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Immediate and long-term effects of testosterone on song plasticity and learning in juvenile song sparrows

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ABSTRACT

Steroid sex hormones play critical roles in the development of brain regions used for vocal learning. It has been suggested that puberty-induced increases in circulating testosterone (T) levels crystallize a bird's repertoire and inhibit future song learning. Previous studies show that early administration of T crystallizes song repertoires but have not addressed whether new songs can be learned after this premature crystallization. We brought 8 juvenile song sparrows (*Melospiza melodia*) into the laboratory in the late summer and implanted half of them with subcutaneous T pellets for a two week period in October. Birds treated with T tripled their singing rates and crystallized normal songs in 2 weeks. After T removal, subjects were tutored by 4 new adults. Birds previously treated with T tended toward learning fewer new songs post T, consistent with the hypothesis that T helps to close the song learning phase. However, one T-treated bird proceeded to learn several new songs in the spring, despite singing perfectly crystallized songs in the fall. His small crystallized fall repertoire and initial lag behind other subjects in song development suggest that this individual may have had limited early song learning experience. We conclude that an exposure to testosterone sufficient for crystallization of a normal song repertoire does not necessarily prevent future song learning and suggest that early social experiences might override the effects of hormones in closing song learning.

1. Introduction

Steroid hormones have important effects on memory formation, including vocal learning, in birds (Saldanha et al., 1999). Both estrogens and androgens have organizational effects on development of the song control system and on song learning behaviors (Gurney and Konishi, 1980; Bottjer and Johnson, 1997; Schlinger, 1997; Fusani and Gahr, 2006). Estrogens may act to promote learning and memorization of songs and may be required for maintaining the brain plasticity necessary for keeping the sensitive phase open (Bottjer and Johnson, 1997; Schlinger, 1997). In contrast, androgens appear to function to promote crystallization of song production (motor stereotypy), and may terminate the song memorization phase (Bottjer and Johnson, 1997; Schlinger, 1997). Each of the major song control nuclei contains androgen receptors, and HVC also contains estrogen receptors (Schlinger and Brenowitz, 2009).

Experiments examining the role of androgens in song crystallization and song learning have been carried out on four species of Emberizine sparrows, song sparrows (*Melospiza melodia*), swamp sparrows (*M. georgiana*), white-crowned sparrows (*Zonotricia leucophyrs*) and dark-eyed juncos (*Junco hyemalis*). In all of these species song learning is completed in the first year – they are 'closed-ended' learners (Beecher and Brenowitz, 2005) – and all or most song memorization occurs in the bird's natal summer (the 'critical period' or 'sensitive phase'). Marler et al. (1988) castrated young song sparrows and swamp sparrows and found that these birds failed to crystallize their songs within the first year as they normally would. Application of testosterone (T) when the birds were more than a year old induced rapid song crystallization and when T implants were removed, the birds regressed to plastic song. Whaling et al. (1995) tape-tutored juvenile white-crowned sparrows during their sensitive period (days 10–100) and treated them with T implants between days 100 and 130, i.e., between the end of the song memorization phase and the beginning of the production phase. T-treated birds crystallized their songs within two weeks, or approximately 5 months earlier than control birds. However, the songs of the T-treated birds were abnormal, resembling those of isolate birds. They were abnormal not only during the early

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T-induced crystallization, but again the following spring, long after T implants had been removed. In contrast, dark-eyed juncos developed structurally normal song in two studies with very different schedules of T administration. Titus et al. (1997) captured juvenile juncos in the field in October and November, long after the sensitive period, and administered T to them in late January during the early part of the motor phase when birds were singing plastic song. Song structure in T-treated birds was normal although these birds had smaller song repertoires and sang less than control birds. Gulledge and Deviche (1998) captured juvenile juncos in the field in mid-September, and administered T implants shortly thereafter, in the silent gap between the end of the memorization phase and beginning of the motor (production) phase. Birds began to sing two weeks after T implants and song structure appeared normal. Finally, one study with zebra finches (*Taeniopygia guttata*), a species in which the memorization phase of song learning is short and is overlapped by a relatively short production phase, found that early T administration induces premature crystallization of incompletely developed song (Korsia and Bottjer, 1991).

The one clear conclusion emerging from these studies is that T administration either ahead of schedule in normal birds, or later in life in castrated birds, produces song crystallization. On the other hand, these studies provide conflicting answers to the question of whether early song crystallization induced by T administration will lead to normal song development: under these conditions, white-crowned sparrows and zebra finches developed abnormal song whereas juncos developed normal song. It is possible that song development proceeded more normally in the junco studies because song memorization had occurred in the field, whereas in the white-crowned sparrow song memorization occurred in the lab with tape-recorded song. There is considerable evidence that birds are more apt to learn from live song tutors than from tape tutors (reviewed in Beecher and Brenowitz, 2005) and in fact, some of the strongest evidence for this comes from these species (Beecher, 2008). A second possible explanation for this difference relates to variation in the time period when T was administered. Zebra finches are the only species in which T was administered during the early sensitive period (among other time periods) and it is clear that the timing of T administration has dramatic effects on crystallized repertoires in this species (Korsia and Bottjer, 1991).

Although these studies have made great strides in elucidating the relationship between T and song crystallization, none of the previous studies addressed the question of whether T-induced premature crystallization also prevents further song learning. Note that in closed-ended learners (all the species considered so far), normal crystallization marks not only the bird's arriving at its species-typical song repertoire, but finalizing that repertoire, i.e., no new songs will be added nor old songs subtracted in subsequent years. In the present study, we investigated the role of T in song crystallization and learning of new songs in juvenile song sparrows. Song sparrows are closed-ended song learners and males develop repertoires of 6–13 song types in the first year of life (Peters et al., 2000). Typically much of the song learning takes place during an early sensitive phase in the natal summer (Marler and Peters, 1987), although song sparrows can continue to learn new songs throughout the first year of life under some circumstances (Nordby et al., 2001; Nulty et al., 2010). In this study, we treated wild-caught juveniles with T for two weeks after the conclusion of the summer sensitive period and exposed them to new live song tutors after removing the implants. We asked five specific questions. First, will T administered to juveniles in the autumn cause birds to prematurely crystallize their song repertoires as predicted by previous work? Second, how quickly will T change song rate and song structure? This process takes several months in unmanipulated wild birds in our field population. Third, will the songs in these early-crystallized song repertoires have normal acoustic structure? Fourth, will these early-crystallized song repertoires be of normal size? Fifth, will early transient T prevent subsequent learning of new songs? That is, will a dose of T sufficient to crystallize a bird's song repertoire also inhibit future song learning? On the basis of the studies reviewed above, we predicted that T would prematurely crystallize song repertoires and this would happen within two weeks. Because T would be administered after early song memorization and because that phase would be taking place under natural field conditions, we also predicted that crystallized songs would have normal acoustic structure. At the same time this early field experience might handicap our test of the fifth question, for once song sparrows have learned songs under normal field conditions, they are much less likely to learn songs in the lab (Beecher and Brenowitz, 2005; Beecher, 2008; Nulty et al., 2010). Nonetheless, we concluded that the benefit of using early field tutors would offset the disadvantages and predicted that if T acts to prevent future song learning, the majority of late song learning from lab tutors should be seen in the control subjects.

2. Methods

2.1. Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of the University of Washington (Protocol Numbers 2207-03 and 2008-06). We released all subjects at the site of capture following the experiments. Since subjects had substantial field experience as juveniles we felt that they might have good chances of survival and we confirmed that at least one subject later obtained a breeding territory at the field site.

2.2. Subjects

We brought eight juvenile birds into the laboratory from our field site (Discovery Park, Seattle, WA) between 28 July and 2 August 2005. All birds were independent of their parents when collected. We estimated the birds to be between 50 and 100 days old. These juveniles had been exposed to song in the field for most or all of the classical sensitive period for song memorization (primarily 20–60 days: Marler and Peters, 1987). Birds were identified as juveniles by their distinctive brown streaky juvenile plumage (Pyle, 1997) and/or their production of subsong. We collected only juveniles with wing chords $\geq$66 mm, to be sure that we had only male subjects, a relatively good measure for song sparrows in our population and in general (Nice, 1937). The sex of each bird was confirmed through genetic testing using PCR sex analysis on a small drop of blood ("Sex Made Easy," Zoogen Inc.; www.zoogen.biz).

In the laboratory each bird was placed in a cage (dimensions: 40 cm $\times$ 23 cm $\times$ 26 cm), and given ad lib. food and water. The cage was placed in a sound attenuating chamber (for details see Beecher et al., 2007) so that each bird was isolated from the vocalizations of all other birds. Each chamber was equipped with two loudspeakers (one on either end of the chamber) and a microphone connected to a PC computer. The computer was programmed to record and save all songs produced by each subject throughout the experiment using the sound detector module in Syrinx (www.syrinxpc.com; John Burt). Because of hardware limitations, only four subjects could be recorded on a given day, so individual birds were recorded every other day. For analyses, we pooled birds sampled in both days. Birds were housed under a natural Seattle photoperiod, which was controlled by an astronomical timer. All subjects were released at the capture site upon completion of the experiment.