Published in final edited form as:

J Voice. 2011 May ; 25(3): 275–282. doi:10.1016/j.jvoice.2009.11.005.

A Novel Model for Examining Recovery of Phonation after Vocal Nerve Damage

Prabhat K. Bhamra, M.D.¹, Allen D. Hillel, M.D.¹, Albert L. Merati, M.D.¹, and David J. Perkel, Ph.D.^{1,2}

¹Department of Otolaryngology – Head & Neck Surgery, University of Washington School of Medicine, 1959 NE Pacific Street, Box 356515, Seattle, Washington 98195 - 6515

²Department of Biology, University of Washington, 1959 NE Pacific Street, Box 356515, Seattle, Washington 98195-6515

Abstract

Objectives/Hypothesis—Recurrent laryngeal nerve injury remains a dominant clinical issue in laryngology. To date, no animal model of laryngeal reinnervation has offered an outcome measure that can reflect the degree of recovery based on vocal function. We present an avian model system for studying recovery of learned vocalizations following nerve injury.

Study Design—Prospective animal study.

Methods—Digital recordings of bird song were made from 11 adult male zebra finches; 9 underwent bilateral crushing of the nerve supplying the vocal organ, and two birds underwent sham surgery. Song from all birds was then recorded regularly and analyzed based on temporal and spectral characteristics using computer software. Indices were calculated to indicate the degree of similarity between pre-operative and post-operative song.

Results—Nerve crush caused audible differences in song quality and significant drops ($p < 0.05$) in measured spectral and, to a lesser degree, temporal indices. Spectral indices recovered significantly (mean=43.0%; SD=40.7; $p < 0.02$), and there was an insignificant trend towards recovery of temporal index (mean=28.0%; SD=41.4; $p = 0.0771$). In 5/9 (56%) birds, there was a greater than 50% recovery of spectral indices within a four week period. Two birds exhibited substantially less recovery of spectral indices, and two had a persistent decline in spectral indices. Recovery of temporal index was highly variable as well, ranging from persistent further declines of 45.1% to recovery of 87%. Neither sham bird exhibited significant ($p > 0.05$) differences in song following nerve crush.

Conclusion—The songbird model system allows functional analysis of learned vocalization following surgical damage to vocal nerves.

Address correspondence/reprint requests/proofs to: Prabhat K. Bhamra, Department of Otolaryngology – Head & Neck Surgery, University of Washington School of Medicine, 1959 NE Pacific Street, Box 356515, BB1165, Seattle, Washington 98195-6515, Tel: (206) 658-7945, Fax: (206) 543-5152, pbhamra@gmail.com.

This work was presented at the American Academy of Otolaryngology – Head & Neck Surgery Annual Conference in Chicago, IL on 9/21-9/24/08

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Recurrent laryngeal nerve injury remains a dominant clinical issue in Laryngology, with significant impacts on voice, airway, and swallowing function. Numerous animals have been used as models for studying the recurrent laryngeal nerve¹⁻⁴. However, a key limitation of animal models has been outcome measurements limited to return of airway patency, anatomical reinnervation, or movement of the vocal cords. No animal model of laryngeal injury has yet offered an outcome measure reflecting recovery of vocalizations.

Oscine songbirds provide an advantageous model for studying phonation. They produce stereotyped, learned vocalizations, allowing measurement of changes to phonation following a vocal tract manipulation. Neuroscientists have shown that songbirds and mammals share similar central nervous system (CNS) pathways involved in vocal learning^{5,6}.

The Songbird Vocal Organ

The syrinx, the songbird vocal organ (Fig.1), is located at the bifurcation of the trachea into the primary bronchi and is composed of several muscles and cartilages⁷. Endoscopic evaluation has revealed that the medial and lateral labia, situated at the cephalic end of each bronchus are responsible for sound production^{8,9}. Two pairs of labial tissues exist – one in each bronchus. Each pair is manipulated by the ipsilateral group of syringeal muscles, which in turn are innervated by the ipsilateral tracheosyringeal branch of the hypoglossal nerve⁷, whose axons arise from nuclei present in the caudal two-thirds of the hypoglossal nucleus (nXII)¹⁰.

Characteristics of Birdsong

Song is a species-specific set of vocalizations used primarily by males for courtship or territory defense¹¹. Zebra finch song is learned during a critical period early in life. Hearing is essential for proper learning¹², and for maintenance of learned song¹³. Moreover, song from zebra finches exhibits high stereotypy (Fig.2). Over a 6 month period, temporal and spectral aspects of song exhibit minimal changes within an individual¹⁴.

Hypothesis

We hypothesized that bilateral tracheosyringeal nerve crush produces an immediate change in song secondary to denervation of the syringeal muscles and that restoration of song occurs, and is measurable audibly and objectively.

Materials and Methods

Pre-operative Recording

Songs from 15 adult male zebra finches (*Taeniopygia guttata*) were digitally recorded at 44.1 kHz and 16-bit accuracy using omnidirectional microphones (Countryman Associates, Inc.). Each bird was permitted to sing until at least 10 motifs were obtained from each individual. Four of the 15 birds (27%) underwent one day of pre-operative recordings, and the remaining 11 (73%) birds underwent multiple days of pre-operative recording.

Surgery

Bilateral Tracheosyringeal Nerve Crush Injury—Thirteen of the 15 birds (87%) underwent bilateral tracheosyringeal nerve crush injury to examine the effect of nerve crush on song. Anesthesia was induced and maintained using inhaled isoflurane.

The head of the bird was extended at the neck and the bird was secured to the operating table. A 1.5 cm midline incision was made on the ventral aspect of the neck. Subcutaneous tissue was cleared from the trachea using blunt and sharp dissection.

The trachea was then visualized and the tracheosyringeal nerves were identified bilaterally along the trachea. The soft tissue overlying each nerve was dissected away, permitting elevation of the nerve from the trachea.

A #5 fine non-serrated forceps (Fine Science Tools, Inc.) was used to crush each nerve using a moderate amount of force for five seconds. Care was taken in each subject to standardize the force of the crush.

After each nerve was crushed, the soft tissue overlying the trachea was replaced, and the skin was closed in a continuous fashion using absorbable suture.

Sham Surgery—Two of the 15 birds underwent sham surgeries to control for the effect of surgery without tracheosyringeal nerve crush on song. The procedure for the sham surgery was identical to the procedure for the crush surgery as described above, except the nerve crush was not performed.

Post-operative Recording

Each bird was recorded on a daily basis following surgery. After the song began to stabilize (as measured audibly by the first author), recording intervals were increased. Recordings were performed using the same methods as pre-operatively. Two birds failed to sing adequately following surgery, and were excluded from further analysis. This was likely due to altered environmental conditions for those two subjects.

Analysis of Song

Motif Isolation and Comparison—Ten motifs free of extraneous cage noises were isolated manually from each recording session using Syrinx software (John Burt, www.syrinxpc.com). In some cases, fewer than 10 acceptable motifs were recorded. In cases of motif variability within a recording session, a proportional number of each version was extracted for each analysis. Prior to analysis, each motif was high-pass filtered at 100 Hz to eliminate any low-frequency disturbance.

Spectral Analysis: Spectral analysis was performed using the Sound Analysis Pro (SAP) software package^{15,16}. This automated software has been used extensively in songbird research for the purpose of comparing songs based on four characteristics: pitch, frequency modulation, spectral continuity, and Weiner entropy. Analysis of these four characteristics between songs yields a *similarity* score and an *accuracy* score, each scored from 0-100 (100 indicating complete similarity or complete accuracy). When compared with a pre-operative song, the similarity score indicates how much of the pre-operative song was found in the post-operative song. The accuracy score represents how well portions of the post-operative song match the pre-operative song¹⁷. The product of the accuracy and similarity scores was then taken to represent both aspects of song comparison and reported as the *SA Index*.

Temporal Analysis: Temporal structure was analyzed using customized MatLab software written by David Perkel following the methods of Roy et al¹⁸. The resulting score, reported as *temporal index*, fell between 0 (indicating no temporal similarity) and 1 (indicating temporally identical motifs).

Comparison Procedure: The maximum number of available song motifs (up to 10) from each day was used for comparison. Pre-operative motifs were compared with one-another to set a baseline. Motifs from each post-operative recording session were then compared to pre-operative song motifs. Interpolation of data was performed to obtain an overall trend for all 9 experimental birds.

Calculation of changes in indices were performed as follows:

Maximum pre-operative index (Pre_{max}) = the maximum pre-operative value

Minimum post-operative index ($Post_{min}$) = the minimum value within 7 days of the bird's first day of singing following surgery

Maximum post-operative index ($Post_{max}$) = the maximum value within 7 days of the bird's last day of recording

Initial Change = $Pre_{max} - Post_{min}$

Final Change = $Pre_{max} - Post_{max}$

Recovery = $Post_{max} - Post_{min}$

% Recovery = $Recovery / Initial\ Change$

Statistics

One sample t-tests were performed to test whether mean SA index and temporal index initial changes and recovery differed from zero. All tests were performed with Prism v 4.03 (GraphPad Software, Inc.).

Role of the Funding Source

This project was supported by NIH grants T32DC000018 and P30DC004661. The funding sources had no involvement in the study design, collection/analysis/interpretation of data, writing of the report, or in the decision to submit the paper for publication.

Results

Controls

The two control birds exhibited no significant ($p=0.28$) changes in the temporal or spectral aspects of song post-operatively (Fig. 3).

Experimental Birds

Of the 11 birds that underwent bilateral nerve crush, 2 (18%) were excluded because of operator technical error. In one bird, the tracheosyringeal nerve was cut rather than crushed, and in the other subject, the thoracic cavity was penetrated.

Eight of the 9 (89%) remaining experimental birds developed a continuous biphasic high-pitched vocalization (akin to stridor) immediately following surgery, which resolved within hours to days (Figs. 4 & 6). Some birds exhibited an intermittent stridor that would return only with agitation. All birds that underwent nerve crush had audible differences in song quality, and exhibited a substantial drop in spectral indices.

After nerve crush, mean SA index in the experimental group dropped by 36.1 (SD=14.9; $p<0.0001$); sham surgery caused no significant effect ($p>0.05$). Mean SA index recovered by 43.0% (SD=40.7; $p<0.02$). In 5/9 (56%) birds, there was a greater than 50% recovery of spectral aspects of song following a four week period (e.g. Figs. 4 & 5). Of these

birds, 3/5 (60%) recovered over 50% of the temporal index, 1/5 (20%) recovered about 4%, and 1/5 (20%) experienced a persistent decline in temporal index. One of the 9 (11%) birds recovered approximately 33% of the spectral and 38% of the temporal aspects of song, and one (11%) bird recovered less than 25% of both spectral and temporal aspects of song. One of 9 (11%) birds exhibited a persistent decline in both spectral and temporal characteristics of song, and one (11%) bird exhibited a persistent decline in spectral indices with about 15% recovery of temporal index. Overall, spectral indices dropped immediately post-operatively and recovered substantially over the following 1-3 weeks (Figs. 8 & 9), (see Table 1).

Overall, temporal index dropped significantly (mean=0.12;SD=0.08;p<0.003) following nerve crush, and remained low in many of the birds. Temporal index did not change as much as the spectral indices (Fig. 8). On average, there was an insignificant (p=0.0771) trend toward initial recovery in TI following nerve crush. It was our subjective impression that many birds experienced a change in the rate of syllable production following nerve crush surgery, and temporal changes were less pronounced than spectral changes.

Discussion

The purpose of this project was to establish a model system to study phonatory recovery following vocal organ injury. The zebra finch has been studied extensively, and many studies pertinent to zebra finch song learning have been published in the neuroscience literature. However, as far as we know, no studies have been performed to monitor recovery of song following a tracheosyringeal nerve insult. Additionally, no studies have been performed in attempts to use the zebra finch as a model for laryngeal study. We attempted to determine the time course and characteristics of song recovery by following the recovery of song after injury of the nerves innervating the syrinx.

Among the birds that exhibited the greatest amount of spectral song recovery (>50%), one actually had a constant decline in temporal index with time. It is unclear why this occurred. The remaining three birds in that group exhibited varying degrees of temporal recovery. Of the two birds that displayed a persistent decline in spectral aspects of song, one also had a decline in temporal aspects of song, which is not surprising, as previous studies have also demonstrated persistent decline in temporal index (termed “decrySTALLIZATION” in the cited paper) 3 weeks following unilateral nerve sectioning¹⁸.

Temporal analysis of song revealed generally less pronounced changes compared with spectral changes. In many birds, a disturbance in the tempo of the song was audible when listening to song immediately following nerve crush, similar to the disturbance in cadence of human speech that occurs following recurrent laryngeal nerve injury.

Several possible explanations could underlie the variation we observed in song recovery. First, although care was taken to apply a consistent amount of pressure during each crush, this process was not objectively standardized.

Second, it is possible that different individual birds, like humans, carry different capacities for specificity of neural repair following injury. Previous studies have demonstrated laryngeal synkinesis rates of approximately 66%-88% following experimental reinnervation, and this incidence is thought to be similar to that which occurs in humans¹⁹⁻²². It is thought that synkinesis is the functional consequence of nonspecific reinnervation²³. The variation in recovery of song is not surprising, since birds that did not fully recovery song could be exhibiting phonatory consequences of syringeal synkinesis.

Advantages of the Model

The zebra finch model has characteristics that enable it to be a useful model for the study of neurolaryngeal injury. They are easy to obtain, breed, house, and maintain. They are also relatively inexpensive. Moreover, a great deal of background information is readily available regarding the species. Additionally, programs to analyze birdsong have been developed and tested^{15,16}. Electromyography and sonomicrometric recordings have also been performed on the zebra finch, and the physiology underlying phonation and resonance has also been described^{24,25}.

The physiology of phonation in the zebra finch is similar to that in mammals⁹. The zebra finch vocal organ has a complex series of muscles innervated by a cranial nerve that are involved in manipulation of apposed membranes. Vibration of these membranes during expiration is the basis of phonation. Variations in airway pressure below the apposed membranes complemented with variable contraction and relaxation of vocal organ muscles can change the characteristics of the phonation⁷. Moreover, resonance in the upper vocal tract is also thought to affect sounds generated in the syrinx²⁶, similar to the process that occurs in humans.

Zebra finches also have qualities that are ideal for the study of neurolaryngology and vocal learning. For instance, only a small number of taxa share the ability to learn their vocalizations, including humans, bats, cetaceans, parrots, hummingbirds, and songbirds^{27,28}. Many species produce unlearned vocalizations, such as a dog barking. Whereas humans are capable of learning an essentially endless combination of learned vocalizations, some songbirds can only produce one type of learned vocalization with a modest degree of variation^{29,30}. Thus, if we anatomically or pharmacologically modify a songbird, any resultant changes in the bird's vocalization can be attributed to our manipulation, and not to the bird's inherently learned variations. And because songbirds learn their vocalizations, this model system also permits us to study the effects of various manipulations on *learned* vocalizations.

The central organization of the song learning system has been described³¹ and mapped out in detail, and many experiments studying the effects of specific manipulations to this system have been performed. Similarities between song learning in birds and speech learning in humans have also been described^{5,32}. This model is therefore ideal for studying the effects of both peripheral *and* central nervous system manipulations on phonation and learned vocalizations.

Drawbacks

Some differences in the zebra finch model system are also evident upon comparison with other existing models of neurolaryngology. For instance, while the zebra finch has a larynx, in songbirds it does not house the vocal organ. Rather, the larynx is a more cephalad located structure in the respiratory tract. Moreover, the zebra finch vocal organ has two sound sources (one in each bronchus)^{8,9}, as opposed to the single sound source in the human. This further complicates study of zebra finch phonation because laterality of sound production must also be considered. In this pilot study, we performed bilateral crush injury to eliminate any variability with regards to laterality that may occur following unilateral nerve manipulation. It will be essential to perform experiments implementing unilateral nerve operations in the future.

As discussed earlier, decrystallization of song may also occur following 3 weeks of perturbed vocal production as a result of unilateral nerve sectioning in some birds, which may complicate analysis of song recovery following a neural insult. However, it is known that certain central nervous system lesions in zebra finches prevent decrystallization of

song¹⁸, which may rectify this issue. Another disadvantage of this model is we are unsure of the mechanism of recovery. Although it is most likely that reinnervation of the syringeal musculature accounted for recovery of song in our experimental subjects, it is also possible that central nervous system (CNS) plasticity permitted “adaptation” of the CNS to the peripheral insult, accounting for song recovery. Nonetheless, tracheosyringeal nerve crush caused a change in phonation, and was followed by a period of song recovery in some birds. Interesting future studies include histological examination of nerve tissue in “recovered” and “non-recovered” birds to clarify physiological mechanisms of recovery.

Future Directions

The unique characteristics of songbirds allow for numerous studies pertinent to vocal learning and functional recovery following neurolaryngeal injury. One possible area of study includes determining the effects of certain behaviors on vocal recovery. For instance, does preventing a bird from singing alter the speed or amount of spectral recovery?

Future studies using the zebra finch model could focus on the effects of various treatment modalities on specificity of reinnervation as measured by anatomic and physiologic measurements, as well as phonatory analysis. For example, do calcium channel blockers augment functional recovery after nerve crush? One recent study demonstrated the beneficial effects of calcium channel blockers on the specificity of reinnervation¹ in rats. This study was limited because the rat model does not permit analysis of functional recovery using phonation, unlike the zebra finch model.

Possible experiments pertinent to vocal learning include simultaneously presenting a juvenile bird with tutor song and white noise to study the effects of distorted song on learning. Having a model that learns its vocalizations also allows us to study the changes that may occur in the central nervous following a peripheral vocal tract injury. Likewise, future studies could be performed to determine the effects of CNS lesioning on vocal recovery following vocal organ denervation.

Prior to embarking on any of the above studies, it is perhaps more prudent to elucidate the mechanisms of vocal recovery following nerve crush that occurred in the zebra finch model, which could be done by histological examination of the nerve crush site in birds that have recovered their song. Electromyographic studies of the syringeal muscles following tracheosyringeal nerve crush would likely contribute useful information regarding the physiology of denervation. Nerve conduction studies are also an option, however, these may be more challenging considering the anatomy of the bird.

Conclusion

Whereas previous animal models used to study vocal production have many benefits, they do not produce a stereotyped, learned acoustic signal. Consequently, they do not permit analysis of recovery of learned vocalizations following an induced deficit. The zebra finch provides the advantages of reliably producing stereotyped, learned vocalizations, thereby allowing for qualitative phonatory analysis following vocal tract manipulations.

Acknowledgments

We thank Ofer Tchernichovski for his assistance with the SAP software, and Jon Meitzen and Sam Gale for their guidance throughout this project. This project was supported by NIH grants T32DC000018 and P30DC004661.

References

1. Hydman J, Remahl S, Björck G, et al. Nimodipine improves reinnervation and neuromuscular function after injury to the recurrent laryngeal nerve in the rat. *Ann Otol Rhinol Laryngol*. 2007; 116:623–30. [PubMed: 17847731]
2. Woodson G. Spontaneous laryngeal reinnervation after recurrent laryngeal or vagus nerve injury. *Ann Otol Rhinol Laryngol*. 2007; 116:57–65. [PubMed: 17305279]
3. Knight M, Birchall M. 29 A neuroanatomical study of the porcine larynx: establishing a model for reinnervation studies. *J Anat*. 2002; 201:425. [PubMed: 17103771]
4. Peterson K, Graves M, Berke G, et al. Role of motor unit number estimate electromyography in experimental canine laryngeal reinnervation. *Otolaryngol Head Neck Surg*. 1999; 121:180–4. [PubMed: 10471854]
5. Brainard M, Doupe A. Auditory feedback in learning and maintenance of vocal behaviour. *Nat Rev Neurosci*. 2000; 1:31–40. [PubMed: 11252766]
6. Doupe A, Perkel D, Reiner A, et al. Birdbrains could teach basal ganglia research a new song. *Trends Neurosci*. 2005; 28:353–63. [PubMed: 15935486]
7. Suthers R, Margoliash D. Motor control of birdsong. *Curr Opin Neurobiol*. 2002; 12:684–90. [PubMed: 12490259]
8. Goller F, Larsen O. A new mechanism of sound generation in songbirds. *Proc Natl Acad Sci U S A*. 1997; 94:14787–91. [PubMed: 9405691]
9. Goller F, Larsen O. New perspectives on mechanisms of sound generation in songbirds. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 2002; 188:841–50. [PubMed: 12471485]
10. Wild J. Neural pathways for the control of birdsong production. *J Neurobiol*. 1997; 33:653–70. [PubMed: 9369465]
11. Searcy WA, Andersson M. Sexual selection and the evolution of song. *Annual Review of Ecology and Systematics*. 1986; 17:507–33.
12. Beecher M, Brenowitz E. Functional aspects of song learning in songbirds. *Trends Ecol Evol*. 2005; 20:143–9. [PubMed: 16701358]
13. Nordeen K, Nordeen E. Auditory feedback is necessary for the maintenance of stereotyped song in adult zebra finches. *Behav Neural Biol*. 1992; 57:58–66. [PubMed: 1567334]
14. Brainard M, Doupe A. Postlearning consolidation of birdsong: stabilizing effects of age and anterior forebrain lesions. *J Neurosci*. 2001; 21:2501–17. [PubMed: 11264324]
15. Tchernichovski O, Nottebohm F, Ho C, et al. A procedure for an automated measurement of song similarity. *Anim Behav*. 2000; 59:1167–76. [PubMed: 10877896]
16. Tchernichovski O, Mitra P, Lints T, et al. Dynamics of the vocal imitation process: how a zebra finch learns its song. *Science*. 2001; 291:2564–9. [PubMed: 11283361]
17. Haesler S, Rochefort C, Georgi B, et al. Incomplete and inaccurate vocal imitation after knockdown of FoxP2 in songbird basal ganglia nucleus Area X. *PLoS Biol*. 2007; 5:e321. [PubMed: 18052609]
18. Roy A, Mooney R. Auditory plasticity in a basal ganglia-forebrain pathway during decrystallization of adult birdsong. *J Neurosci*. 2007; 27:6374–87. [PubMed: 17567798]
19. Siribodhi C, Sundmaker W, Atkins JP, et al. Electromyographic studies of laryngeal paralysis and regeneration of laryngeal motor nerves in dogs. *Laryngoscope*. 1963; 73:148–64.
20. Tashiro T. Experimental studies on the reinnervation of larynx after accurate neurotomy. *Laryngoscope*. 1972; 82:225–35. [PubMed: 5014389]
21. Dedo H. Electromyographic and visual evaluation of recurrent laryngeal nerve anastomosis in dogs. *Ann Otol Rhinol Laryngol*. 1971; 80:664–8. [PubMed: 4938993]
22. Crumley R, McCabe B. Regeneration of the recurrent laryngeal nerve. *Otolaryngol Head Neck Surg*. 1982; 90:442–7. [PubMed: 6817274]
23. Crumley R. Mechanisms of synkinesis. *Laryngoscope*. 1979; 89:1847–54. [PubMed: 502707]
24. Goller F, Cooper B. Peripheral motor dynamics of song production in the zebra finch. *Ann N Y Acad Sci*. 2004; 1016:130–52. [PubMed: 15313773]

25. Franz M, Goller F. Respiratory units of motor production and song imitation in the zebra finch. *J Neurobiol.* 2002; 51:129–41. [PubMed: 11932954]
26. Daley M, Goller F. Tracheal length changes during zebra finch song and their possible role in upper vocal tract filtering. *J Neurobiol.* 2004; 59:319–30. [PubMed: 15146548]
27. Nottebohm F. The Origins of Vocal Learning. *The American Naturalist.* 1972; 106:116.
28. Jarvis E, Ribeiro S, da Silva M, et al. Behaviourally driven gene expression reveals song nuclei in hummingbird brain. *Nature.* 2000; 406:628–32. [PubMed: 10949303]
29. Catchpole, C.; Slater, PJB. *Bird song : biological themes and variations.* Cambridge [England] New York: NY Cambridge University Press; 1995. p. viii-248.
30. Farabaugh, SM.; Dooling, RJ. Acoustic communication in parrots: Laboratory and field studies of budgerigars, *Melopsittacus undulatus*. In: Kroodsma, DE.; Miller, EH., editors. *Ecology and evolution of acoustic communication in birds.* Ithaca, N.Y.: Comstock Pub. : Cornell University Press; 1996. p. xx-587.
31. Nottebohm F, Stokes T, Leonard C. Central control of song in the canary, *Serinus canarius*. *J Comp Neurol.* 1976; 165:457–86. [PubMed: 1262540]
32. Doupe A, Kuhl P. Birdsong and human speech: common themes and mechanisms. *Annu Rev Neurosci.* 1999; 22:567–631. [PubMed: 10202549]
33. Suthers R, Zollinger S. Producing song: the vocal apparatus. *Ann N Y Acad Sci.* 2004; 1016:109–29. [PubMed: 15313772]

FIGURE 1.

The syrinx is the vocal organ of the songbird located at the tracheobronchial bifurcation. (A) ventrolateral view of syrinx (B) coronal section through a syrinx ABBREVIATIONS: T, trachea; ST, sternotrachealis muscle; LL, lateral labium; ML, medial labium; TL, tracheolateralis muscle; vS, syringealis ventralis muscle; vTB, tracheobronchialis ventralis muscle; dTB, tracheobronchialis dorsalis muscle; dS, syringealis dorsalis muscle.

Nomenclature is from Suthers and Zollinger, 2004.

Adapted from: Suthers R, Zollinger S. Producing song: the vocal apparatus. *Ann N Y Acad Sci* 2004;1016:109-29.

FIGURE 2.

Zebra finch song exhibits high stereotypy, which can be visualized. Each spectrogram above represents a motif, a repetitive element of song, from an individual zebra finch. The first two examples illustrate song from different days, and the last two examples are non-consecutive motifs from the same day. Note the high degree of similarity between all motifs.

FIGURE 3. Spectral and temporal analysis of “Bird #29” demonstrating nearly no change in spectral or temporal aspects of song following sham surgery. Day 0 is the operative date.

FIGURE 4.

Spectrograms from “Bird #15” demonstrating degeneration of song following nerve crush, and nearly complete recovery of song within two weeks. Each song recording illustrates two motifs. During POD #1, the bird exhibited biphasic vocalizations, but was able to sing (intermittently) later in the day. Note that syllables two and three have lost some of their temporal definition and now are blended together on POD #1. During recovery, the bird began to exhibit greater discrimination between syllables two and three, and by POD #10, song was nearly completely recovered. At this point, objective indices were also recovered to nearly pre-operative levels, and there were no detectable audible differences in song.

FIGURE 5.

Spectral and temporal analysis of “Bird #15” demonstrating nearly full recovery of song following bilateral tracheosyringeal nerve crush. Day 0 is the operative date.

FIGURE 6.

Spectrograms from “Bird #6” demonstrating degeneration of song following tracheosyringeal nerve crush followed by nearly no recovery. Each recording demonstrates two motifs with introductory notes between the motifs. On POD#4, the bird was stridulous. Song on POD #19 shows substantial spectral differences and mild temporal changes, and by POD #96, syllable differentiation was nearly complete, but with persisting spectral differences when compared with pre-operative song. By POD #112, song exhibited only mild recovery, and objective indices were also similar to immediate post-operative levels, with persistent detectable audible differences in song.

FIGURE 7. Spectral and temporal analysis of “Bird #6” demonstrating no recovery of song following bilateral tracheosyringeal nerve crush. Day 0 is the operative date.

FIGURE 8.

SA Index, similarity, accuracy, and temporal index interpolated from all birds. The SEM is depicted with error bars. Day 0 is the operative date. Following nerve crush, spectral and temporal indices dropped substantially and then partially recovered. Temporal index then began to decline. The continuous drop in SA index from POD 2 to 6 is a result of interpolation, which created the illusion of a steady drop from the preoperative SA index to the first post-operative index. In reality, many birds were unable to sing in the immediate post-operative period. The first post-operative index was plotted when the bird was first able to sing, thereby excluding any attempts to vocalize prior to that time.

FIGURE 9.

Changes in SA index for experimental birds. Initial Change = $\text{Pre}_{\text{max}} - \text{Post}_{\text{min}}$ Final Change = $\text{Pre}_{\text{max}} - \text{Post}_{\text{max}}$

TABLE 1

Percentage recovery of song for all experimental birds. A negative number represents a consistent decline in similarity over time.

Bird #	Spectral Recovery (%)	Temporal Recovery (%)
22	-18.4	-45.1
26	-4.99	15.2
6	4.96	22.0
7	32.5	37.6
13	56.3	54.0
20	72.3	4.44
5	75.7	-1.24
14	76.6	78.2
15	92.2	87.0