

S14-5 Studies of song behavior in European starlings: interrelationships among testosterone, neuroanatomy and immune function

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Abstract Female starlings pair preferentially with males that produce song organized into long bouts. Females exhibit immediate early gene responses in the auditory forebrain that are biased towards longer bout songs. In male starlings, length of song bout correlates with variation in the volume of two key brain areas controlling song production, the HVC and the robust nucleus of the archistriatum (RA). Length of song bout and song rate are correlated positively with variation in humoral and cell-mediated immunity respectively. Testosterone stimulates female-directed song and the volume of the song control nuclei, but inhibits (perhaps indirectly) immune responses. This hormone is thus positioned as an important endogenous signaling mechanism that coordinates several aspects of male physiology and behavior relevant to reproductive success in starlings.

Key words Brain control, Testosterone, Immune response, European starling

1 The function of male song in relation to female choice

In this paper we review recent studies that interrelate endocrine state, brain mechanisms for song perception and production, features of song, and immune measures in European starlings (*Sturnus vulgaris*), considering first the adaptive significance of male song in that species. Several lines of evidence indicate that male starling song influences female choice. Male starlings paired with a female will increase their rate of singing just prior to copulation (Eens and Pinxten, 1990) and stop singing only after the clutch is complete, whereas unpaired males continue singing throughout the breeding season (Kluyver, 1993; Eens, 1997). In fact, male song output is closely related to different stages in the female breeding cycle. There is a rapid decrease in male singing activity after pairing (Eens et al., 1994; Hindmarsh, 1984), and then an increase 2 to 4 days prior to egg laying, after which song rates remain elevated until the end of the laying period before nearly ceasing altogether (Eens et al., 1994).

Likewise, male song rate is negatively correlated with the date of clutch initiation (Mountjoy and Lemon, 1996; Wright and Cuthill, 1992). The post-pairing period of high song output coincides, moreover, with the presumed fertile period for female starlings (Birkhead et al., 1987), and (albeit roughly) with the most rigorous period of mate guarding for the male (Pinxten et al., 1987). In the field, copulations between starlings are almost always preceded by bouts of male song (Eens et al., 1989; Eens and Pinxten, 1995;

Mountjoy and Lemon, 1996); and when presented with a conspecific female, unmated captive male starlings sing many more song bouts than when confronted with a conspecific male (Eens et al., 1993). The number of songs sung in the nest box also increases significantly with the introduction of a female, both for captive male starlings (Eens et al., 1993) and those in the field (Eens et al., 1994).

Among males, both repertoire size and song bout length are directly correlated with age and mating success (Eens et al., 1991). In the laboratory, moreover, female behavioral preferences can be directly controlled by varying the mean length of male song bouts. That is, females will spend more time listening to long bouts of male song than to shorter ones, and will preferentially track the position of longer bouts coming from different locations (Gentner and Hulse, 2000).

2 Testosterone and male starling song

Like many temperate zone songbirds, starlings are seasonal breeders and exhibit marked seasonal variation in hormone concentrations in the plasma (Dawson, 1983; Ball and Wingfield, 1987). As noted above, song activity peaks just prior to and during the egg-laying period which means that high rates of song roughly correlate with high concentrations of plasma testosterone (T) in field-caught males (Dawson, 1983; Ball and Wingfield, 1987). However, it is not the case that male starlings sing only when breeding. Song can be heard in the fall as well, and even in late summer during molting (Feare, 1984; Eens, 1997). Song in the fall and early winter, in contrast to breeding song, does not

seem to be influenced by the presence or absence of a female. When males held in outdoor aviaries with nest boxes are presented with a female, they do not change their song rates, whereas males housed in similar conditions in the spring increase song rates dramatically (Riters et al., 2000). Experimental studies involving the administration of exogenous T indicate that T does enhance the rate of song production in male starlings but it is specifically female-directed song that is enhanced (DeRidder, Pinxten and Eens, 2000; Pinxten et al., 2002).

3 Variation in the song control system and song behavior in starlings

Starlings, like all songbirds, possess a specialized forebrain circuit involved in the learning and production of song (Brenowitz et al., 1997). Captive studies involving the manipulation of photoperiod, as well as studies of starlings housed in outdoor aviaries experiencing natural fluctuations in photoperiod, indicate that a key forebrain nucleus, HVC, changes seasonally in volume (Bernard and Ball, 1995; Riters et al., 2002). An important nucleus for the control of courtship behavior, the preoptic medial nucleus (POM), also exhibits variation in volume and is largest during the breeding season (Riters et al., 2000). These brain changes are regulated, at least in part, by seasonal variation in testosterone concentration in plasma (Bernard and Ball, 1995;

Riters et al., 2000). Within older birds, the size of the HVC and another brain nucleus involved in song production, the robust nucleus of the archistriatum (RA), correlates with variation in length of song bout (Bernard et al., 1996) — Fig. 1. It is not known whether this intra-specific variation is related to variation in concentration of plasma testosterone. Thus features of male neuroanatomy appear to be driven by behavioral attributes that females use in choosing males.

4 Neural correlates of song perception in female starlings

It was discovered about 10 years ago that conspecific song can induce a marked expression of immediate early genes in the auditory forebrain of male and female songbirds (Mello et al., 1992). In particular, many neurons in the caudomedial neostriatum (NCM) and the caudal ventral hyperstriatum (cHV) show a rapid up-regulation of the immediate early gene ZENK in response to the presentation of conspecific songs (Mello et al., 1992). This gene expression is tuned to the acoustics of particular conspecific song syllables (Ribiero et al., 1998). Interestingly, the genomic response also habituates to the repeated presentation of the same conspecific song (Mello et al., 1995). In starling females, it was found that the number of cells expressing the ZENK protein in the ventral NCM were significantly higher in those females exposed to longer songs, whereas variation in the total duration of song exposure had no significant effect (Gentner et al., 2000). These results suggest that subregions of NCM in female starlings are tuned to variation in male song length. Thus females exhibit biases in gene expression that correlate with song attributes to which they attend when choosing males.

5 Why choose males that sing longer bout songs?

One attractive theory for female choice is that females attend to characters that predict male quality (Andersson, 1994). Recently much work has focused on traits that correlate positively with variation in immune responses as a basis for female choice (Folstad and Karter, 1992). In male starlings, measures of cell-mediated and humoral immunity were assessed via cutaneous swelling responses to T-cell mitogen phytohaemagglutinin and via antibody responses to a novel antigen, keyhole limpet haemocynin. Song rate and length of song bout were positively correlated with cell-mediated and humoral immunity respectively. Thus features of male song chosen by females experimentally do predict variation in immunocompetence (Duffy and Ball, 2002).

6 The role of testosterone

In European starlings it is clear that there is interplay between female choice and various aspects of male behavior, neuroanatomy and physiology. The gonadal hormone T is involved in the regulation of all these aspects of the male phenotype. It stimulates song behavior during the breed-

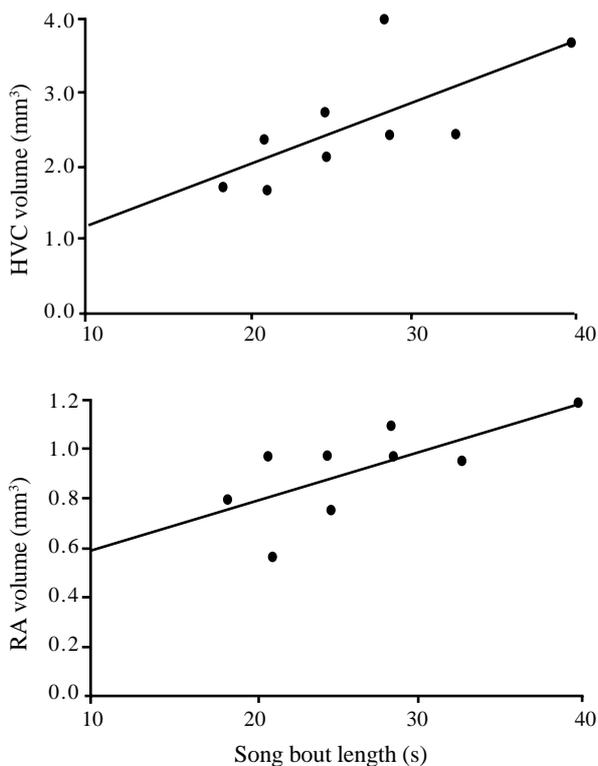


Fig. 1 Scattergrams showing the positive correlation between length of song bout (in seconds) and the volume of song nuclei HVC (top) and robust nucleus of the archistriatum (RA) (bottom) in the brain of European starlings

From Bernard et al. (1996).

ing season (Pinxten et al., 2002) and it promotes the growth of song nuclei such as HVC and RA (Bernard and Ball, 1995). It also inhibits cell-mediated and humoral immune measures, although this may be through increased corticosterone secretion (Duffy et al., 2000). Further studies are needed to assess exactly how this hormone may coordinate the disparate but related aspects of the male phenotype that are so important for reproductive success.

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