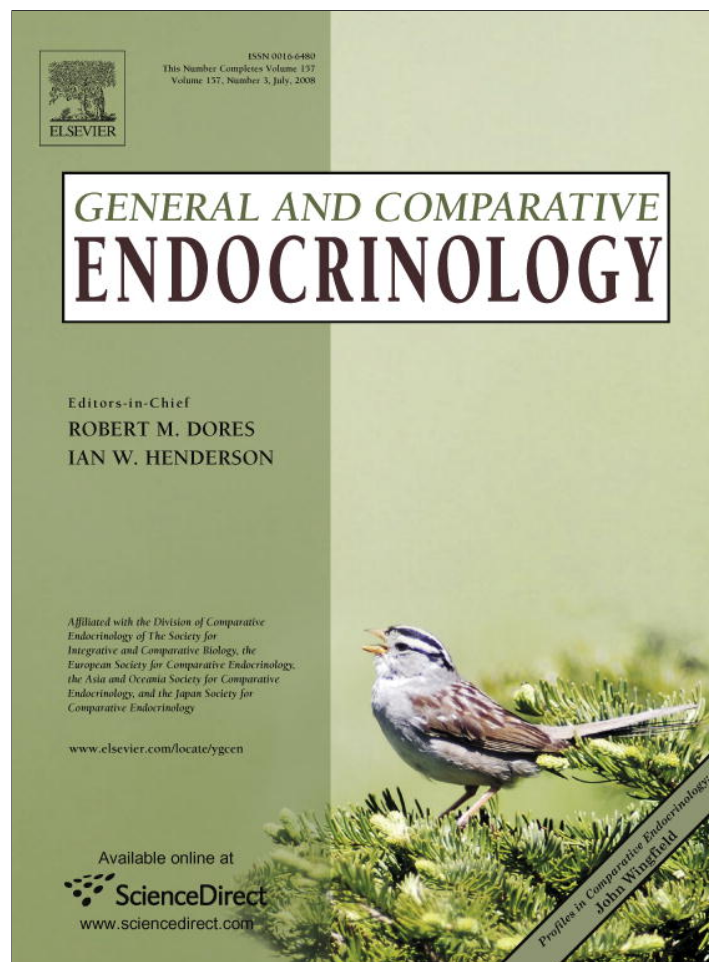


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## Minireview

## Seasonal-like growth and regression of the avian song control system: Neural and behavioral plasticity in adult male Gambel's white-crowned sparrows

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## ABSTRACT

Birdsong is regulated by a series of discrete brain nuclei known as the song control system. In seasonally-breeding male songbirds, seasonal changes in steroid sex hormones regulate the structure and electrophysiology of song control system neurons, resulting in dramatic changes in singing behavior. Male songbirds can be brought into the laboratory, where circulating levels of steroid hormone and photoperiod can be abruptly manipulated, providing controlled conditions under which rapid "seasonal-like" changes in behavior and morphology can be carefully studied. In this mini-review, we discuss the steroidal and cellular mechanisms underlying seasonal-like growth and regression of the song control system in adult male Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*), and its impact on song behavior. Specifically, we discuss recent advances concerning: (1) the role of androgen and estrogen receptors in inducing seasonal-like growth of the song control system; (2) how photoperiod modulates the time course of testosterone-induced growth of the song control system; (3) how bilateral intracerebral infusion of androgen and estrogen receptor antagonists near the song control nucleus HVC prevents seasonal-like increases in song stereotypy but not song rate; and (4) the steroidal and cellular mechanisms that mediate rapid regression of the song control system. Throughout this mini-review we compare data collected from white-crowned sparrows to that from other songbird species. We conclude by outlining avenues of future research.

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## 1. Introduction

Plasticity in brain and behavior is fundamental for animals to react to changing environmental demands. An organism's ability to adapt to seasonal changes in the environment is critical to reproductive success, and breeding typically happens during the season with the highest probability of successfully rearing offspring. It is thus not surprising that seasonal plasticity in brain and behavior has been found in every vertebrate taxon (Tramontin and Brenowitz, 2000). These seasonal changes in the environment are often signaled to the brain via changes in hormone levels, which can trigger massive restructuring of the neural substrates that regulate behavior. In this mini-review we survey recent advances in understanding the proximate steroidal and cellular mechanisms underlying an example of adult seasonal plasticity: the seasonal growth and regression of the song control system (Fig. 1) and the resulting changes in song (a reproduction-related vocal behavior) in the male Gambel's white-crowned sparrow.

White-crowned sparrows are age-limited learners (Marler and Tamura, 1964) that as adults exhibit substantial seasonal changes

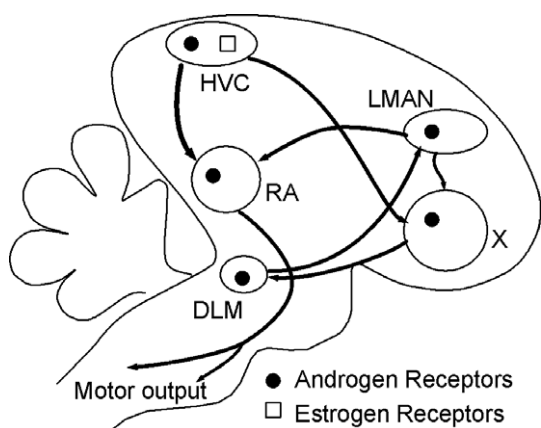
in the song control system and song behavior. Gambel's white-crowned sparrows are long-distance migrants that winter along the west coast and western interior of the contiguous United States, and breed in Alaska and Canada, unlike some other white-crowned sparrow subspecies such as the non-migratory Nuttall's (Farner and Lewis, 1973; Chilton et al., 1995). In the spring, longer day lengths induce an increase in plasma testosterone (T) level, which in turn triggers anatomical and electrophysiological changes in the nuclei of the song control system and in white-crowned sparrow song behavior, most notably increases in song rate, song stereotypy, and duration (Nottebohm, 1981; Smith et al., 1995; Tramontin et al., 2000; Brenowitz, 2004; Park et al., 2005; Meitzen et al., 2007a). The volumes of the song control nuclei HVC, Area X, and RA all increase, and the cellular mechanisms underlying these increases vary by nucleus. The increase in HVC volume is largely driven by an increase in neuron number (Fig. 2A), whereas growth of RA (Fig. 2B) and Area X (Thompson and Brenowitz, 2005) is driven by changes in neuron size and density. The morphological changes that underlie growth of the song control system nuclei have been recently reviewed in detail elsewhere (Brenowitz, 2004), so we will not discuss this issue further.

Changes in the song control system and singing behavior can be induced in the laboratory using the appropriate environmental and hormonal cues, allowing carefully controlled experiments. To mi-

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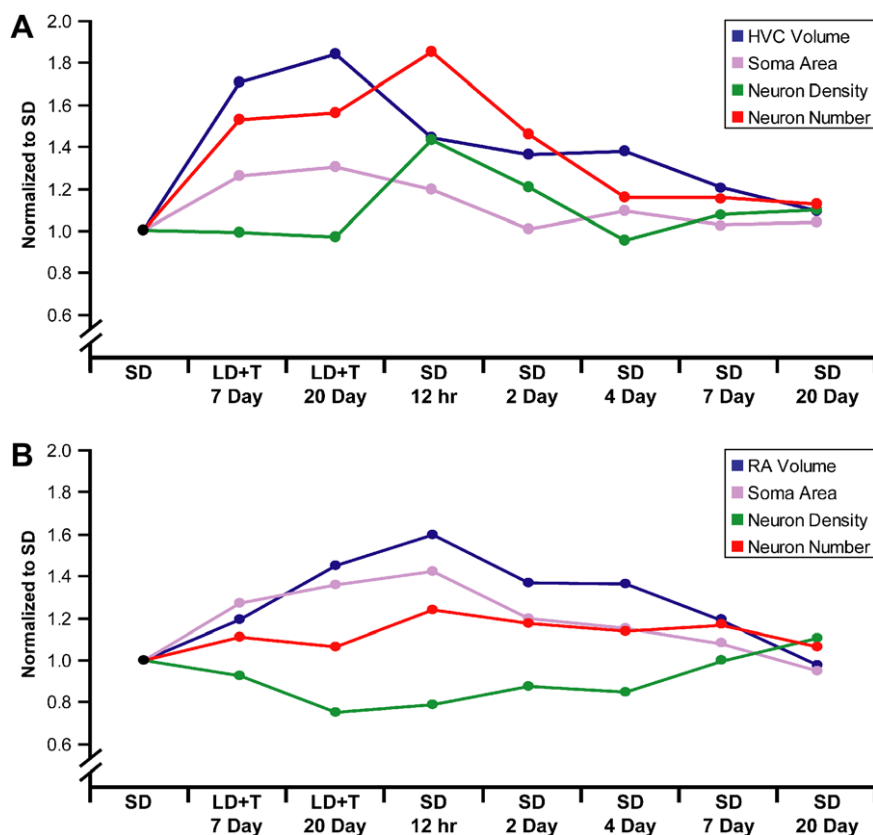
**Fig. 1.** Simplified schematic of the avian song control system showing the distribution of steroid receptors. The nuclei HVC and RA comprise the main descending motor circuit. RA projects to several brainstem nuclei (including nXIIIts) that contain motoneurons that project to the muscles controlling respiration and the sound production organ, the syrinx. Both brainstem motoneurons and the syrinx express androgen receptors. HVC, X, DLM, and LMAN comprise the anterior forebrain pathway (AFP), which is necessary for song learning. HVC, used as a proper name; DLM, the medial portion of the dorsolateral nucleus of the anterior thalamus; LMAN, the lateral magnocellular nucleus of anterior nidopallium; RA, the robust nucleus of the arcopallium; nXIIIts, the tracheosyringeal portion of the hypoglossal nucleus; X, Area X, a subdivision of the medial striatum. Nomenclature used here follows Reiner et al. (2004).

mic the spring and summer breeding season, we expose birds to long-day (LD) photoperiod (20 h light, 4 h dark; typical of their Alaskan breeding grounds) and a systemic T implant. The T implant insures that plasma T levels are within the physiological breeding range. To mimic the transition to non-breeding conditions, typically seen in the fall and winter, we expose birds to short-day (SD) photoperiod (8 h light, 16 h dark). In addition, we remove the subcutaneous T pellet and castrate the birds, to insure rapid and synchronous withdrawal of circulating sex steroids. This “seasonal-like” plasticity provides controlled conditions under which proximate mechanisms underlying changes in behavior and morphology can be carefully studied, which then informs field studies of non-captive animals (Just one example: Park et al., 2005; Meitzen et al., 2007b).

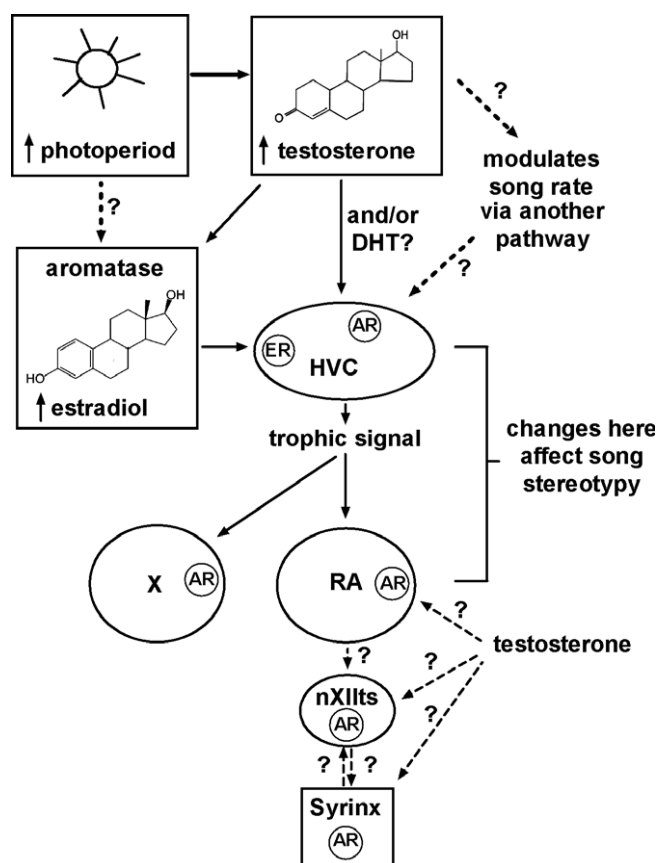
## 2. Mechanisms of growth

### 2.1. Photoperiod increases plasma T level

Seasonal-like plasticity in the song control system is modulated by changes in photoperiod and circulating steroid sex hormones, with important contributions coming from both of these factors (Fig. 3). Very early in the breeding season, increasing day length activates the hypothalamic–pituitary gonadal axis, which stimulates growth of the testes and elevates circulating levels of T. This increase in circulating T primarily regulates song control system



**Fig. 2.** Schematic, normalized representation of seasonal-like changes in morphology of HVC and RA. These representations are based upon the means of data taken from Tramontin et al. (2000) and Thompson et al. (2007). We made these data sets congruent using the one time point shared between both sets (long-day photoperiod and systemic testosterone (LD + T) for 20 days) and then normalized them to the short-day photoperiod (SD) time point. Birds were exposed to at least 10 weeks of SD photoperiod in both studies to insure photosensitivity. (A) HVC grows within 7 days after the transition to LD + T, driven largely by changes in neuron number. After the transition from LD + T to SD photoperiod, HVC volume regresses within 12 h, initially driven by a sharp increase in neuron density followed by a slower decrease in neuron number. (B) RA volume increases significantly within 20 days after the transition to LD + T, largely driven by an increase neuronal soma area and a decrease in density. After the transition from LD + T to SD photoperiod, RA volume regresses significantly by 20 days, with significant changes in density and soma area by 2 days by continuing to significantly change over the entire time course.



**Fig. 3.** Working model illustrating how steroid hormones induce changes in the song control system and song behavior. Increasing photoperiod stimulates an increase in plasma testosterone (T) concentration. In the brain, T can be metabolized into other androgens and 17- $\beta$  estradiol (E2), an estrogen. The enzyme aromatase converts T into E2. T and E2 act on HVC to increase neuron number and nucleus volume. Steroid receptor binding in HVC is necessary and sufficient to create a trophic signal that is transported by HVC's afferents to RA and X. Androgen receptor binding in RA may be permissive for morphological and electrophysiological change to occur in response to the trophic signal released by HVC. The mechanism triggering growth of the syrinx and nXllts is unclear, but one possibility is direct activation of the androgen receptors present in both.

growth; T induces growth under both SD and LD photoperiod conditions (Smith et al., 1997; Bernard et al., 1997; Gulledge and Deviche, 1997; Meitzen et al., 2007a; further reviewed in Brenowitz, 2004; for an alternate view see Ball et al., 2004), and T levels below breeding maxima can induce growth of the song system (Tramontin et al., 2001). LD photoperiod and systemic T induces song control system growth even when the bird has been deafened and sings at a very low rate (Brenowitz et al., 2007).

### 2.2. Androgens and estrogens are both necessary for song control system growth

Both androgen and estrogens may be necessary for maximal growth of the song control system. In the brain, T can be metabolized into other androgens, such as 5 $\alpha$ -dihydrotestosterone (DHT), or estrogens, such as 17- $\beta$  estradiol (E2). These metabolites have been implicated in song control system growth. In white-crowned sparrows, systemic exposure to either DHT (a non-aromatizable androgen) or E2 alone for 21 days results in significant growth of song control nuclei (Tramontin et al., 2003), but not RA spontaneous firing rate or soma area (Park et al., 2005; Meitzen et al., 2007a). Systemic exposure to both an androgen and an estrogen, however, produce full growth of song nuclei volumes and maximal

song rate (Tramontin et al., 2003), RA soma area, and RA spontaneous firing rate (Park et al., 2005; Meitzen et al., 2007a). Exposure to the aromatase inhibitor ATD blocks the increase in RA soma area and spontaneous firing rate induced by LD photoperiod and systemic T (Meitzen et al., 2007a). Similarly, in wild, free-ranging male song sparrows, exposure to the aromatase inhibitor fadrozole during the breeding season decreased HVC volume (Soma et al., 2004). Additional evidence comes from adult male canaries, where E2 promotes the survival of new neurons in HVC (Hidalgo et al., 1995). In castrated male canaries exposed to SD photoperiod for only 11 days, systemic T, but not DHT or E2 alone, increases HVC volume and song rate (Sartor et al., 2005).

### 2.3. Androgens and estrogens act on HVC to stimulate growth of RA and X

Studies using systemic hormone implants implicate both androgens and estrogens in regulating growth of the song control nuclei but provide no information on where the hormones act. The next step in building a useful model of how T and its metabolites induce growth of the song control system is to determine which steroid receptors in which song system nuclei are necessary and sufficient for T-induced growth. Experiments have focused on HVC because it is the first song control nucleus to grow in response to elevated systemic T (Tramontin et al., 2000), and because it expresses estrogen receptors (ER) Fig. 1; (Gahr et al., 1993; Bernard et al., 1999; Metzdorf et al., 1999; Fusani et al., 2000). Androgen receptors (AR) or their mRNA are expressed in every song control nucleus and the syrinx, the avian vocal organ Fig 1; (Arnold et al., 1976; Gahr et al., 1993; Nastiuk and Clayton, 1995; Smith et al., 1996; Bernard et al., 1999; Metzdorf et al., 1999; Soma et al., 1999b; Fusani et al., 2000; Kim et al., 2004). AR immunoreactivity is elevated in the HVC of Gambel's white-crowned sparrows captured in the spring; AR immunoreactivity in other song control nuclei was not quantified (Soma et al., 1999b). Androgens and estrogens could be activating activate different processes in different HVC cell neuron types. Studies using steroid autoradiography and retrograde labeling found that X-projecting neurons accumulated estrogens (Johnson and Bottjer, 1995), and RA-projecting neurons accumulated androgens (Johnson and Bottjer, 1993) in the canary. In the zebra finch, both RA-projecting and X-projecting neurons accumulated androgens (Sohrabji et al., 1989). Determining which neuron types in HVC express ER and AR in the white-crowned sparrow is a critical next step.

Several experiments validate the hypothesis that T and its metabolites initially act on HVC, which then stimulates growth of the other song control nuclei. Unilateral lesions of HVC in LD photoperiod and systemic T-treated white-crowned sparrows block growth of RA and X on the ipsilateral, but not contralateral, hemisphere (Brenowitz and Lent, 2001). Small T implants unilaterally implanted near HVC in SD photoperiod-treated white-crowned sparrows increase the volumes of HVC, RA and X in the ipsilateral, but not contralateral, hemisphere, while T implants near RA have no effect (Brenowitz and Lent, 2002). Similarly, unilateral infusion of DHT and E2 near HVC in SD photoperiod exposed male white-crowned sparrows increases soma size and spontaneous firing rate in the ipsilateral, but not contralateral, RA, but DHT and E2 infused near RA have no effect (Meitzen et al., 2007a). Unilateral infusion of androgen and estrogen receptor antagonists near HVC block the LD photoperiod and systemic-T induced increase in soma size and spontaneous firing rate in the ipsilateral, but not contralateral, RA (Meitzen et al., 2007a). Together, these studies provide strong evidence that androgens and estrogens act on HVC to stimulate not only its own growth, but also that of RA and X. For a working model (Fig. 3), we thus hypothesize that in HVC, AR and ER activation enhances incorporation of new RA-projecting neurons into



HVC (new neurons in HVC are thought to be RA-projecting, [Scotto-Lamasse et al., 2007](#)) and increases synthesis of a transsynaptic trophic signal that drives changes in intrinsic activity and morphology in RA and Area X. The exact nature of the trophic signal that HVC provides to RA and Area X is unclear, but it could involve neurotrophin release and/or changes in activity (further reviewed in [Brenowitz, 2004](#)). Given that increases in Area X and RA nucleus volume occur after HVC grows ([Tramontin et al., 2000](#)), the trophic signal produced by HVC may either take time to be produced and transported, and/or new RA-projecting neurons may need to be recruited to HVC and establish synapses on the increased dendritic arbors of RA neurons ([Hill and DeVoogd, 1991](#)). AR in RA may be permissive for RA neurons to respond to or amplify the incoming trophic signal from HVC, as exposing RA neurons to the AR antagonist flutamide prevents the increase in firing rate and soma size induced by LD photoperiod and systemic T in three of five birds ([Meitzen et al., 2007a](#)). Further studies of the hypothesized permissive role of AR in RA are required.

#### 2.4. Do androgens act directly on nXIIIs and the syrinx?

The steroidal mechanism underlying growth of the brainstem motoneurons and of the syrinx (the avian vocal organ) is unclear, although it may not depend upon activation of steroid hormone receptors in either HVC or RA. When small intercerebral T implants are unilaterally placed near either HVC or RA, neither nXIIIs nor the syrinx increase in volume or mass, respectively ([Brenowitz and Lent, 2002](#)). Lesions of HVC prevented the growth of nXIIIs ([Brenowitz and Lent, 2001](#)). Since both nXIIIs and the syrinx contain AR ([Smith et al., 1996](#)), one reasonable hypothesis is that androgens act individually and directly on them to increase growth. Other hypotheses include but are not limited to: (1) retrograde support of nXIIIs growth from the syrinx, (2) afferent support of nXIIIs from either or both RA, and the dorsomedial portion of the intercollicular nucleus (ICo), which both contain AR, and (3) afferent support of syringeal growth from nXIIIs. These hypotheses remain to be tested.

#### 2.5. Photoperiod may modulate the growth of the song control system

Photoperiod might also modulate song control system growth independently of stimulating the production of T by the testes (recently reviewed in [Ball et al., 2004](#); [Brenowitz, 2004](#)). In castrated male starlings, American tree sparrows, and dark-eyed juncos and Gambel's white-crowned sparrows, LD photoperiod is sufficient to promote at least partial growth of some song control system nuclei ([Bernard et al., 1997](#); [Smith et al., 1997](#); [Bentley et al., 1999](#); [Dloniak and Deviche, 2001](#)). In castrated male house finches, LD photoperiod is sufficient to induce growth of RA but not HVC ([Strand and Deviche, 2007](#)). More recent data testing this hypothesis comes from Gambel's white-crowned sparrows, where LD photoperiod accelerates the systemic T-induced increase in spontaneous firing rate and soma size in the nucleus RA ([Meitzen et al., 2007a](#)). This acceleration can be blocked by systemic exposure to 1-4-6-androstatrien-3,17-dione (ATD), an aromatase inhibitor; aromatase is the enzyme that converts T into its estrogenic metabolite E2 ([Meitzen et al., 2007a](#)). Conversely, exposure to systemic E2 accelerates the increase of spontaneous firing rates and soma size in SD photoperiod and systemic T or DHT treated-birds ([Park et al., 2005](#); [Meitzen et al., 2007a](#)). These results suggest that photoperiod might regulate aromatase expression or activity. Regional aromatase activity can change seasonally in starlings and song sparrows ([Riters et al., 2001](#); [Soma et al., 2003](#)), but has only been measured in Gambel's white-crowned sparrows captured in the winter ([Schlinger et al., 1992](#)). Aromatase is positioned to have a critical influence on T-induced growth of the song control system.

In song sparrows (*Melospiza melodia*), a species closely related to white-crowned sparrows ([Carson and Spicer 2003](#)), the nidopallium surrounding HVC expresses aromatase mRNA, but HVC itself does not ([Soma et al., 2003](#)), similar to other songbird species ([Forlano et al., 2006](#)). Aromatase has been observed in pre-synaptic terminals within HVC of zebra finches ([Peterson et al., 2005](#)). Aromatase could thus modulate local concentrations of neurosteroids (reviewed in [Forlano et al., 2006](#)).

#### 2.6. Changes in song stereotypy and song production may be differentially regulated

LD photoperiod and systemic T induce dramatic changes in Gambel's white-crowned sparrow song behavior. These changes include: an increase in song rate ([Smith et al., 1995](#); [Meitzen et al., 2007a](#)), an increase in syllable stereotypy ([Smith et al., 1995](#); [Tramontin et al., 2000](#); [Meitzen et al., 2007a](#)), and an increase in overall song length and buzz length ([Smith et al., 1995](#)). White-crowned sparrows are closed-ended learners that do not learn new syllables seasonally ([Marler and Tamura, 1964](#)), unlike some other species ([Brenowitz and Beecher, 2005](#)). While numerous studies have shown strong correlations between changes in song behavior and song control nuclei, few have tested the causal relationship between them. Recent studies have begun to provide evidence regarding which seasonal changes in the song control system mediate particular changes in song behavior. When song system growth is blocked by infusing AR and ER antagonists near HVC in white-crowned sparrows exposed to LD photoperiod and systemic T, song stereotypy differs between treatment groups, but song rate does not ([Meitzen et al., 2007a](#)). This result suggests that activation of sex steroid receptors in HVC is not necessary for the systemic T and LD photoperiod-induced increase in song rate, and instead mediates changes in song stereotypy, and furthermore, that increases in song rate are not sufficient for seasonal changes in the song control system. In support of this, deafened white-crowned sparrows greatly reduce song production, yet their song control systems still grow and song becomes more stereotyped in response to LD photoperiod and systemic T ([Brenowitz et al., 2007](#)).

### 3. Mechanisms of regression

#### 3.1. Transition to non-breeding conditions is not typically driven by decreasing photoperiod

As detailed above, an increase in day length stimulates male white-crowned sparrows to enter breeding condition, leading to an increase in circulating T and growth of the song control system. In contrast, much less is known about the mechanisms regulating the transition from breeding to non-breeding conditions. In the wild, once birds mate, establish nests, and start brooding, T levels decline to basal levels within a matter of weeks ([Wingfield and Farner, 1978](#)). This decline happens prior to the summer solstice while photoperiods are still increasing. Thus, the transition to non-breeding conditions is not actively driven by decreasing photoperiod. Instead, male white-crowned sparrows, like other seasonally-breeding songbird species undergo absolute photorefractoriness; their reproductive axis becomes insensitive to the stimulating effects of long-day photoperiods. This is in contrast to relative photorefractoriness, which is dependent upon a decrease in photoperiod. The mechanisms regulating the transition to photorefractoriness come from studies of various songbird species including Gambel's white-crowned sparrows ([Dawson and Goldsmith, 1983](#); [Wilson, 1985](#); [Saldanha et al., 1994](#); [Meddle et al., 1999](#)). Photorefractoriness is characterized by a decrease of GnRH expression and/or re-

lease, followed by a decrease in circulating levels of luteinizing hormone and follicle stimulating hormone, which ultimately lead to regression of the testes. The onset of photorefractoriness is determined weeks earlier when individuals enter breeding condition in response to the initial exposure to LD photoperiod, given that the transition to photorefractoriness is relative to the onset of LD and largely independent of subsequent changes in photoperiod (Dawson and Goldsmith, 1984; Bentley et al., 1997; Dawson, 2001; Dawson et al., 2001; Dawson, 2004). Ultimately, the decrease in circulating T removes steroidal trophic support for an enlarged song control system, which then regresses via neurodegenerative mechanisms.

It is not yet known if changes in photoperiod are sufficient to induce steroid-independent regression of the song control system in photostimulated males with high levels of circulating T. Despite the fact that the onset of photorefractoriness is not dependent upon a transition to SD photoperiod, we typically expose birds to SD photoperiod at the same time circulating T is withdrawn to insure that any stimulatory signal LD photoperiod may contribute to maintaining a breeding-state song control system is removed (Thompson et al., 2007). This manipulation is not the same as a transition to photorefractoriness, because male white-crowned sparrows are probably still photostimulated after just 3–4 weeks of LD and high levels of circulating T. Yet the manipulation abruptly removes two factors known to promote enlarged song control system nuclei: high levels of circulating T and LD photoperiod. The purpose of the manipulation is to remove as much exogenous trophic support for the song control system as possible so that it resembles that which is seen in birds held non-breeding conditions. Regardless, there are many unanswered questions regarding the steroid-independent contributions of photoperiod to song control system plasticity.

### 3.2. Steroidal mechanism of regression

Acute withdrawal of circulating T induces a significant decrease in HVC volume within 12 h (Thompson et al., 2007). This decrease occurs before the birds experience a change in photoperiod, which demonstrates that, at the very least, the initial regression of HVC volume is driven largely by the withdrawal of circulating sex steroids and not by physiological changes driven by photoperiod. This suggests that regression, like growth, of the song control system in male white-crowned sparrows is largely driven by changes in circulating levels of sex steroids.

### 3.3. Changes in neuronal morphology that underlie song control system regression

In general, long-term changes in HVC volume result from changes in neuron number. The initial decrease in HVC volume within 12 h of T withdrawal, however, results from an increase in neuron density (Fig. 2A, Thompson et al., 2007). HVC essentially collapses in on itself as the space between neurons decreases. Over the next four days, neuron number and soma area (a measure of neuron size) significantly decrease (Thompson et al., 2007). At this point, the rate of neuron loss offsets the decrease in neuron spacing, and neuron density significantly decreases. Thus, the increase in neuron density lasts only a few days.

The downstream nuclei of HVC, RA and Area X, regress more slowly than HVC, taking days to weeks instead of hours to days. The volumes of Area X and RA are not significantly regressed until seven and 20 days, respectively, following the transition to non-breeding conditions (Thompson et al., 2007). RA soma area and neuron density significantly regress by two days after the transition to non-breeding conditions and continue to regress for at least 20 days (Fig. 2B, Thompson et al., 2007). Thus the neuronal mech-

anism mediating regression is the same as that underlying the growth of RA: changes in soma area and neuron density with no change in neuron number.

### 3.4. Neurodegenerative mechanisms of HVC regression and steroidal neuroprotection

In male white-crowned sparrows, the loss of HVC neurons during song control system regression is mediated by caspase-dependent cell death pathways. Apoptotic-like processes are to be expected, given that HVC neuron number decreases by nearly 30% following a seasonal-like transition to non-breeding conditions (Thompson et al., 2007). HVC cells positive for activated caspase-3 are apparent three days after the transition to non-breeding conditions (accepted for publication, C.K. Thompson). In addition, infusion of a cocktail of caspase inhibitors near HVC in male Gambel's white-crowned sparrows transitioned to non-breeding conditions seems to prevent a decrease in HVC volume, neuron number, neuron spacing, and soma area (accepted for publication, C.K. Thompson).

T and/or its metabolites act within HVC to both induce and maintain its seasonal growth (discussed above). This suggests that T and/or its metabolites could play a neuroprotective role in rescuing HVC from the regression induced by the withdrawal of systemic T and exposure to SD photoperiod, not unlike the hormone-mediated neuroprotection that is seen in several *in vivo* animal models of neurodegenerative insult (Ramsden et al. 2003; Pike et al. 2006). Consistent with this, we found that direct intracerebral infusion of T near HVC unilaterally in castrated male white-crowned sparrows transferred to SD photoperiod and systemic T-withdrawal seems to ameliorate neurodegeneration of the ipsilateral HVC (unpublished data, C.K. Thompson). These results suggest that T and/or its metabolites directly act on HVC neurons to protect them from degenerative mechanisms induced by withdrawal of circulating sex steroids and photoperiod shift. Seasonal-like rapid regression of the song control system therefore may serve as an excellent model to further elucidate the molecular mechanisms that underlie hormone-mediated neuroprotection.

### 3.5. Behavior

Male white-crowned sparrows stop singing immediately following the withdrawal of circulating T (Meitzen et al., unpublished data). This observation strongly suggests that T is rapidly cleared from the blood stream. Though a time course for the clearance of circulating T following castration is not known in male white-crowned sparrows, intramuscular injections of highly concentrated T propionate dissolved in peanut oil into male song sparrows is cleared within 90 min (Soma et al., 1999). In male house finches, removal of subcutaneous T pellets results in significantly reduced circulating levels of T 24 h later (Deviche et al., 2006). If a similar time course for clearance of circulating T applies to white-crowned sparrows, it would suggest that abrupt cessation of singing in males is driven by the rapid withdrawal of T independent of photoperiod.

## 4. Future directions

There are many unanswered questions about seasonal-like plasticity of the song control system in male white-crowned sparrows, and many ways that the model presented here could be further tested, refined, and expanded. In addition to the questions raised above, outstanding questions include: (1) what are the downstream molecular cascades that are turned on or off by changes in circulating sex steroids? (2) Do the various neuron

types in HVC (RA-projecting, Area X-projecting, and interneurons) and RA (glutamatergic projection neurons and GABAergic interneurons) contribute to song control system growth and regression in different ways? (3) How does the absence of metabolites of T contribute to regression of the song control system? (4) How do rates of proliferation, migration, and incorporation of new neurons into HVC change during seasonal-like growth and regression? (5) What other electrophysiological properties, in addition to the ones described here, change during the growth and regression of the song control nuclei? (6) What changes occur in the syrinx during the transition to breeding conditions and to non-breeding conditions? (7) How similar are the proximate mechanisms in Gambel's white-crowned sparrow seasonal plasticity to other steroid-sensitive models? These and other questions illustrate that study of seasonal plasticity within the avian song control system will continue to serve as a useful model for hormone-mediated neural plasticity.

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