



# Hypothalamic gene switches control transitions between seasonal life history states in a night-migratory photoperiodic songbird



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## ABSTRACT

This study investigated photoperiodic plasticity in hypothalamic expression of genes implicated in the photoperiodic light perception (*rhodopsin*, *melanopsin*, *neuropsin* and *peropsin*), transduction (*pax6*, *bmal1*, *clock*, *per2* and *casr*), induction (*eya3*, *tshβ*, *dio2* and *dio3*, *gnrh* and *gnih*) and metabolism (*NPY*, *sirtuin1*, *foxO1*, *hmgcr*, *citrate synthase* and *dehydrogenases*) in photosensitive and photorefractory redheaded buntings. There was a significant increase in *eya3*, *tsh β*, *dio2*, *pax6* and *rhodopsin* and decrease in *dio3* mRNA expression at hour 15 and/or 19 on the day photosensitive buntings were subjected to a 13- or 16 h, but not to 8- and 11 h light exposure. Downstream reproductive and metabolic gene expression was not altered, except for an increase in those genes coding for succinate and malate dehydrogenase enzymes involved in lipogenesis. Photorefractory buntings had high *dio3* mRNA expression which significantly declined after 1 short day exposure, suggesting possible involvement of *dio3* in the maintenance of photorefractoriness. Positive correlation of *rhodopsin* on *eya 3* and *tshβ* indicates its role in photoperiodic timing, perhaps involving the *peropsin* and *pax6* genes. These results suggest that rapid switching of hypothalamic gene expression underlies photoperiod-induced seasonal plasticity and regulates transitions from photosensitive to photostimulated and from photorefractory to photosensitive states in migratory songbirds.

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## 1. Introduction

Many birds inhabiting both high and low latitudes use annual photoperiodic changes in controlling their seasonal events, particularly the processes linked with the reproduction. In long day species, increasing day lengths during the spring and summer periods initiate gonadal maturation, but post-reproduction birds become refractory to long day effects and regress their gonads (Dawson et al., 2001; Kumar, 1997; Kumar et al., 2010). Photorefractory individuals need to pass through shortening autumn and winter day lengths to become re-photosensitive to the stimulatory effects of increasing photoperiods in the forthcoming spring (Dawson et al., 2001; Kumar, 1997; Kumar et al., 2010).

Light is perceived in all probability by the encephalic photoreceptors (Oliver and Bayle, 1982), which have been shown to express mRNA and/or protein of multiple photopigment molecules, viz. neuropsin (Nakane et al., 2010), rhodopsin (Wang and Wingfield, 2011), melanopsin (Chaurasia et al., 2005; Kang et al., 2010), and vertebrate ancient opsin, VA-opsin (Halford et al., 2009). The duration of perceived light is interpreted as a long day when the light

period extends into the inductive phase of the entrained circadian rhythm of photoinducibility; the converse is true for the interpretation of light period as a short day (Kumar et al., 2010). A neuroendocrine response is triggered with rapid reciprocal switching between types 2 and 3 deiodinase (*dio2* and *dio3*) mRNA expression in the hypothalamus (Yoshimura et al., 2003), with an increase and decrease in *dio2* expression with the onsets of photoperiodic induction and regression and photorefractoriness, respectively; the converse is true for *dio3* expression (Watanabe et al., 2007; Yasuo et al., 2005; Yoshimura et al., 2003). The deiodinase mRNA expression is further reported to be under the control of 'gene switches' in the pars tuberalis, PT. The inductive process is "switched on" as soon as hour 14 on the first long day with an enhanced *Tshβ* (thyroid stimulating hormone-beta subunit) transcription in the PT thyrotrophs (Nakao et al., 2008). *Tshβ* coupled with *Tshα* forms Tsh, which binds to its receptors in ependymal cells lining ventrolateral walls of the third ventricle and initiates *dio2* transcription by hour 18 of the first long day (Nakao et al., 2008). *Dio2* enzyme mediates the conversion of T4 into T3, and in turn GnRH (gonadotropin releasing hormone) secretion from the preoptic area results in the synthesis and release of pituitary gonadotropins. At hour 14, *eye absent 3* (*eya3*) gene is also activated and acts perhaps upstream in the photoperiodic induction pathway (Majumdar et al., 2014). This molecular cascade seems to be a critical step in the photoperiodic control of seasonal cycles in birds and mammals (Dardente et al.,

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2010; Dupre et al., 2010; Masumoto et al., 2010; Nakao et al., 2008; Surbhi and Kumar, 2014).

However, a study that compared molecular response with a single long day between Swedish and German populations of great tit (*Parus major*) found a changed mRNA expression of genes coding for deiodinase enzymes in the hypothalamus of Swedish population only, although photoperiod induced gonadotropin secretions occurred with a temporal difference in both the populations (Perfito et al., 2012). Also, *Dio2*, not *Dio3* expression varied seasonally but was correlated neither with the seasonal photoperiodic changes nor with the GnRH levels or testis size in European starlings, *Sturnus vulgaris* (Bentley et al., 2013). Thus, the concept of deiodinase based mechanism for seasonal timing might not be as universally applicable as was originally envisaged. There could be real differences in the photoperiodic timing between species, or differences in results are the reflection of the experimental paradigm used to examine changes in the hypothalamic gene expression. It could also be that photoperiodic plasticity in hypothalamic gene expression, which is reported to begin with the changes in *Tshβ*, in fact begins with changes in the transcription of genes upstream, e.g. in the photoperiodic perception and/or transduction pathways. If yes, there would be concomitant changes in the mRNA expression of genes involved in the photoperiodic perception, transduction and induction pathways. This could be addressed by the measurement of expression pattern of both upstream and downstream genes along with deiodinase mRNA expression in more bird species, possibly inhabiting different kinds of photoperiodic environment during its annual life history, and subjected to a lighting protocol that is in some way different from the ones that have been previously used. Therefore, the goal of this study was to examine hypothalamic gene expression in long distance migratory redheaded bunting (*Emberiza bruniceps*), which in its travel across latitudes is exposed to consistent changes in the environmental photoperiod twice-a-year. Also, buntings rapidly respond to photostimulation as shown by increased mRNA and protein levels of genes implicated in photoperiodic induction in the first hour of day following 16 h of light exposure (Majumdar et al., 2014). Here, we exposed photosensitive buntings to a single day of photoperiods around the critical day length (CD, minimum daily light period that will induce a response in half of the test population of a species; Dunn and Sharp, 1990), and similarly photorefractory buntings to acute short days. We first measured the expression of genes that are implicated in the photoperiodic induction (*eya3*, *tshβ*, *dio2*, *dio3*, *gnrh*, *gnih*). Then, as a measure of upstream molecular response, we measured mRNA expression of genes involved in the photoperiodic light perception (*rhodopsin*, *melanopsin*, *neuropsin*, *peropsin* and *rgropsin*) and activation of *tshβ* (*clock*, *bmal1* and *per2*; Unfried et al., 2009) and *eya3* (*pax6*; Xu et al., 1997), and calcium homeostasis (e.g. gene encoding for calcium sensing receptor, *casr*). Finally, downstream molecular response was examined by changes in the mRNA levels of genes involved in the energy homeostasis and metabolism (glucose metabolism: *neuropeptide Y*, *sirtuin 1*, *foxO1*; lipid and cholesterol metabolism: *hmgr*) and Krebs's (TCA) cycle (*citrate synthase*, *α-ketoglutarate dehydrogenase*, *succinate dehydrogenase*, *malate dehydrogenase*). We expected a strong positive correlation of photoperiod length on the expression pattern of genes implicated in the photoperiodic light perception and induction in photosensitive birds. Similarly, a negative correlation was expected of photoperiod length on genes linked with regression and reproductive inactivity. Further, it was expected that photorefractory birds would show a decreased mRNA expression of genes implicated in the maintenance of gonadal regression and/or reproductively inactive state on acute exposure to short days. In addition, there would be a parallel change in mRNA levels of genes regulating downstream seasonal physiology unless these genes were activated by a cumulative effect of one or several upstream genes over a period of photoperiodic exposure.

## 2. Materials and methods

### 2.1. Animals and maintenance

Redheaded bunting is a Palearctic-Indian latitudinal migrant songbird, which migrates in between its breeding grounds in west Asia and south-east Europe (~40°N) and overwintering grounds in India (Ali and Ripley, 1974). It is a photoperiodic species and exhibits differences in photoperiod induced cycles in fat deposition and weight gain, and gonadal maturation during long-term exposure to long day lengths (Rani et al., 2005). The present study was carried out at the University of Lucknow, Lucknow, India, as per guidelines of the Institutional Animal Ethics Committee, using male buntings that were procured from their overwintering flocks around in the late February. These birds were first acclimated to natural light and temperature conditions (NDL) for a week in an outdoor aviary (size = 3 × 2.5 × 2.5 m) and then placed indoors in a photoperiodic room (size = 2.2 × 1.8 × 2.8 m) providing programmed photoperiods and constant temperature conditions (22 ± 2 °C) until used in the experiment.

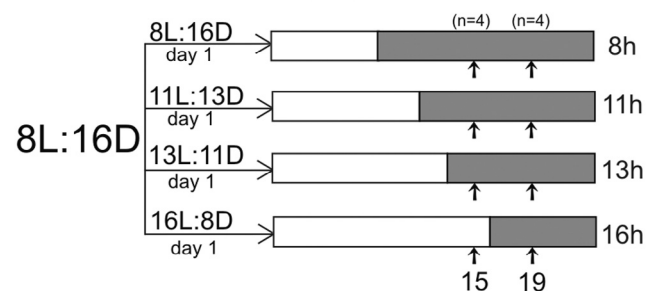
### 2.2. Experiment

Two experiments were performed, one each with photosensitive (experiment 1) and photorefractory (experiment 2) male buntings, as per the experimental design shown in Fig. 1.

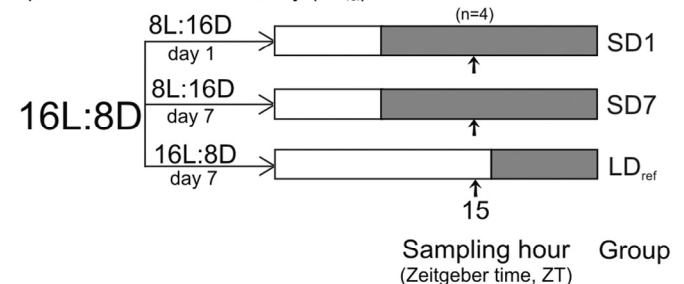
#### 2.2.1. Experiment 1: exposure of photosensitive birds to single increasing light periods

This experiment was carried out on photosensitive birds that were maintained on short days (8 h light: 16 h darkness, 8L:16D), in which buntings do not fatten (body mass = 22–24 g) and maintain small reproductively immature testes. These are called photosensitive birds, for they remain responsive to the stimulatory effects of long day

#### Experiment 1: Photosensitive (Pse)



#### Experiment 2: Photorefractory (LD<sub>ref</sub>)



**Fig. 1.** Experimental design. Experiment 1: Photosensitive (Pse) redheaded buntings maintained on short days (8 h light: 16 h darkness; 8L:16D) were exposed to a single 11- 13- and 16 h light or retained on 8 h light (controls). Experiment 2: Photorefractory (LD<sub>ref</sub>) buntings maintained on long days (16L:8D) were exposed to one (SD1) or seven (SD7) cycles of short days or retained in 16L:8D. They ( $n = 4$ ) were sampled on ZT 15 and/or 19 for the measurement of a number of genes linked with the regulation of reproduction and metabolism (for details see Figs 2–5).

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