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## Age-related changes in the Bengalese finch song motor program

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### Abstract

It is well established that there are remarkable similarities between song learning in oscine birds and acquisition of speech in young children. Human speech shows marked changes with senescence, but few studies have evaluated how song changes with advanced age in songbirds. To investigate the effect of old age on song, we compared song of old Bengalese finches (*Lonchura striata domestica*) with that of middle-aged birds. The main observed difference was a decrease in the song tempo, largely due to an increased inter-syllable duration. Aging also affected the acoustic characteristics of the song, causing a decrease in pitch and in the range of frequency modulations. Gross morphological measurements of selected vocal muscles did not show detectable changes over this age range, suggesting that song deterioration may be due to neural deterioration. The age-induced temporal and acoustic changes in song parallel the acoustic changes that occur in human speech, suggesting songbirds as a suitable model for aging studies on learned vocal behavior.

### Keywords

Birdsong; speech; respiration; timing; sensorimotor integration

## 1. Introduction

Motor performance of humans and other animals declines substantially during senescence (Faulkner et al. 2007, Doherty et al. 2003), but the reasons for this decline are not fully understood. Aging effects on motor behavior have been shown at multiple levels, from central neural control to muscle and feedback mechanisms (e.g., loss of motoneurons, changes at the neuromuscular junction, in muscle composition, and in muscle spindles; Luff

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1998; Kim et al. 2007). It is therefore not surprising that human speech undergoes marked changes with senescence. Speech production requires complex central control of precise movements of multiple motor systems as well as complex integration of motor control with sensory feedback information. The most striking age-induced changes in speech include a slowing of the respiratory tempo and a sex-dependent change in the fundamental frequency (Linville 2001).

Song production in oscine birds shares many parallels with human speech, among which developmental processes are the most extensively studied (e.g., Doupe and Kuhl, 2008). Age-dependent changes to adult song have been investigated in the zebra finch (*Taeniopygia guttata*). Zebra finch song only changes slightly after completion of ontogeny (Lombardino and Nottebohm, 2000; Pytte et al. 2007), so experimental manipulation of acoustic feedback or sound production has been used to reveal adult song plasticity (e.g., Leonardo and Konishi, 1999; Lombardino and Nottebohm, 2000; Cooper and Goller, 2004). Young adults show more drastic changes to song that occur after a shorter period of experimental manipulation than was observed in older adult males (Lombardino and Nottebohm 2000; Cooper and Goller, 2004), indicating that with increasing adult age the song motor program becomes more stabilized. However, these studies were confined to the time period of early adulthood and therefore do not address the question of whether or not song characteristics change in senescence.

Bengalese finches (*Lonchura striata domestica*) sing less stereotyped songs and following deafening they show rapid deterioration of song syntax and acoustic structure (Okanoya and Yamaguchi 1997; Woolley and Rubel, 1997). The degradation of song for Bengalese finches can begin one week following deafening, which is faster than zebra finches another close-ended learner. For middle-aged zebra finches the first evidence of song degradation begins at 4–6 weeks post surgery (Lombardino and Nottebohm, 2000). These characteristics are useful in studying the effects of aging on song. Here we analyze the effects of senescence on the songs of Bengalese finches and show the first evidence that song in old Bengalese finches undergoes marked changes in rhythm and acoustic structure.

## 2. Methods

### Subjects

Adult male Bengalese finches were purchased from Magnolia Bird Farms (Anaheim, CA) in 2001 and 2004. Songs from 4 birds purchased in 2001 were recorded in December of 2007. At the time of recording, these males were at least six years old and at the end of the average life span (“aged” group). Three of these four birds survived until August of 2008 at which point they were recorded again. Of the four male birds in the aged group, two had also been recorded earlier (one bird in 2001 and another bird in 2004). These recordings are used as data points for the “middle-aged” group and provide a direct within-male comparison. Songs from 3 additional male Bengalese finches (recorded between 2005 and 2007, birds were between 1–3 years old) provided additional data points for the middle-aged group.

### Recording

Acoustic recordings were made with an omnidirectional Audiotechnica microphone (AT3032) in small sound attenuating chambers (26 cm × 70 cm × 30 cm). The microphone signal was amplified (100x) and digitized at 44.1 kHz using a National Instruments (Austin, TX) A/D board and Avisoft Recorder software (Avisoft Bioacoustics; Berlin, Germany).

## Data analysis

Syllable duration and the inter-syllable interval were measured from the oscillogram of the microphone recording using SASLAB Pro (Avisoft Bioacoustics). Pitch and frequency modulation were measured using Sound Analysis Pro (SAP, courtesy of O. Tchernichovski, Tchernichovski et al., 2000). In a longitudinal analysis, songs of the same individual were compared between middle age and aged conditions. Syllables were retained across the lifespan and measurements on song characteristics were compared with a paired t-test. A second, cross-sectional analysis consisted of a between-group comparison of means of individuals using a two-tailed t-test. Five birds provided data for the middle-aged group and four birds for the aged group.

## Muscle fiber typing

Syringeal (*m. syringealis ventralis*) and inspiratory muscles (*m. scalenus*) were harvested from 3 aged and 3 middle-aged male Bengalese finches. Fiber types were characterized following protocols described elsewhere (McFarland and Meyers, 2008). We then compared the fiber type composition of *m. scalenus*, an inspiratory muscle, and syringeal muscles. We also measured fiber diameters of syringeal muscles.

## 3. Results

In the 2 birds recorded at middle and old age visual inspection showed that the syllable repertoire of songs remained largely unchanged, allowing for direct comparison of individual syllables across the age-span. In aged song, syllables were repeated fewer times within a song than at middle age, and this was more pronounced for syllables sung with a high repetition rate (Fig. 1). The most striking change to song structure was a slowing of the song tempo at old age. A significant increase in the inter-syllable intervals in the old birds accounts for most of this effect (Fig. 2A and B). Duration of matched syllables did not change significantly, but the duration of the matched inter-syllable intervals increased as birds aged ( $t_{(6)} = -4.83$ ,  $p < .003$ ). Consistent results for the syllable duration and inter-syllable intervals were also obtained by the cross-sectional analysis (Fig. 2E). As is evident in the frequency distribution of the inter-syllable intervals (Fig. 2F), old birds ( $n = 4$ ) could produce short intervals but did so less frequently than middle-aged birds ( $n = 5$ ).

The acoustic characteristics of the different syllables were also compared for the two age groups by longitudinal and cross-sectional analyses (Fig. 2C and D). In the longitudinal analysis, pitch of matched syllables decreased significantly with age ( $t_{(6)} = -3.6$ ,  $p < .01$ ), as did the range of frequency modulation ( $t_{(6)} = -3.2$ ,  $p < .012$ ). In the cross-sectional analysis, pitch and frequency modulation approached significance (pitch:  $t_{(7)} = -2.2$ ,  $p = .064$ ; FM:  $t_{(7)} = -2.0$ ,  $p = .085$ ). In this latter analysis, variable song syllables of different individuals result in large intra-group variability, which reduces statistical power.

Another striking change in the songs of old birds was a drastic reduction in sound amplitude for song. To normalize the sound amplitude measurements across different recording conditions, we compared the ratio between the amplitude of song divided by the distance call amplitude. The ratio was 0.29 in the middle-aged birds and 0.18 in aged birds. Song amplitude decreased even further at the end of the recording period, such that no acoustic signal was recorded while the bird still assumed normal song posture despite the fact that calls remained audible.

Are differences in song caused by changes in muscle composition and structure? To assess whether age-related changes in acoustic structure were caused by differences in muscle fiber types, we compared the ratio of superfast/fast muscle fibers in the ventral syringeal muscles. Content of superfast fibers did not differ significantly between the two age groups (old:

74.7±1.7; middle-aged: 69±4.5%; t-test,  $p>0.05$ ). To assess whether the change in the intersyllable intervals was caused by deterioration of the inspiratory muscles, fiber type composition was measured in *m. scalenus*. Differences in fiber type composition were not observed between middle-aged and aged birds. Diameters of superfast and fast oxidative fibers did not differ between syringeal muscles of aged (means for the 3 birds range from 23.5–30.7  $\mu\text{m}$  for superfast fibers and 19.4–21.8  $\mu\text{m}$  for fast oxidative fibers) and middle-aged birds (superfast: 26.6–35.6  $\mu\text{m}$ ; fast oxidative 16.5–22.5  $\mu\text{m}$ ; t-tests  $p>0.05$ ). Also, the total mass of the syrinx (estimated by the cross-sectional area of the muscle) was not different between groups (aged:  $3.467 \pm 0.016 \text{ mm}^2$ ; middle-aged:  $3.838 \pm 0.394 \text{ mm}^2$ , t-tests  $p>0.05$ ). These results indicate that no detectable changes in muscle composition occurred between middle age and aged groups.

#### 4. Discussion

This study provides a first quantification of the effects of aging on vocal production in a songbird. Vocal motor control is a highly complex sensorimotor task that requires the coordination of respiratory, vocal and supravocal motor systems. Similar to the effects of old age on human speech, temporal and acoustic changes occur in the song of old Bengalese finches.

In humans, the fundamental frequency of female speech decreases with age, whereas in men it increases (Linville, 2001). In Bengalese finches, old birds showed a decrease in both pitch and frequency modulation of the song syllables. These acoustic changes may be caused by physical changes in the histological structure of the vibrating labia. In aged songbirds, the tempo was slowed through an increase in the silent intersyllable intervals, which correspond to short and deep inspirations (Suthers et al., 1999). It is possible that this effect is caused by changes in peripheral motor control. However, neither the main inspiratory nor the syringeal muscles showed a significant shift towards slower muscle fibers or a decrease in fiber diameter or mass. These data speak against a marked change in muscle architecture as the cause of age-related song changes. It may be more likely that the observed changes occur at the motor or pre-motor level of central control. Slowed song tempo could reflect changes in central control, like an age-related change in the avian basal ganglia. Both, peripheral and neural changes, including loss of dopaminergic function in the basal ganglia, are thought to underlie age-related changes in human speech (Linville, 2001) and other motor deficits (Watanabe, 1987). The decline in song amplitude in old birds may also indicate neural mechanisms. It is possible that the input signal from telencephalic areas for song production is not able to drive the respiratory and syringeal systems during senescence or that the high coordination necessary for song production is no longer present.

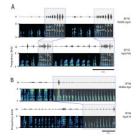
In summary, the age-induced temporal and acoustic changes in song parallel changes that occur in human speech. This parallel adds to the well-characterized similarities between song production and human speech. More detailed studies will show to what degree vocal behavior in songbirds can provide an animal model system for exploring how age-related changes in peripheral physiology and central nervous system control cause the slowing and acoustic deterioration of learned vocalizations.

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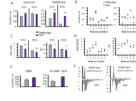
## References

- Cooper BG, Goller F. Partial muting leads to age-dependent modification of motor patterns underlying crystallized zebra finch song. *J Neurobiol.* 2004; 61(3):317–332. [PubMed: 15389688]
- Doherty TJ. Aging and sarcopenia. *J Appl Physiol.* 2003; 95:1717–1727. [PubMed: 12970377]
- Doupe, AJ.; Kuhl, PK. Birdsong and human speech: common themes and mechanisms. In: Zeigler, HP.; Marler, P., editors. *Neuroscience of Birdsong.* Cambridge Univ. Press; Cambridge: 2008. p. 5-31.
- Faulkner JA, Larkin LM, Claflin DR, Brooks SV. Age-related changes in the structure and function of skeletal muscles. *Clin Exp Pharmacol Physiol.* 2007 Nov; 34(11):1091–6. [PubMed: 17880359]
- Kim GH, Suzuki S, Kanda K. Age-related physiological and morphological changes of muscles spindles in rats. *J Physiol.* 2007; 582(2):525–538. [PubMed: 17495047]
- Linville, SE. *Vocal Aging.* Singular, San Diego: CA; 2001.
- Leonardo A, Konishi M. Decrystallization of adult birdsong by perturbation of auditory feedback. *Nature.* 1999; 399(6735):466–70. [PubMed: 10365958]
- Lombardino AJ, Nottebohm F. Age at deafening affects the stability of learned song in adult male zebra finches. *J Neurosci.* 2000; 20(13):5054–5064. [PubMed: 10864963]
- Luff AR. Age-associated changes in the innervation of muscle fibers and changes in the mechanical properties of motor fibers. *Ann N Y Acad Sci.* 1998; 854:92–101. [PubMed: 9928423]
- McFarland J, Meyers RA. Anatomy and histochemistry of hindlimb flight posture in birds. 1. The extended hindlimb posture of shorebirds. *J Morphol.* 2008; 269:967–979. [PubMed: 18506762]
- Okanoya K, Yamaguchi A. Adult Bengalese finches (*Lonchura striata var. domestica*) require real-time auditory feedback to produce normal song syntax. *J Neurobiol.* 1997; 33:343–356. [PubMed: 9322153]
- Pytte CL, Gerson M, Miller J, Kirn JR. Increasing stereotypy in adult zebra finch song correlates with a declining rate of adult neurogenesis. *Dev Neurobiol.* 2007
- Suthers RA, Goller F, Pytte C. The neuromuscular control of birdsong. *Philos Trans R Soc Lond B Biol Sci.* 1999; 354(1385):927–39. [PubMed: 10382225]
- Tchernichovski O, Nottebohm F, Ho CE, Pesaran B, Mitra PP. A procedure for an automated measurement of song similarity. *Anim Behav.* 2000; 59:1167–1176. [PubMed: 10877896]
- Watanabe H. Differential decrease in the rate of dopamine synthesis in several dopaminergic neurons of the aged rat brain. *Exp Gerontol.* 1987; 22(1):17–25. [PubMed: 3297754]
- Woolley SMN, Rubel E. Bengalese finches *Lonchura striata domestica* depend upon auditory feedback for maintenance of adult song. *J Neurosci.* 1997; 17(16):6380–6390. [PubMed: 9236246]
- Woolley SM, Rubel EW. Vocal memory and learning in adult Bengalese finches with regenerated hair cells. *J Neurosci.* 2002; 22(17):7774–7787. [PubMed: 12196601]



**Figure 1.**

Syllables with a high repetition rate are repeated less as the bird ages. A) Spectrogram of the song for BFY68 as a middle-aged adult and as an old bird. B) Spectrogram of the song for BFY50 as a young adult and as an old bird. As the bird ages, there was an 8% (for BFY68) and a 60% (for BFY50) decrease in the duration of the highlighted syllable sequence. In BFY50, the mean number of repetitions of the syllables decreased from 8 in the middle-aged songs to 7 repetitions in the aged songs (for BFY50). In BFY68, the mean number of syllable repetitions decreased from 12 in the middle aged songs to 6 repetitions in the aged songs.

**Figure 2.**

Song tempo and acoustics analysis. (A) Longitudinal analysis of syllable duration and inter-syllable intervals. Matched syllables did not change significantly in duration, whereas the duration of the matched inter-syllable intervals increase significantly as birds aged ( $t_{(6)} = -4.83$ ,  $p < .003$ ). (B) The individual syllables contributing the group averages show that syllable duration did not change systematically, whereas inter-syllable duration consistently increased in duration. (C) Cross-sectional analysis of syllable duration and inter-syllable intervals. Syllable duration did not change significantly, whereas inter-syllable intervals were significantly longer at old age ( $t_{(7)} = -4.15$ ,  $p < .004$ ). (D) Frequency distributions illustrate that age birds could produce short inter-syllable intervals but did it less frequently than young adult birds. (E) In the longitudinal analysis, pitch of matched syllables decreased significantly with age ( $t_{(6)} = -3.6$ ,  $p = .012$ ), as did the range of frequency modulation ( $t_{(6)} = -3.2$ ,  $p < .012$ ). (f) Variations in pitch and in frequency modulation for individual syllables.