Animal Behaviour 86 (2013) 25–35

Contents lists available at SciVerse ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Early-life stress affects song complexity, song learning and volume of the brain nucleus RA in adult male song sparrows

Kim L. Schmidt [a,b,*], S. Drew Moore [a], Elizabeth A. MacDougall-Shackleton [a,b], Scott A. MacDougall-Shackleton [a,b,c]

[a] Department of Biology, University of Western Ontario, London, ON, Canada
[b] Advanced Facility for Avian Research, University of Western Ontario, London, ON, Canada
[c] Department of Psychology, University of Western Ontario, London, ON, Canada

ARTICLE INFO

Article history:
Received 11 January 2013
Initial acceptance 19 February 2013
Final acceptance 18 March 2013
Available online 2 May 2013
MS. number: A13-00042

Keywords:
developmental indicator
glucocorticoid
Melospiza melodia
nutritional stress
sexual selection
songbird
song control system
song sparrow
vocal performance

The developmental stress hypothesis proposes that the honesty of birdsong is maintained by costs incurred during development, such that song in adulthood reflects exposure to early-life stressors. We determined the effects of early-life (7–60 days of age) food restriction or corticosterone (CORT) treatment on adult song production and neuroanatomy in male song sparrows, *Melospiza melodia*. When males were adults, we quantified song type repertoire size, syllable repertoire size, song-learning accuracy, trill deviation (the speed of frequency modulation in a trill) and song type stereotypy. We also analysed the volumes of the song control nuclei HVC, the robust nucleus of the arcopallium (RA), area X and the number of neurons in HVC. Song type and syllable repertoire sizes of food-restricted and CORT-treated birds were smaller than those of controls. Food restriction, but not CORT treatment, also reduced song-learning accuracy. We observed no effects of either treatment on trill deviation or song type stereotypy. However, trill deviation was significantly related to paternal repertoire size, suggesting a heritable component to some aspects of vocal learning. The volume of RA was smaller in food-restricted birds than in control or CORT-treated birds. Neither treatment affected the volumes of HVC or area X, or the number of neurons in HVC. Our results suggest that song complexity and song-learning accuracy may be honest indicators of a male song sparrow's early ontogeny and that early-life stress has long-lasting effects on the song control system in this species. However, measures of vocal performance (trill deviation, song type stereotypy) do not appear to be significantly affected by early-life stress in song sparrows.

In many species of songbirds, the species-specific characteristics of song are acquired early in life by learning from adult tutors. Male songbirds typically sing for two primary reasons: to defend territories and to attract females (Catchpole & Slater 1995). Therefore, variation in a male's ability to learn song could have important fitness consequences. When selecting a mate, female songbirds may attend to a variety of features of song (Nowicki & Searcy 2004). For example, females may prefer males that sing more complex song (MacDougall-Shackleton 1997), which is typically determined by the number of song types or syllables in their repertoire. In addition, females may attend to features of song that reflect the quality of song learning, for example preferring songs types characteristic of the natal population (MacDougall-Shackleton et al. 2001) or songs that contain fewer invented notes (Nowicki et al. 2002a). Another feature of song that may be important for attracting females is vocal performance, for example, the ability of a male to produce physically challenging notes such as trills that require rapid and precise coordination of vocal tract movement (Podos 1997; Ballentine et al. 2004).

The learning and production of song are controlled by a series of interconnected brain nuclei that are collectively referred to as the song control system. The nuclei that make up the song control system exhibit rapid growth during the period of song acquisition (Bottjer et al. 1985). Variation in the size of the song control nuclei in adulthood may be related to variation in the quality of song production. For example, the volume of HVC may be positively correlated with song complexity between species (DeVoogd et al. 1993) and the volume of both HVC and the robust nucleus of the arcopallium (RA) may be positively correlated with song complexity within species (Garamszegi & Eens 2004). However, this relationship may not hold true for all species. For example the

* Correspondence: K. L. Schmidt, Department of Biology, Advanced Facility for Avian Research, University of Western Ontario, London, ON N6A 5B8, Canada.
E-mail address: kschmi5@uwo.ca (K. L. Schmidt).

0003-3472/$38.00
http://dx.doi.org/10.1016/j.anbehav.2013.03.036

size of HVC is not related to song complexity in red-winged blackbirds, *Agelaius phoeniceus* (Kirn et al. 1989). In addition to song complexity, the volume of the song control nuclei may be related to other song features such as the length of song phrases (Airey & DeVoogd 2000) or song bouts (Bernard et al. 1996) and the stereotypy of note structure (Smith et al. 1997a).

As mentioned above, female songbirds may attend to features of song when selecting a mate. Females may benefit by attending to particular song attributes when they accurately reflect the phenotypic quality of potential mates. For example, results from field studies suggest that song complexity may indicate a male's physiological condition (Pfaff et al. 2007), ability to successfully defend a territory (Hiebert et al. 1989), or future parental effort (Buchanan & Catchpole 2000). The developmental stress hypothesis, originally called the nutritional stress hypothesis, posits that learned song may honestly advertise the phenotypic quality of the singer, because song learning and the development of the song control system occur early in life when young birds are exposed to a variety of environmental stressors, such as food restriction (Nowicki et al. 1998, 2002b). One prediction derived from this hypothesis is that males who are exposed to more developmental stressors should show poor development of the song control system and as a result should produce a low-quality song in adulthood. In addition to the song control system and song production, early-life stress may have widespread effects on the development of other physiological and neural systems (Buchanan et al. 2003; Verhulst et al. 2006; Spencer et al. 2009; Farrell et al. 2011). Therefore, males exposed to early-life stress may both produce low-quality song and be of poorer phenotypic quality in general. Furthermore, some males may be more resistant to stressors such that song could indicate both the quality of a male's early-rearing environment and/or his genotype.

In recent years, evidence for the developmental stress hypothesis has accumulated (reviewed in MacDougall-Shackleton & Spencer 2012). For example, exposing nestling zebra finches, *Taeniopygia guttata*, to food restriction or treatment with the glucocorticoid hormone corticosterone (CORT) reduces the growth of HVC (Buchanan et al. 2004) and results in shorter songs with fewer syllables (Spencer et al. 2003). However, another study of zebra finches found no effect of food restriction on syllable repertoire size, although food restriction decreased the accuracy of song learning (Brumm et al. 2009). Similarly swamp sparrows, *Melospiza georgiana*, exposed to food restriction early in life show reduced song-learning accuracy and smaller volumes of RA relative to the size of the telencephalon (Nowicki et al. 2002b). Song type repertoire size was not affected in this study, however, swamp sparrows have small song repertoires (two to four song types) with little variation among males in a population, which could make it difficult to detect an effect of stress on this aspect of song (Nowicki et al. 2002b). Indeed, most studies examining the effects of stress on song complexity to date have focused on species with relatively small song type or syllable repertoire sizes such as zebra finches, Bengalese finches, *Lonchura striata domestica*, and swamp sparrows. However, European starlings, *Sturnus vulgaris*, which have large song type repertoires, sang fewer song types in adulthood after exposure to an unpredictable food supply in the fledgling period (Spencer et al. 2004). In addition, most studies have focused on measures of song complexity and song-learning accuracy and little is known about how early-life stress affects measures of vocal performance.

In the current study, we examined the effect of early-life food restriction or CORT treatment on song sparrows, *Melospiza melodia*. Song sparrows are closed-ended song learners meaning that they do not alter their song repertoires in adulthood (Nordby et al. 2002). Song sparrows have complex repertoires that typically consist of 5–12 song types composed of 20–50 unique syllables (Pfaff et al. 2007; Stewart & MacDougall-Shackleton 2008). In addition, there is some evidence that song complexity is a sexually selected trait in this species that may influence female mating preferences and/or male–male competition (Searcy 1984; Hiebert et al. 1989; Reid et al. 2004; although see Beecher et al. 2000). Our first objective was to determine whether early-life stress affects several song features including song complexity (number of song types and syllables), song-learning accuracy and measures of vocal performance not previously tested, including trill deviation (the speed of frequency modulation within a trill, thought to be constrained by a trade-off between trill rate and frequency bandwidth; Podos 1997) and song type stereotypy. The second objective was to determine whether early-life stress affects the song control system including the volumes of HVC, RA and area X, as well as the number of neurons in HVC. HVC, RA and area X are important for the learning and production of song. We chose to measure the number of neurons in HVC because CORT affects the total number of neurons as well as the number of proliferating cells in this region in adult song sparrows (Newman et al. 2010). Lastly, we also recorded the song repertoires of the fathers of the experimental subjects to determine whether paternal song quality is related to the offspring's adult song quality following exposure to developmental stress. If a particular song feature is resistant to the effects of early-life stress and has a significant relationship with paternal song production (despite the fact that nestlings were not reared by their parents), then this feature may be more strongly influenced by hereditary factors than by environmental factors. If so, then certain song features may indicate a male's genetic quality and ability to resist stressors as opposed to the amount of stress experienced during development. Consistent with previous studies, we predicted that early-life stress would affect features of song that are linked to sensory learning during song acquisition (song complexity, song-learning accuracy), as well as the song control system. However, because development of vocal performance is believed to occur later in ontogeny during the sensorimotor phase of song learning (Podos et al. 2009), which in song sparrows can occur throughout the first year of life and long after the stress treatments were terminated, early-life stress might not affect vocal performance in this species.

METHODS

Subjects and Experimental Treatments

All animal procedures followed guidelines set by the University of Western Ontario and the Canadian Council on Animal Care, and were approved by our institutional Animal Use Subcommittee (protocol 2007-089 to S.A.M.-S.). We located song sparrow nests near Newboro, Ontario (44°38′N, 76°20′W) in May and June 2010 (Schmidt et al. 2012). We recorded the complete song repertoire of the resident male associated with each nest (see below for details on song recording) to quantify paternal song complexity and trill deviation for each experimental subject. Since extrapair paternity is low in this population (consistently <10% of nestlings; Potvin & MacDougall-Shackleton 2009), the resident male was presumed to be the genetic father of nestlings collected from each nest. In total, 23 male nestlings from 12 broods were hand-reared in the current study (control: $N = 9$; food restriction: $N = 8$; CORT treatment: $N = 6$). Males were age 3 days ($N = 18$), 4 days ($N = 4$) and 7 days ($N = 1$) posthatch when they were brought into captivity (mean ± SE age = 3.35 ± 0.19 days posthatch). Sex was determined by PCR amplification of genes located on the sex chromosomes (Griffiths et al. 1998).

Nestlings collected for use in this study were also used in another study addressing the effects of early-life stress on growth