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Exploring vocal recovery after cranial nerve injury in Bengalese finches

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HIGHLIGHTS

- Bengalese finches produce sounds ≥2.2 kHz with the left side of their vocal organ.
- Denervation of the left vocal organ transiently eliminated higher frequency sound production.
- Higher frequency sound production recovered within 30 days following nerve injury.
- Nervous system damage, not altered auditory feedback, increased song stereotypy.
- This animal model can be used to study recovery of vocal control after nerve injury.

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ABSTRACT

Songbirds and humans use auditory feedback to acquire and maintain their vocalizations. The Bengalese finch (*Lonchura striata domestica*) is a songbird species that rapidly modifies its vocal output to adhere to an internal song memory. In this species, the left side of the bipartite vocal organ is specialized for producing louder, higher frequencies (≥2.2 kHz) and denervation of the left vocal muscles eliminates these notes. Thus, the return of higher frequency notes after cranial nerve injury can be used as a measure of vocal recovery. Either the left or right side of the syrinx was denervated by resection of the tracheosyringeal portion of the hypoglossal nerve. Histologic analyses of syringeal muscle tissue showed significant muscle atrophy in the denervated side. After left nerve resection, songs were mainly composed of lower frequency syllables, but three out of five birds recovered higher frequency syllables. Right nerve resection minimally affected phonology, but it did change song syntax; syllable sequence became abnormally stereotyped after right nerve resection. Therefore, damage to the neuromuscular control of sound production resulted in reduced motor variability, and Bengalese finches are a potential model for functional vocal recovery following cranial nerve injury.

1. Introduction

Humans and songbirds exhibit similar patterns of vocal learning, and research has used birdsong as a model for both fluent and dysfluent vocal behavior [3,19]. Songbirds produce a diverse array of sounds through their bifurcated vocal organ (syrinx). Each side of the syrinx possesses an independent sound source, and the mechanisms of songbird and human sound production are similar [12]. Passing airflow sets semi-obstructive vocal tissue into an oscillatory state that generates the frequencies of the song. In songbirds, however, the muscles on each side of the syrinx are independently controlled by the ipsilateral hemisphere via the tracheosyringeal portion of the hypoglossal nerve (NXIIts). Bilateral NXIIts damage in zebra finches (*Taeniopygia guttata*) was used as a model for measuring vocal recovery following cranial nerve injury (CNI) [2]. An animal model for studying CNI is advantageous. In humans, damage to the recurrent laryngeal nerve (RLN) is the most common complication following thyroid surgery [6]. The RLN innervates the larynx (mammalian vocal organ) and is a branch of the vagus nerve (NX). RLN damage impairs respiration, swallowing and vocal control [4]. As such, mammalian models for vocal recovery following RLN injury are complicated by a concomitant respiratory dysfunction. Similarly, bilateral NXIIts damage can induce “wheezing” and difficulty breathing during periods of arousal. However, unilateral syringeal denervation does not induce respiratory distress.

Here, we evaluate Bengalese finches (*Lonchura striata domestica*) as a model system for recovering learned vocalizations following unilateral NXIIts damage. Traditionally, denervation of either the left or right NXIIts was utilized to explore lateralized control of song production. For example, in the zebra finch, right denervation resulted in the elimination of higher frequency syllables [5,20]. Conversely, in Waterslager canaries (*Serinus canaria*), left denervation caused the loss of over 67% of their original syllables

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[7]. However, a left denervation shortly after canaries hatched resulted in nearly complete reversal of hemispheric control of song production in adulthood [8]. We have recently demonstrated that Bengalese finches produce their higher frequency vocal range (≥2.2 kHz) solely with the left side of the syrinx [14]. Immediately after left denervation, higher frequency notes within the song are abolished and birds sing only lower amplitude, abnormally structured vocalizations. In contrast, denervation of right syringeal muscles has little effect on the acoustic structure of the song.

Moreover, Bengalese finches rely on auditory feedback to maintain their song. Bengalese finches adjust syllable phonology in response to real-time manipulations of auditory feedback to minimize perceived acoustic error [15]. If hearing is transiently impaired, their song deteriorates rapidly and recovers when hearing is restored [21]. The current pattern of data suggests that Bengalese finches are monitoring their vocalizations during song and making regular adjustments to vocal output. Bengalese finches are thus ideal for studies of vocal recovery because of their sensitivity to auditory feedback and lateralized acoustic production. Therefore, we sought to determine whether they could recover higher frequency notes following left NXIIts damage.

2. Methods

2.1. Subjects

Seventeen Bengalese finches were used in this experiment with approval from the Texas Christian University Institutional Animal Care and Use Committee (#0805). Ten of these birds were used in a previous experiment to evaluate the transient effects of lateralized NXIIts damage [14].

2.2. Surgical and data collection procedures

The surgical and recording procedures used in this study were identical to previous work [14]. Briefly, left ($n = 5$) or right ($n = 5$) syringeal muscles were denervated via resection of the ipsilateral NXIIts nerve. Right denervation provides a comparison group for exploring effects of nerve damage after a more modest acoustic change. Songs were recorded prior to surgery, continuously for the first month (PS 1 M), and for a period of three to five days after eight to nine months (PS 9 M). As described in Secora et al. [14], the majority of individual syllables were too phonologically unstable following left denervation to identify syllables across song bouts. Syllable identification is necessary for syntax analyses, therefore analyses were not possible until PS 1 M when all subjects demonstrated phonological consistency in their song structure. Because of unexpected syntax changes observed following right denervation (described in Section 3.4), we then sought to determine whether syntax change was caused by altered auditory feedback or CNI. To compare the effects of neural denervation versus altered acoustic feedback, the right syringeal sound source was devocalized in three birds [for description of procedures and acoustic effects following devocalization, see 14]. These devocalized birds were recorded prior to surgery and at PS 1 M. Each recording session encompassed three to five days. Four additional birds were used for histologic analysis of syringeal muscle lateralization in non-manipulated birds (Section 2.6). All surgical procedures were performed under isoflurane anesthesia (1–2%), and efforts were made to minimize pain and discomfort.

2.3. Acoustic analyses

Using the Sound Analysis Pro similarity scoring module [17], the degree of acoustic degradation was quantified by calculating a similarity score between pre- and post-surgery songs. Sound Analysis Pro uses acoustic features to quantify similarity. Bengalese finch song contains segments of 2–4 syllables that are consistently sung in a linear order ("syllable chunks") [9]. Ten iterations of a syllable chunk were selected per time point for the similarity analysis based on the greatest visual similarity between recording time points. Pre-surgery syllable chunks were compared to one another to set a similarity baseline. In one case where acoustic degradation was so severe that a match was not possible, we selected the first syllable chunk in the song after the introductory notes. Similarity was then compared across all recording time points using a 2×3 (nerve resection by time point) mixed model ANOVA, using Fisher's least significant difference (LSD) post hoc analyses.

2.4. Higher versus lower frequency syllable production

We also assessed whether the denervation led to systematic changes in the production of higher or lower frequency syllables. We analyzed 20 songs for each time point. Individual syllables from pre-surgery, PS 1 M, and PS 9 M songs were analyzed using a fast-Fourier transform to capture the fundamental frequency (f_o) of a 300 ms segment of a harmonic stack. The 300 ms segment of a syllable used for analysis began about 7 ms after syllable onset because this initial period of the syllable had a broadband, noisy acoustic structure [14]. The f_o was defined as the lowest integer multiple of the peaks in the frequency spectrum; syllables were then categorized according to lower frequency ($f_o < 2.2$ kHz) and higher frequency ($f_o \geq 2.2$ kHz), and a total number of syllables was tallied for each category. Changes in these syllable distributions after denervation were then analyzed with a 2×3 (frequency range $\times$ time point) Chi-square test.

2.5. Syntax analysis

Syntax features were defined by calculating the stereotypy, linearity and consistency of syllable-to-syllable transitions [for review, see 13]. The Songinator (courtesy of S.W. Bottjer, University of Southern California) was used to calculate syntax scores. The scores range from 0 to 1 and a song that possesses only one syllable sequence would have a score of 1. Syllables from 20 songs from each recording time point were analyzed. All syntax analyses were based on the songs recorded within each recording time point. Scores for sequence stereotypy and sequence linearity were compared between pre-surgery, PS 1 M, and PS 9 M using a 2×3 ANOVA, using LSD post hoc analyses.

2.6. Histology

At the end of the experiment, all denervation subjects were euthanized and their syringes were extracted. Left or right syringeal mass was measured (left = 2; right = 4) or muscle tissue was histologically verified (left = 3; right = 1). Due to expense of reagents and tissue processing, birds from the left nerve resection group were used preferentially in the histological analyses because they recovered higher frequency syllables and tissue sections were more likely to provide a better estimate of muscular atrophy. Syringes from four non-manipulated birds were harvested and histologically processed to serve as a control group. Syringeal cross-sections were sliced in 5 μm sections, stained with a modified Morris Pentachrome stain, and digitally photographed using previously described methods [10]. With this technique, muscle is stained brown. Using ImageJ software (Bethesda, MD), images were converted into a RGB stack and color thresholds were adjusted to identify brown pixels. The area fraction was defined as the percentage of selected pixels out of the total number of pixels in images of equal dimensions (1344×576 pixels). A ratio of denervation was calculated by dividing the muscle mass or area fraction of