied to the puncture site for 1 min with an alcohol-soaked cotton wool swab to staunch bleeding. Blood samples were stored at 4 °C for up to 8 h until centrifuged at 10,000 rpm for 10 min. The resultant blood plasma was then kept frozen in micro-centrifuge tubes until assayed. The sex of the birds was determined from blood cells using the molecular methods described by Griffith et al. (1999).
2.3. Photoperiod experiment

Tree sparrows used in the photoperiod experiment were captured in December. Birds were captured at Minzu University, an urban site, and Beilangzhong, a rural site. Individuals captured at each site were kept in separate aviaries and acclimatized to captivity in a room in which the temperature and photoperiod were similar to those outdoors. During this acclimatization period all birds were subject to a short photoperiod (8L:16D) and a temperature of 10 °C ± 2 °C, but the birds from the urban site were exposed to an additional 6 lux of light (the mean value of all the lux readings at Minzu university) during the dark phase of the photoperiod. After one week of this acclimatization regime, the ambient temperature was increased to 20 ± 2 °C and the birds were divided into four groups; two control groups comprised of 10 urban birds and 10 rural birds that were not exposed to light during the dark phase of the photoperiod, and two treatment groups comprised of 10 urban and 10 rural birds that were exposed to 6 lux of light during the dark phase of the photoperiod. The sex ratio of each group was 1:1. The photoperiod of all groups was lengthened by 2 h per week until the fourth week when the experiment concluded. All birds received the same food and water. 50 μl of blood was taken from each bird for LH concentration analysis when they were first captured and at the end of each week of the experiment.

2.4. Hormone assay

Tree sparrow plasma LH, T, and E2 levels were measured using chicken enzyme immunoassay kits from MyBioSource (cat #MBS165746). Assay Design (cat #ADI-901-065 and -174, respectively), according to the manufacturer’s instructions. We followed the method used by Chastel et al. (2005) and Washburn et al. (2007) to dilute a tree sparrow plasma pool. Because the LH, T and E2 dilution curves of tree sparrow plasma were parallel to the standard ELISA Kit curves, we concluded that these kits can reliably assess relative levels of LH, T, and E2 in tree sparrow plasma. The respective inter- and intra-plate coefficients of variation for LH, T, and E2 were 7.02% and 6.8%, 9.3% and 7.8%, 8.3% and 2.1%.

2.5. Data analysis

The significance of any differences in blood hormone levels between the two urban sites and between the two rural sites were determined using independent sample t-test. If there were no significant between-site differences, data from the two urban or two rural sites were pooled for subsequent analysis. For free-living birds, the effects of LAN, sex and date on LH, and the effect of LAN and date on testosterone and estradiol concentrations, were analyzed with LMMs. For LH, LAN, sex, date, and the interactions between these factors, were modeled as fixed factors with sample site as a random factor. For testosterone and estradiol, LAN, date, and the interactions between these factors, were modeled as fixed factors with sample site as a random factor. Recaptures were excluded from the analysis, so all samples were independent.

Differences in the plasma LH levels of different treatment groups in the laboratory experiment were analyzed with a LMM in which LAN, sex, treatment week, sample site (urban or rural), and the interactions between these factors, were modeled as fixed factors and individual bird was as a random factor. Plasma LH, testosterone and estradiol concentrations were log transformed to correct for departures from normality and homogeneity of variance. The significance level in all tests was α = 0.05. All data analyses were performed in SPSS 16.0.

3. Results

3.1. Plasma LH, T and E2 concentration of free-living tree sparrows

There were no significant differences in LH, T and E2 concentration between either the two urban sites or the two rural sites (independent sample t-test, P > 0.05), therefore data from sites in the same habitat type (urban or rural) were pooled for subsequent analysis.

The results of a LMM indicate that LAN, date and the interaction between these two factors significantly influenced the LH concentration of free-living tree sparrows, whereas sex and all other interaction terms were insignificant (Table 1). The mean plasma LH concentrations of tree sparrows at urban and rural sites are shown in Fig. 3. Generally, the LH levels of birds first increased then decreased. Visual inspection of Fig. 3 indicates that mean LH levels of urban birds peaked on the 30th of March, earlier than those of the rural birds which peaked on the 6th of April. The peak mean LH value of urban birds was higher than that of the rural birds. The results of LMM for T indicate that date, and the interaction between date and LAN, significantly influenced T concentration (Table 1). The mean plasma T concentrations of male birds from urban and rural sites are shown in Fig. 4. Visual inspection of Fig. 4 shows that mean T levels of birds from urban and rural sites all peaked on the 12th of April and the peak mean T value of the urban birds was higher than that of the rural birds. The LMM for E2 show that date, LAN and the interaction between these two factors, significantly influenced E2 concentration (Table 1). The mean plasma E2 concentrations of female tree sparrows sampled at urban and rural sites are shown in Fig. 4. Visual inspection of Fig. 4 indicates that mean E2 levels at urban and rural sites all peaked on the 12th of April and the peak mean E2 level of the urban birds was higher than that of the rural birds.

3.2. Plasma LH concentration of captive tree sparrows during a controlled photoperiod experiment

Mean plasma LH concentrations of the four experimental groups of birds are shown in Fig. 5. Visual inspection of Fig. 5 indicates that mean plasma LH concentrations of all four groups of birds peaked at the end of the second week of the experiment and began to decrease after the third week. The mean plasma LH concentrations of the urban and rural treatment groups were higher in the second week, and lower in the third and fourth week, than those of the corresponding control groups. The results of a LMM indicate that LAN, week, and the interaction between these two factors, significantly affected LH concentration, whereas sex,

<table>
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<td>Sex</td>
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<td>Date</td>
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sample site and all other interaction terms were insignificant (Table 2).

4. Discussion

Many studies of seasonally breeding songbirds, such as house finches (Carpodacus mexicanus), European starlings (Sturnus vulgaris), white-crowned sparrows (Zonotrichia leucophrys) and dark-eyed juncos (Junco hyemalis), have revealed a general pattern of photoperiodism (Dawson, 2003; Dawson et al., 2001). Briefly, as spring approaches and day length increases, birds increase secretion of GnRH and gonadotropins (LH and FSH) which stimulate gonadal growth. Several weeks of exposure to long days usually results in the eventual onset of “photorefractoriness” (Nicholls et al., 1988), in which the HPG axis is no longer stimulated by long days, resulting in the decline of GnRH and gonadotropic secretion and eventual gonadal involution. Our results show that LH secretion in free-living tree sparrows in both urban and rural habitats follow this general pattern.

Our analysis of blood samples collected from free-living tree sparrows indicates that whereas urban populations activated the HPG axis earlier than rural ones, the rural birds attained higher peaks in LH levels. A similar pattern was also evident in the results of our laboratory experiment in which birds from both rural and urban sites that were exposed to artificial light during the dark phase of an artificial photoperiod began to secrete LH earlier, but had lower peak LH levels, than control birds. A Linear mixed model of the effects of exposure to LAN, date and sex on LH in free-living birds indicates that LAN was an important factor influencing variation in LH levels. These results suggest that exposure to artificial light during the usual hours of darkness can both advance the onset of HPG axis activity and reduce peak LH levels.

The LH levels of urban tree sparrows decreased earlier than those of rural birds, this time shift was not, however, evident in the laboratory experiment in which the LH levels of the treatment groups (i.e. exposed to LAN) all began to decrease by the third week of the experiment and were significantly lower than those of their respective control groups.

Although LH secretion began earlier at urban than at rural sites, there was no corresponding pattern in T and E2 secretion. On the contrary, peak T and E2 levels at urban sites were lower than those at rural sites. The earlier LH peak of urban birds could be due to a number of factors, including stimulation of the hypothalamus by artificial light sources. Nonetheless, our results are consistent with those of Partecke et al. (2005) and Schoech et al. (2013), and corroborate those of Dominoni et al. (2013a) in that increased exposure to anthropogenic light sources advanced LH secretion.

Exposure to anthropogenic light may cause the hypothalamus to initiate GnRH-I synthesis, thereby causing LH secretion to begin earlier than in rural habitats. LAN can also affect melatonin secretion and thereby circadian rhythm and photosensitivity (Dominoni et al., 2013b), which may explain the earlier decline and lower peak LH levels of urban tree sparrows compared to those from rural sites.

When considering potential mechanisms that might underlie the depression of LH levels in the urban tree sparrow population, one might hypothesize that the ‘stress’ of living in an urban...
environment might play a role. Elevated levels of corticosterone (CORT) have negative effects upon the reproductive axes of mammals and birds (Sapolsky, 1992; Wingfield and Sapolsky, 2003). However, while an earlier study found that baseline CORT levels of tree sparrows at Minzu University were higher than those of birds at rural sites, the same study found that baseline CORT levels of tree sparrows at Zhongshan Park were not significantly higher than those from rural sites (Zhang et al., 2011). In the current study, the temporal pattern of LH secretion at urban sites was similar, suggesting that baseline CORT is not responsible for the observed difference in the temporal pattern of LH secretion between urban and rural populations.

In addition to anthropogenic light sources, temperature is another environmental factor that can differ between urban and rural habitats due to the “heat island effect” (Song and Zhang, 2003). For example, high temperatures can enhance follicular development in the female white-crowned sparrow (Partecke et al., 2005; Dominoni et al., 2013a) and western scrub-rural habitats due to the “heat island effect” (Song and Zhang, 2003). For example, high temperatures can enhance follicular development in the female white-crowned sparrow (Partecke et al., 2005; Dominoni et al., 2013a) and white-crowned sparrows (Zonotrichia leucophrys pugetensis) (Wingfield et al., 1997) and testicular growth rate and LH secretion in the great tit (Parus major) (Silverin et al., 2008). Colder montane temperatures slowed testicular growth rate in mountain song-sparrows (Melospiza melodia morpha) (Wingfield, 1993). Conversely, temperature had no effect on testicular development in male white-crowned sparrows (Wingfield et al., 1997) or common starlings (Sturnus vulgaris) (Dawson, 2005), testicular development and LH secretion in two Scandinavian populations of great tits (Silverin et al., 2008), or LH secretion in the mountain white-crowned sparrow (Z. l. oriantha) (Wingfield et al., 2003). The above experiments compared significantly different temperatures (5, 10 and 25 °C), whereas in our study the urban sites were only about 1–2 °C warmer than the rural sites (Fig. 2). Therefore, we think it unlikely that this small difference in temperature was responsible for the earlier secretion and lower levels of LH in urban tree sparrow populations.

Compared with the available results on the European blackbird (Partecke et al., 2005; Dominoni et al., 2013a) and western scrub-jay (Schoech et al., 2013), our results not only show that urban, free-living tree sparrows had lower LH, T, and E2 than their rural counterparts, but also confirmed in a laboratory experiment that this difference was caused by exposure to anthropogenic light. In addition, although the peak LH levels of urban birds were lower than those of rural birds, free-living urban birds initiated endocrine production earlier than rural birds. We suggest that a long term comparative study of the reproductive parameters (e.g. laying dates) of urban and rural tree sparrow populations be conducted to evaluate the ecological effects of exposure to anthropogenic LAN.

5. Conclusion

Our results provide further support for the hypothesis that anthropogenic light sources can stimulate the HPG axis of urban birds. Such light sources appear to advance the production of reproductive endocrine hormones in urban birds, but also lower general LH, and consequently the T and E2, levels of urban birds relative to their rural counterparts.

Acknowledgments

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References


Table 2

Results of a linear mixed model of the effects of exposure to anthropogenic light at night (LAN), sex, sample site (site) and treatment week (week) on the plasma LH concentration of tree sparrows during a controlled laboratory experiment.

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