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Research report

Daily, circadian and seasonal changes of rhodopsin-like encephalic photoreceptor and its involvement in mediating photoperiodic responses of Gambel's white-crowned Sparrow, *Zonotrichia leucophrys gambelii*

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ABSTRACT

Extra-retinal, non-pineal, encephalic photoreceptors (EP) play important roles in mediating development of the reproductive system by the annual change in day length (photoperiodic gonadal response – PGR) in birds. However, the distribution of rhodopsin-like EPs and their functional daily, circadian and seasonal changes are still unclear in the avian brain. This study identifies two novel groups of rhodopsin-immunoreactive cells in the nucleus paraventricularis magnocellularis (PVN) of the hypothalamus and in the medial basal hypothalamus (MBH) in a seasonally breeding species, Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*). In the PVN, rhodopsin-ir cell number showed both daily and circadian changes with more labeled cells apparent in the night phase in photosensitive birds, while only circadian changes were observed involving fewer labeled cells in the night phase in photorefractory birds. Single long day photo-stimulation significantly decreased the rhodopsin-ir cell number only in photosensitive birds, coincident with a rise in plasma levels of luteinizing hormone (LH). In the MBH, rhodopsin-ir cell number did not show daily, circadian or single long day induced changes in either photoperiodic states. But, overall these rhodopsin expressing neurons significantly increased from photosensitive to photorefractory states. In the median eminence (ME), more intense rhodopsin-ir was detected in photorefractory birds compared to photosensitive birds. For expression of GnRH and vasoactive intestinal polypeptide (VIP), seasonal differences were found with opposite relationships, consistent with previous studies. Our results suggest different roles of the two groups of rhodopsin-like EPs in the regulation of PGR in white-crowned sparrows.

1. Introduction

Unlike mammals, birds possess extra-retinal, non-pineal photoreceptors in the brain to convey light information such as day/night cycles and seasonal changes in photoperiod, to neuroendocrine systems that regulate seasonal morphological, physiological and behavioral changes. Previous studies had established the existence of functional extra-retinal photoreceptors and it has been generally accepted that encephalic photoreceptors (EP) play a critical role in mediating photoperiodic gonadal responses (PGR) of seasonally breeding birds (Benoit, 1935; Menaker et al., 1970; Menaker, 1971; Homma and Sakakibara, 1971; Wilson, 1990; Siopes and Wilson, 1974; Yokoyama et al., 1978; Simpson and Follett, 1981; Simpson et al., 1983; Cassone et al., 2009; Kumar, 2015). Accordingly, remarkable progress has been made on the identifications of different EPs that appear to be involved in PGR. For example, melanopsin-like EPs have been detected in the lateral septum (SL), preoptic area (POA), premammillary nucleus (PMH), optic tectum and cerebellum in chickens

Abbreviations: C_oS, nucleus commissuralis septi; CT, circadian time; EP, encephalic photoreceptors; GnRH, gonadotropin-releasing hormone; INF, nucleus infundibulum; LH, luteinizing hormone; LV, lateral ventricle; LZT, single long day stimulated zeitgeber time; MBH, medial basal hypothalamus; ME, median eminence; nCP_a, nucleus commissurea palli; PGR, photoperiodic gonadal response; POA, preoptic area; PT, pars tuberalis; PVN, nucleus paraventricularis magnocellularis; SL, nucleus septalis lateralis or lateral septum; SM, nucleus septalis medialis; V, ventriculus; VIP, vasoactive intestinal polypeptide; ZT, zeitgeber time.

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(*Gallus domesticus*) and turkey hens (*Meleagris gallopavo*) (Bailey and Cassone, 2005; Chaurasia et al., 2005; Kang et al., 2010). The phylogenetically ancient vertebrate (VA) opsin-like EPs have been shown to be widely distributed in the anterior and mid hypothalamus (including MBH) in birds (Halford et al., 2009) and VA opsin appear to play a key role in regulating photoperiodic responses (Davies et al., 2011). Moreover, a mammalian neural tissue opsin (OPN5) has been identified in paraventricular organ (PVO) of Japanese quails (*Coturnix japonica*) (Nakane et al., 2010).

Based on these studies, three models of EP-mediated PGR have been proposed: a melanopsin-mediated pathway (Kang et al., 2010), a VA opsin-mediated pathway (Halford et al., 2009), and an OPN5-mediated pathway (Nakane et al., 2010). Furthermore, rhodopsin-like EPs have been observed in both the lateral septum (SL) and mediobasal hypothalamus (MBH) in ringdoves (*Streptopelia risoria*), Japanese quails, Cayuga ducks (*Anas platyrhynchos*) (Silver et al., 1988) and house sparrows (*Passer domesticus*) (Wang and Wingfield, 2011). A fourth model via a rhodopsin-mediated pathway can also be proposed based on the distribution of rhodopsin-like EP in avian brains (Silver et al., 1988; Wang and Wingfield, 2011).

In general, all four putative EPs have two major characteristics in common: they have fibers projecting to the median eminence (ME), and have an association with ventricles (Silver et al., 1988; Kang et al., 2010; Halford et al., 2009; Nakane et al., 2010). In addition, all these EP-mediated models are similar except for the identity of photo-pigment and location of the photoperiodic clock, that in birds is suggested to reside either in the MBH (Yasuo et al., 2003; Halford et al., 2009) or in the EP (Kang et al., 2010; Wang and Wingfield, 2011).

The molecular mechanisms of PGR induced by long days in Japanese quail has been explored by Yoshimura and his colleagues (Yasuo et al., 2003; Yoshimura et al., 2003; Nakao et al., 2008; Ono et al., 2008). Photoperiod information is transmitted by EPs to the pars tuberalis (PT), where thyroid-stimulating hormone (TSH) expression is stimulated by long days. TSH released from the PT acts on tanycytes in the ME to induce type 2 deiodinase (DIO2) gene expression. DIO2 converts the prohormone thyroxine (T4) to bioactive 3,5,3′-triiodothyronine (T3). Up-regulation of T3 could cause morphological changes in the proximity of gonadotropin-releasing hormone (GnRH) nerve terminals to basal lamina in ME, which in turn facilitates GnRH release thus mediating long-day induced PGR. However, Bentley et al. (2013) were unable to show DIO2 expression was related to gonadal development in European starlings, (*Sturnus vulgaris*) exposed to natural changes in day length. The investigations on Japanese quail employed an increase in day length from short to long days overnight, which possibly enhanced DIO2 expression compared to a gradual change in photoperiod on a natural schedule (Bentley et al., 2013).

Moreover, the DIO2 gene activation mechanism cannot explain PGR regulation during photorefractoriness, a condition in which reproductive system is switched off indefinitely until it is dissipated by short day length in many avian species (Nicholls et al., 1988; Bentley et al., 2013). For example, in house sparrows, photorefractory birds showed continuously high levels of Dio2 expression under extended long days, while the hypothalamo-pituitary-gonad (HPG) axis was inhibited (Watanabe et al., 2007). In Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*), the adenohypophysis of male photorefractory birds contained LH β-subunit mRNA (Kubokawa et al., 1994) and has been shown to be sensitive to GnRH, because a single intra venous injection resulted in a surge of circulating LH (Wingfield et al., 1979). In addition, injection of the excitatory amino acid glutamate agonist N-methyl-D-aspartate (NMDA) induces significantly high level of plasma LH release in photorefractory white-crowned sparrows (Meddle et al., 1999). Taken together, the evidence suggests that the molecular mechanisms of photorefractoriness involve regulation of GnRH release at both perikarya levels and terminal field levels, and this mechanism is different from the T3-mediated photoperiodic response. Some studies suggested that onset of photorefractoriness was probably regulated by EPs in avian species such as American tree sparrows (*Spizella arborea*) and European starlings (Turek, 1975; Dawson and Goldsmith, 1982; Wilson, 1989). Meanwhile, rhodopsin-like EPs have been shown to interact directly with GnRH neurons at both the perikarya and terminal field levels (Saldanha et al., 2001), which may imply a functional role of these EPs in the PGR including photorefractoriness.

Onset of photorefractoriness has also been shown to involve prolactin secretion and vasoactive intestinal peptide (VIP) content. VIP is considered to be the releasing factor for prolactin in avian species (Mauro et al., 1989; Sharp et al., 1989; El Halawani et al., 1997; Maney et al., 1999). Immunohistological studies suggest that the hypothalamic content of VIP has the opposite seasonal pattern to that of GnRH (Saldanha et al., 1994; Deviche et al., 2000). High hypothalamic VIP content often marks the period of photorefractoriness while prolactin secretion peaks at the onset of photorefractoriness and decreases thereafter (Mauro et al., 1989; Saldanha et al., 1994; Chaiseha et al., 1998; Deviche et al., 2000). However, the onset of photorefractoriness is not always associated with up-regulated hypothalamic VIP content. For example, in turkey hens, VIP receptor mRNA was expressed throughout the hypothalamus including the INF (nucleus infundibulum) with a strong signal in the ependymal region lining the third ventricle as well as in the PT. Decreased VIP receptor mRNA expression was observed in the INF area of photosensitive and photorefractory hens in comparison to photostimulated hens (Chaiseha et al., 2004). Central infusion of VIP into laying turkeys lowered their circulating LH levels and terminated egg-laying activity (Pitts et al., 1994). In European starlings, active immunization against endogenous VIP delayed onset of photorefractoriness and extended reproductive activity (Dawson and Sharp, 1998, Dawson et al., 2002). Taken together this evidence from VIP receptor mRNA expression (Kansaku et al., 2001; You et al., 2001; Chaiseha et al., 2004) as well as VIP administration (Pitts et al., 1994; Dawson and Sharp, 1998) suggest that VIP may play a role in modulating GnRH release in the ME via neuron communication or vasodilatory effects in the hypophysial portal vessels. In view of the reported co-expression of rhodopsin and VIP-containing neurons in the INF, and in the external layer of ME (Silver et al., 1988; Wang and Wingfield, 2011), hypothalamic VIP-containing neurons may have important roles in decoding photoperiodic information and maintaining photorefractoriness.

In order to explore whether seasonal changes in the putative hypothalamic machinery for the PGR to occur between photosensitive and photorefractory states, we used immunocytochemistry to investigate both daily and circadian changes of hypothalamic rhodopsin-like EP, and daily changes of GnRH-containing neurons and VIP-containing neurons in both photosensitive (held in experimental chambers under 10L: 14D), and in photorefractory white-crowned sparrows (held under 12L: 12D). In addition, we challenged the birds with a single long day (18 h day length) to examine immediate responses of all three types of neurons in both photoperiodic states. The plasma level of LH was used as a marker for activation of the HPG, and the response of LH level to a single long day was used to validate photoperiodic status.

2. Results

2.1. Plasma LH

Plasma LH level remained low at ZT2 (zeitgeber time morning) and ZT18 (midnight) in both photosensitive birds held on 10 h light