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Regulation of vernal migration in Gambel's white-crowned sparrows: Role of thyroxine and triiodothyronine

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ABSTRACT

Appropriate timing of migratory behavior is critical for migrant species. For many temperate zone birds in the spring, lengthening photoperiod is the initial cue leading to morphological, physiological and behavior changes that are necessary for vernal migration and breeding. Strong evidence has emerged in recent years linking thyroid hormone signaling to the photoinduction of breeding in birds while more limited information suggest a potential role in the regulation of vernal migration in photoperiodic songbirds. Here we investigate the development and expression of the vernal migratory life history stage in captive Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*) in a hypothyroidic state, induced by chemical inhibition of thyroid hormone production. To explore possible variations in the effects of the two thyroid hormones, triiodothyronine and thyroxine, we subsequently performed a thyroid inhibition coupled with replacement therapy. We found that chemical inhibition of thyroid hormones resulted in complete abolishment of mass gain, fattening, and muscle hypertrophy associated with migratory preparation as well as resulting in failure to display nocturnal restlessness behavior. Replacement of thyroxine rescued all of these elements to near control levels while triiodothyronine replacement displayed partial or delayed rescue. Our findings support thyroid hormones as being necessary for the expression of changes in morphology and physiology associated with migration as well as migratory behavior itself.

Introduction

Increasing photoperiod in spring triggers a cascade of events leading to the expression of the vernal life history stages that include prenuptial molt, migration, and breeding in migratory songbirds such as the white-crowned sparrow (Ramenofsky and Wingfield, 2006; Wingfield et al., 1990). Since Rowan's (1925) pioneering discovery of photoinduction of migratory behavior in captive dark-eyed juncos (*Junco hyemalis*) our understanding of the suite of vernal events triggered by photoperiod has increased greatly. However, the neural and hormonal mechanisms regulating the development of migratory readiness and expression of migratory behavior remain incomplete. Of the numerous endocrine candidates, thyroid hormones offer great promise although their role is poorly studied. Early investigations showed histological increases in thyroid gland activity in white-crowned sparrows with season and temperature (Oakeson and Lilley, 1960; Wilson and Farner, 1960). Building on these observations, increases in plasma levels of thyroid hormones were found in Canada geese (*Branta canadensis*; John & George 1978), Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*; Smith, 1982), and rosy pastor (*Sturnus roseus*; Pathak and Chandola, 1984) during the period preceding vernal migration. Furthermore, Wingfield et al. (1996) found significant increases in thyroid hormones from wintering levels 10 to 30 days following photostimulation with 20L:4D (Light:Dark). However, work on red-headed buntings (*Emberiza bruniceps*) showed direct involvement of thyroid hormones via thyroidectomy, which suppressed mass gain and expression of nocturnal restlessness (Pant and Chandola-Saklani, 1993). Furthermore, they demonstrated that administration of thyroid hormones partially reinstated both mass gain and nocturnal restlessness (Nair et al., 1994). Taken together these studies provide evidence that thyroid hormones may have a critical in role vernal migration.

More recently, the hypothalamic-pituitary-thyroid axis has been strongly implicated to regulate the photoinduction of breeding (reviewed in: Nakane and Yoshimura, 2010). Thyroid hormones acting within the brain have been identified as a key component in the pathway leading from transduction of photoperiodic information into the gonadotropin-releasing hormone (GnRH) cascade ultimately responsible for the onset of breeding (Nakane and Yoshimura, 2010; Nakao et al., 2008a; Nakao et al., 2008b). Recent work by Wang et al. (2013) showed that Gambel's white-crowned sparrows exposed to long photoperiods under low-intensity light (green light: 510 nm), displayed

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normal patterns of migratory fattening and nocturnal restlessness, previously characterized for the species (Agatsuma and Ramenofsky, 2006), but no gonadal growth. These findings suggest independent neural regulation of migration from breeding as noted previously for this species (Ramenofsky, 2011; Stetson and Erickson, 1972; Yokoyama, 1976) and may indicate involvement of thyroid hormones in the transduction of photoperiodic information in the regulation of multiple pathways.

Given the effects of thyroidectomy on migratory behavior and its role in the induction of the hypothalamic-pituitary-gonadal axis, we hypothesized that thyroid hormones may play a similar role in mediating the response to photoperiod that triggers the onset of the vernal migratory life history stage. If this is the case, it is possible that local thyroid signaling, analogous to that involved in breeding, is responsible for the photoperiodic response of the vernal migration life history stage. Here we present our findings from two experiments designed to address the role thyroid hormones in vernal migration in a seasonally breeding songbird.

First we sought to establish the validity of chemical inhibition of thyroid hormone production as a reversible alternative to surgical or radiation based methods of thyroidectomy in wild birds as well as appropriate dosages (Weng et al., 2007). We further sought to confirm abolishment of major vernal events following chemical inhibition of thyroid hormones. To investigate the observed effects of the thyroid inhibition were due to the absence of circulating thyroid hormones and not pharmacological effects of the anti-thyroid agent methimazole, we conducted a thyroid replacement experiment. The replacement experiment allowed for disassociation between effects of 3,5,3′-triiodothyronine (T3), classically considered the active form of the hormone (higher receptor affinity), and thyroxine (T4) the primary product of the thyroid gland. We present two predictions: first, that inhibition of thyroid hormone production would abolish pre-migratory fattening, mass gain, increases in muscle size and the onset of nocturnal restlessness. Second, that thyroid replacement would restore all changes in morphology/physiology and behaviors to levels observed in control birds.

Methods

Study species

The Gambel's white-crowned sparrow (*Zonotrichia leucophrys gambelii*) is a seasonally breeding, long distance, nocturnal migrant. In preparation for the onset of migration, birds undergo a pre-migratory development phase (Ramenofsky and Wingfield, 2007; Wingfield and Farner, 1978a). During this phase both wild and captive birds exhibit hyperphagia, deposition of subcutaneous fat stores, hypertrophy of pectoralis flight muscles, and changes in metabolic enzymes (Bairlein and Simons, 1995; Bauchinger and Biebach, 2005; Price and Guglielmo, 2010; Price et al., 2010; Ramenofsky, 2011). These changes serve to support endurance flight during migration (Ramenofsky, 2011; Ramenofsky et al., 2012). The transition between the development of migratory capability and expression of migration is poorly characterized in the wild, while in captivity, the behavior of migratory passerines has been well documented (Agatsuma and Ramenofsky, 2006). Captive white-crowned sparrows show a characteristic pattern of twilight quiescence followed by nocturnal restlessness, nighttime movements including wing flapping and bill up behaviors; these are considered to be an active expression of the urge to migrate and are typically seen during the first portion of the night (Agatsuma and Ramenofsky, 2006; Coverdill et al., 2008; Coverdill et al., 2006). All research was conducted in compliance with Institutional Animal Care and Use Committee guidelines under protocol #17812, the *Guidelines to the Use of Wild Birds in Research*, and National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experiment 1: chemical thyroid inhibition

Eleven male Gambel's white-crowned sparrows were caught in the vicinity of the University of California, Davis, California (N 38.554, W 121.738) in the late autumn of 2012 and brought into captivity. Sex determination was conducted in the field using a wing length cut-off of ≥75 mm to identify males (Fugle and Rothstein, 1985) and confirmed via laparotomies at the end of the experiment. Birds were acclimated to captivity in flight aviaries for two weeks prior to the start of the experiment, at which point they were moved to individual registration cages. Each cage was equipped with an infrared photodetection system to record locomotor activity and transfer the output to a Mini Mitter Acquisition System – Vital View (Sunriver, OR) per the methods of Ramenofsky and Németh (2014). This system monitors breaks of an infrared beam crossing the perch and has been successfully used to tie raw activity to nocturnal restlessness behavior (Agatsuma and Ramenofsky, 2006; Ramenofsky and Németh, 2014). Birds were held on natural photoperiod (~38.5° N), at 21–24 °C, and given water and food ad libitum.

Birds were randomly assigned to two groups: control implant ($n = 4$) and treatment ($n = 7$). All birds in the study received two 14 mm silastic implants, sealed with silicone sealant at both ends, inserted subcutaneously beginning on February 21, 2013 and replaced every two weeks until the termination of the study. The implants for the treatment groups were packed with the anti-thyroid agent methimazole (M8506, Sigma-Aldrich, St. Louis MO) and the vehicle controls were left empty. Methimazole acts to block the production of thyroid hormones within the thyroid gland, targeting the enzyme thyroid peroxidase. In addition to administration of silastic implants, treatment birds were also given filter sterilized methimazole orally (500 mg/L; per Weng et al., 2007) in their drinking water. The combination of implants and oral administration was chosen to ensure sufficient dosage of methimazole to achieve inhibition of thyroid hormone, as implants alone were determined to be insufficient (Pérez, unpublished data).

Experiment 2: thyroid replacement

Thirty-two adult white-crowned sparrows were caught near the campus of the University of California, Davis in early winter 2014. Sex was again confirmed at the end of the experimental period by necropsy; 29 males and 3 females had been included. Inclusion of the females did not affect the results of analyses for all variables examined thus they were not removed from final analyses presented here. Birds were housed and monitored as described previously for Experiment 1.

Birds were randomly assigned to four treatment groups ($n = 8$): control, methimazole treated (M), methimazole + T3 (MT3), and methimazole + T4 (MT4). Two 14 mm silastic implants packed with methimazole, along with oral administration (500 mg/mL) were begun on Feb. 20, 2014. Additionally, the MT3 birds received a single 10 mm subcutaneous silastic implant packed with T3 (T2877, Sigma Aldrich, St. Louis MO) while the MT4 birds received a 12 mm silastic implant packed with T4 (T2376, Sigma Aldrich, St. Louis MO) inserted on March 6th or 7th, 2014. T3 and T4 implants were punctured twice with a needle to enhance diffusion. Emptying rates for both T3 and T4 implants had been previously determined to be slow enough that implants were only replaced once halfway through the experimental period to ensure continuous delivery, as thyroid hormone remained in all implants when removed.

Measurements & sampling

Bi-monthly morphometrics of subcutaneous fat were scored on a scale from 0 (no fat) to 5 (greatly bulging with fat) in both the furcular and abdominal regions (Wingfield and Farner, 1978b), and pectoralis muscle size were visually scored (Bairlein and Simons, 1995; Salewski et al., 2009). Mass was measured by Pesola spring scale to the nearest 0.1 g. Blood samples for hormonal analysis were collected monthly.