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Breeding-context-dependent relationships between song and cFOS labeling within social behavior brain regions in male European starlings (*Sturnus vulgaris*)

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Abstract

Male European starlings (*Sturnus vulgaris*) sing throughout the year, but the social factors that motivate singing behavior differ depending upon the context in which song is produced. In a non-breeding context (when testosterone concentrations are low), starlings form large, mixed-sex flocks and song is involved in flock cohesion and perhaps maintenance of social hierarchies. In contrast, in a breeding context (when testosterone concentrations are high), male song plays a direct role in mate attraction. How the nervous system ensures that song production occurs in an appropriate context in response to appropriate stimuli is not well understood. The song control system regulates song production, learning, and, to some extent, perception; however, these nuclei do not appear to regulate the social context in which song is produced. A network of steroid hormone sensitive nuclei of the basal forebrain and midbrain regulates social behavior. The present study used the immediate early gene cFOS to explore possible involvement of these regions in context-dependent song production. Numbers of cFOS-labeled cells in the medial bed nucleus of the stria terminalis, anterior hypothalamus, and ventromedial nucleus of the hypothalamus related positively only to song produced in a breeding context. In contrast, numbers of cFOS-labeled cells in three zones of the lateral septum related positively only to song produced in a non-breeding context. Taken together, these data suggest differential regulation of male starling song by social behavior nuclei depending upon the breeding context in which it is produced.

Keywords: Songbird; Season; Context; Vocal communication; Immediate early gene; cFOS; Lateral septum; Bed nucleus of the stria terminalis; Anterior hypothalamus; Ventromedial nucleus of the hypothalamus

Introduction

In songbirds, as in other social vertebrates, the function of vocal behavior shifts depending upon the context in which it occurs. Song production by adult male songbirds is typically used to attract mates and to defend territories and highly influenced by social and environmental factors including the presence of potential mates/rivals and season (e.g. Catchpole and Slater, 1995; Eens, 1997; Eens et al., 1990). Many studies have focused on how the songbird brain regulates various aspects of singing behavior such as song learning, production, and perception (e.g. Brenowitz et al., 1997; Margoliash, 1997; Nottebohm et al., 1976; Wild, 1997). However, how the nervous system ensures that song production occurs in the appropriate context in response to appropriate stimuli has not been well examined. With this study, we explore the possibility that the proximate mechanisms regulating singing behavior in songbirds differ depending upon the context in which it occurs.

European starlings (*Sturnus vulgaris*) are an ideal model system in which to explore the context-dependent neural regulation of singing behavior. Male starlings sing throughout the year, but the function of song and the factors that motivate song production differ depending upon the context in which it occurs. Within a breeding context, circulating concentrations of the steroid hormone testosterone (T) are elevated (Ball and Wingfield, 1987; Riters et al., 2002), and starling song can be highly sexually motivated. Possession of a nest site is critical for successful mate attraction (see Gwinner et al., 2002). In the absence of female conspecifics, males both with and without

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nest sites sing at similar, relatively low levels (Riters et al., 2000). However, only males with nest sites increase their song rate in response to the introduction of a female (Riters et al., 2000). Once these individuals pair with a female, song production occurs immediately prior to almost every copulation (Eens and Pinxten, 1990; Eens and Pinxten, 1995; Pinxten and Eens, 1997). Thus, song in males with nest sites is considered highly sexually motivated. In contrast, males without nest sites do not increase their song rate in response to the introduction of a female conspecific but continue to sing at relatively low levels (Riters et al., 2000). What motivates singing behavior in males without nest sites is not clear. However, given that female presence does not dramatically alter song rate, it does not seem to be highly sexually motivated or to function in immediate mate attraction.

In a non-breeding context, in fall and winter, starlings neither occupy nor defend nest sites, and the introduction of a female conspecific does not stimulate male song output (Riters et al., 2000). At this time, circulating concentrations of T are basal (Ball and Wingfield, 1987; Riters et al., 2002), and song does not appear to play any direct role in mate attraction. Rather, birds are typically found feeding and roosting in large, mixed-sex flocks (Feare, 1984), and non-breeding context song is suggested to play a prominent role in flock cohesion and perhaps the establishment and/or maintenance of dominance hierarchies within the flock (Hausberger et al., 1995; Summers et al., 1987). As such, in contrast to breeding context song, singing behavior in a non-breeding context can be considered more broadly socially motivated.

In songbirds, song is controlled by a well-defined series of cytoarchitecturally distinct, interconnected brain nuclei known as the song control system. The brain regions HVC (used as a proper name), robust nucleus of the acropallium (RA), lateral magnocellular nucleus of the anterior nidopallium (lMAN), and Area X (among several others) regulate song learning, production, and to some extent perception (e.g. Brenowitz et al., 1997; Margoliash, 1997; Nottebohm et al., 1976; Wild, 1997). Data indicate that activity within the song control nuclei Area X, RA, and lMAN can be context-dependent. For example, activity within the song system can differ depending upon whether a bird directs song towards a conspecific or sings alone (Hessler and Doupe, 1999; Jarvis et al., 1998) or whether a bird is singing within or outside a breeding context (Heimovics and Riters, 2005; Liu and Nottebohm, 2005; Riters et al., 2004). Taken together, these studies indicate that the regulation of singing behavior by the song control system can be strongly influenced by the context in which it occurs. However, to date, no data implicate song nuclei in regulating motivational aspects of vocal communication in songbirds. Lesion studies suggest that damage to song nuclei results in deficits in song production, but lesioned birds continue to adopt singing postures and display motor patterns associated with singing behavior, indicating an intact motivation to communicate (Bottjer et al., 1984; Nordeen and Nordeen, 1993; Nottebohm et al., 1976; Sohrabji et al., 1990). A growing body of research implicates nuclei outside of the classically defined song control system as being involved in regulating the motivation to sing and suggests that these regions might influence singing behavior in a context-dependent manner.

Vocal communication can be central to the mediation of social interactions between conspecifics. The lateral septum (LS), medial bed nucleus of the stria terminalis (BSTm), preoptic area (POA), anterior hypothalamus (AH), ventromedial hypothalamus (VMH), and areas of the tegmentum (including the ventral tegmental area [VTA] and midbrain central gray [GCt]) are components of a proposed vertebrate 'social behavior network' (Newman, 1999). These interconnected steroid hormone sensitive nuclei appear central to the control of all types of vertebrate social behavior including communication, sexual behavior, and aggression (Goodson, 2005).

Recently, brain regions within the social behavior network have been directly implicated in the regulation of singing behavior in songbirds. For example, in starlings, the numbers of cFOS-labeled cells (an indirect marker of neural activity) within the medial preoptic nucleus (POM) and VTA relate positively to song produced within, but not outside of, a breeding context (Heimovics and Riters, 2005). In song sparrows, cFOS labeling in VTA and GCt relates positively to singing behavior in response to territorial intrusion, suggesting that midbrain regions influence agonistic song production (Maney and Ball, 2003). Lesions to the lateral septum (LS) either facilitate or inhibit courtship song depending upon whether the species examined is territorial or gregarious (Goodson et al., 1999), suggesting that septal modulation of song production is influenced by social context. Taken together, these data suggest that nuclei involved in social behavior might differentially regulate song within different social contexts.

Here, we used immunocytochemistry for the protein product of the immediate early gene (IEG) cFOS (an indirect marker of neuronal activity) to explore a possible role for these regions in the context-dependent regulation of male starling singing behavior. We compared numbers of cFOS-labeled cells within social behavior network regions of males singing within versus outside of a breeding context. Because these nuclei are known to mediate multiple forms of social behavior and are steroid-hormone-sensitive, the relationships between cFOS and aggression, nest box status, and breeding condition were also examined.

Methods

Animals

In fall 2002 and early winter 2003, forty-two adult male and four adult female starlings were captured on a single farm north of Madison, Wisconsin using fly-in traps. These are the same birds used in a previous study examining cFOS immunolabeling within a separate group of brain regions not reported here (Heimovics and Riters, 2005). Birds were immediately housed indoors after capture in single sex cages (91 cm × 47 cm × 47 cm) in the University of Wisconsin Department of Zoology animal facilities. Photoperiodic and hormone manipulations were used to place birds into either a breeding (i.e. spring-like with high circulating testosterone (T)) or a non-breeding (i.e. fall/winter-like with low T) condition. Protocols used for bird acquisition, surgery, and behavioral testing were in adherence to guidelines approved by the 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) as well as the University of Wisconsin-Madison Research Animal Resource Committee (RARC).