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Plasticity of the Rufous-winged Sparrow, *Aimophila carpalis*, song control regions during the monsoon-associated summer breeding period

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Abstract

In most temperate zone songbirds, exposure to increasing photoperiod in the spring stimulates the reproductive system and induces reproductive behaviors. Additionally, the brain regions that control singing (song control regions; SCRs) are larger during the breeding season, thus paralleling changes in the activity of the reproductive system. However, in some birds, environmental factors other than photoperiod initiate breeding. For example, free-living male Rufous-winged Sparrows develop their testes in March due to increasing photoperiod, but have relatively low plasma T until after they begin to breed, usually in July, during the monsoon period when day length is declining. We tested the hypothesis that SCRs grow and singing behavior increases after the monsoon rains begin. We captured adult male Rufous-winged Sparrows in July 2002, 7 days before and 20 days after the monsoon rains began, euthanized birds in the field, collected their brains, and measured SCR volumes from sections immunostained for the neuronal marker NeuN. In June and July 2006, we measured song rates in the field before and after the monsoon rains. SCR volumes were larger and singing behavior increased after the onset of the monsoon rains, coinciding with the initiation of breeding. Unlike in other species studied so far, SCR volumes grew as day length was decreasing. Comparative studies utilizing species that do not breed when day length is increasing may provide information on the relative contributions of various environmental factors to SCR neuroplasticity.

Keywords: Neuroplasticity; HVC; Testosterone; Singing; Songbird; Day length; Testes; NeuN

Introduction

The avian song control system is one of the best-studied models available to investigate interactions between gonadal steroids and environmental factors on brain plasticity. In many northern temperate zone songbirds, the brain regions controlling singing behavior (song control regions; SCRs) are larger in the spring than in the fall, coinciding with the breeding season and increases in photoperiod, circulating testosterone (T) and singing behavior (Nottebohm, 1981; Brenowitz et al., 1991, 1998; Smith, 1996; Gulledge and Deviche, 1997; Smith et al., 1997b). In these species, the primary cue that stimulates the reproductive system and initiates breeding behaviors is the increase in photoperiod, although other cues such as temperature, food availability or social factors often play a role in determining the exact timing of breeding (Wingfield and Kenagy, 1991). It is hypothesized that, in males, the increase in photoperiod stimulates gonadal T secretion, which in turn increases singing behavior and SCR volumes (Smith et al., 1997b; Nottebohm, 2002). However, in some species, SCRs grow in late winter or early spring, before maximum plasma T concentrations are reached and before singing behavior increases (Tramontin et al., 2001; Caro et al., 2005). Additionally, increasing photoperiod can increase the size of some SCRs independent of its effect on T secretion (Bernard et al., 1997; Smith et al., 1997b; Whitfield-Rucker and Cassone, 2000, Dloniak and Deviche, 2001; Strand and Deviche, 2007). Furthermore, social interactions can also influence the effectiveness of T treatment or photoperiod on SCR growth (Tramontin et al., 1999; Boseret et al., 2006). Thus, an increase in circulating T may play a major role in the control of SCR plasticity, but other factors contribute independently to this regulation. Identifying the extent to which these factors

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influence SCR growth may help elucidate mechanisms and functions of adult neuroplasticity.

The effects of photoperiod on SCR growth may depend on reproductive condition. Many temperate zone songbirds cease to breed during the summer although photoperiod is still longer than needed to stimulate their reproductive system in the spring. At this time, birds lose sensitivity to photoperiod as a reproductive stimulus, become reproductively quiescent and are said to be absolutely photorefractory (Hamner, 1968; Nicholls et al., 1988; Deviche et al., 2000; Dawson et al., 2001). Absolute photorefractoriness is associated with a decrease in SCR sizes. For example, adult male European Starlings, *Sturnus vulgaris* (Bernard and Ball, 1995), and House Sparrows, *Passer domesticus* (Whitfield-Rucker and Cassone, 2000), have a larger HVC when photostimulated than when photorefractory and exposed to long days. Investigating the relative contributions of photoperiodic condition and gonadal hormones to SCR growth in such species is complicated by the fact that photorefractoriness induces regression of the reproductive system and low secretion of these hormones. Therefore, studies on this subject may benefit from the use of species that show seasonal changes in gonadal hormone secretion but in which these changes are not primarily driven by photoperiod. For example, Moore et al. (2004) found in an equatorial songbird, the Rufous-collared Sparrow, *Zonotrichia capensis*, that SCRs grow during the breeding season. As these birds are naturally exposed to an almost constant photoperiod, the study allowed the researchers to eliminate photoperiod as a primary factor controlling SCR growth and to conclude that other environmental cues are associated with this response.

Little information on changes in SCRs in free-living, northern latitude, flexibly breeding birds is available. MacDougall-Shackleton et al. (2001) found in captive White-winged Crossbills, *Loxia leucoptera*, that exposure to long photoperiod stimulated the reproductive system and induced SCR growth. However, crossbills are flexible breeders and have an extended annual breeding season, the duration and timing of which are strongly influenced by food availability rather than photoperiod (Deviche and Sharp, 2001). MacDougall-Shackleton et al. (2001) did not determine whether cues other than photoperiod (i.e., food availability) stimulate SCR growth in crossbills and it remains unknown whether free-living, flexibly breeding birds have large SCR volumes when in breeding condition, independent of the time of year or natural day length.

The Rufous-winged Sparrow, *Aimophila carpalis*, is a resident species of the Sonoran Desert of Northern Mexico and Southeast Arizona (Lowther et al., 1999). Adult males are paired and maintain territories year-round and pairs rarely leave their territory, even in times of extreme drought (Ohmart, 1969; Lowther et al., 1999). These birds are flexible breeders that can breed any time between March and August, although in most years breeding occurs during the summer monsoon (July and August) and birds breed in the spring only following an unusually wet winter (Ohmart, 1969; Lowther et al., 1999; Deviche et al., 2006b; Small et al., 2007). Males sing year-round and although song rates are higher in the spring than in winter, song rates are highest during breeding (Groschupf, 1983; Lowther et al., 1999). The gonads of free-living adult male Rufous-winged Sparrows develop in the spring in anticipation of breeding, but plasma T remains relatively low until summer breeding begins (Deviche and Small, 2005; Deviche et al., 2006b). At this time, plasma T rises to a seasonal maximum level within 2 to 4 weeks of the onset of the monsoon, then returns to levels similar to those before the monsoon within 1 to 2 weeks and drops to basal levels in September when breeding activities cease and gonads regress (Deviche et al., 2006b).

The main objective of the present study was to determine whether SCRs grow and singing behavior increases after the monsoon rains begin in free-living Rufous-winged Sparrows. We collected adult males before and during the monsoon and measured SCR volumes and neuron number. We also determined song rates before and after the beginning of the monsoon to correlate changes in this behavior with changes in SCRs and plasma T. If SCR growth is controlled by the same factors that initiate breeding behaviors, we predicted that SCRs would grow after the onset of monsoon rains. Alternately, if increasing photoperiod and/or submaximal levels of plasma T initiate SCR growth in the spring, as is the case in some northern latitude songbirds (Tramontin et al., 2001; Caro et al., 2005), we predicted that there would be little or no growth of the SCRs after the onset of monsoon rains because at this time SCRs would already be maximally enlarged. This possibility is supported by the fact that in other flexibly breeding songbirds, increasing photoperiod in captive birds induces SCR growth, possibly through an increase plasma T or a combination of these two factors (MacDougall-Shackleton et al., 2001). The present data are the first to demonstrate seasonal neuroplasticity in northern latitude, adult male songbirds that breed primarily in response to environmental cues other than photoperiod.

Materials and methods

Animals

On July 2 ($n=6$) and July 29 ($n=6$), 2002, adult male birds were caught at the University of Arizona's Santa Rita Experimental Range (31°46′N/110°50′W, elevation approximately 900 m) using Japanese mist nets and conspecific song playback recordings. Day length on July 2 was 14.18 h and on July 29 was 13.75 h, a difference of 26 min. In 2002, the monsoon (defined by the first of at least three consecutive days when the dew point averages 12.8 °C or higher) began on July 9, and the first rain since March 19 occurred on July 10 [National Climatic Data Center's Green Valley, AZ weather station (31°54′N/111°00′W; COOP # 023668)]. All birds were caught between 5:30 am and 1:30 pm. The Arizona State University Institutional Animal Care and Use Committee pre-approved all experimental protocols. Scientific collecting permits were secured from the Arizona Game and Fish Department and the United States Fish and Wildlife Service before birds were captured. The study was carried out in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals (DHEW Publication 80-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205).

Blood sampling, hormone assay, and cloacal protuberance measurements

Within 3 min of capture, a blood sample (approximately 200 μl) was collected from a wing vein of each bird into heparinized microhematocrit capillary tubes. Samples were kept on ice until centrifuged on the same day as collection. Plasma was collected and stored at −80 °C until assayed for T