THE SEARCH FOR NEURAL LATERALIZATION

IN THE BENGALESE FINCH HVC

by

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<u>ACKNOWLEDGEMENTS</u>

Fresh Finch of the Vive (To the tune of Fresh Prince of Bel-air)

Now this is the story all about how
My HVC got lesioned, turned upside down
And I'd like to take a minute just perched right there
I'll tell you how I become the subject of a neuroscience fair
In Anaheim, CA born and raised
In the birdcage is where I spent most of my days
Eating corn, chirping, relaxing all cool
And all singing some crystallized songs inside of the school
When a couple of grad students, they were up to no good
Started performing surgeries in my neighborhood
I got one does of isoflourane and I got scared
And was told "You're moving to a cooler with increased bird care!"
I whistled for other birds but they didn't come near
By day 2 I realized I couldn't sing and lost all cheer
If anything I could say this song was rare
But I thought nah, forget it, yo home to my new lair!
They turned on the light around seven or eight
And I chirped to the Daily Skiff "Yo, homes smell ya later!"
Looked at my foam-cooler I was finally there
To sit on my perch as the subject of this lair

INTRODUCTION

Lateralization is a phenomenon in which one hemisphere of the brain or body is dedicated to executing control of a particular behavior over the other. Humans exhibit lateralized behaviors, such as speech production and handedness. Lateralization of complex behaviors is not a uniquely human phenomenon. It can also be found in a multitude of other species including fish, birds, rodents, and chimpanzees. For example, corvids and chimpanzees prefer one limb over the other for tool use (Rutledge & Hunt, 2003). The tern, a seabird, prefers to feed chicks from a specific side of the beak (Grace, 2004) and both Australian parrots and glossy black-cockatoos prefer to use one foot over the other when manipulating food (Magat, 2009). Brain asymmetry can even be traced back through the fossil record to some of the most primitive chordates (Vallortigara, 1999). It is unclear why we have evolved dominance for one side over the other when it comes to performing complex tasks, but it has been proposed that this allows for greater efficiency and may provide a learning advantage for species capable of hemispheric localization.

Speech production in humans is a complex, learned behavior that is controlled by the left hemisphere of the brain. This has been demonstrated through fMRI techniques which measure blood as it flows through the brain while a behavior is being performed. More active brain areas in the left hemisphere showed an increase in blood flow while the subject was speaking (Baynes & Gazzaniga, 2005). Paul Broca was the first to identify the left hemisphere's role in human speech production with the discovery of Broca's area, located in the left frontal cortex, which controls the production and initiation of speech

(Broca, 1861). Since this initial discovery, there have been other areas documented through case studies and research.

Songbirds have been used as an animal model for exploring hemispheric dominance underlying vocal learning and production. All songbirds produce song with two sound sources, each controlled by the ipsilateral hemisphere which makes them an ideal model organism for experimental study. Research has shown some species such as canaries and finches have a dominant hemisphere for normal song production. Waterslager canaries (*Serinus canaria*) show right hemispheric preference for higher frequency song elements (Halle et al., 2003). In the zebra finch (*Taeniopygia guttata*), the right hemisphere is also somewhat dominant in the production of some high frequency components (Goller & Cooper, 2004). Brown thrashers (*Toxostoma rufum)* show lateralization of the motor production of song, higher frequency notes, and syllables produced with a rapid amplitude modulation are right side generated (Goller & Suthers, 1995).

Humans and songbirds have several similar characteristics when it comes to the ways in which they learn and maintain vocalizations. Songbirds require a tutor to learn to sing properly, much as humans learn to speak by repeating and mimicking sounds produced by other humans. Songbirds reared without a male tutor, termed isolates, do not develop their species-specific song and instead develop an extremely abnormal song (Thorpe, 1958). Additionally, songbirds acquire their song early in development and maintain it for the rest of their lives once they reach adulthood just as human speech acquisition occurs early in life and remains well maintained (Doupe & Kuhl, 1999). Song learning and maintenance also requires auditory feedback which is similar to how

humans prevent the deterioration of their speech. In humans, those that become deaf after learning how to speak experience deterioration of their speech due to the loss of auditory feedback (Plant & Hammarberg, 1983). Similarly, after adult male Bengalese finches (*Lonchura striata domestica*) were deafened, their song elements showed signs of deterioration within five days (Okanoya & Yamaguchi, 1997). These similarities are what make songbirds such an intriguing model species to study; however, there are some noted differences between vocalization in songbirds and humans.

Both humans and songbirds do exhibit vocal learning and lateralized speech production, but the bi-phonation of the songbirds vastly different from the single set of vocal folds found in humans. Songbirds generate vocalizations using the avian vocal organ known as a syrinx. The syrinx is a bifurcated vocal organ possessing two independently controlled sets of vocal tissue or labia. Each set of labia is controlled by the ipsilateral hempisphere of the brain and can produce sound independently or simultaneously from the other sound source (Suthers, 1997). Each side of the syrinx contributes unique acoustic components to song which varies between species. The Bengalese finch is unique as compared to other songbirds because the Bengalese finch produces and discriminates song with left hemispheric dominance similar to the left hemispheric dominance humans have over speech production (Okanoya, 2004). Selectively devocalizing the left and right sound generators provided further evidence to support this point. In the Bengalese finch, the left and right sides of the syrinx have very distinct contributions to each syllable. The song of a Bengalese finch is characterized by louder, higher-frequency components produced by the left side of the syrinx (>2.2 kHz), and lower amplitude, lower frequency (<2.2 kHz) sounds are

Figure 1:
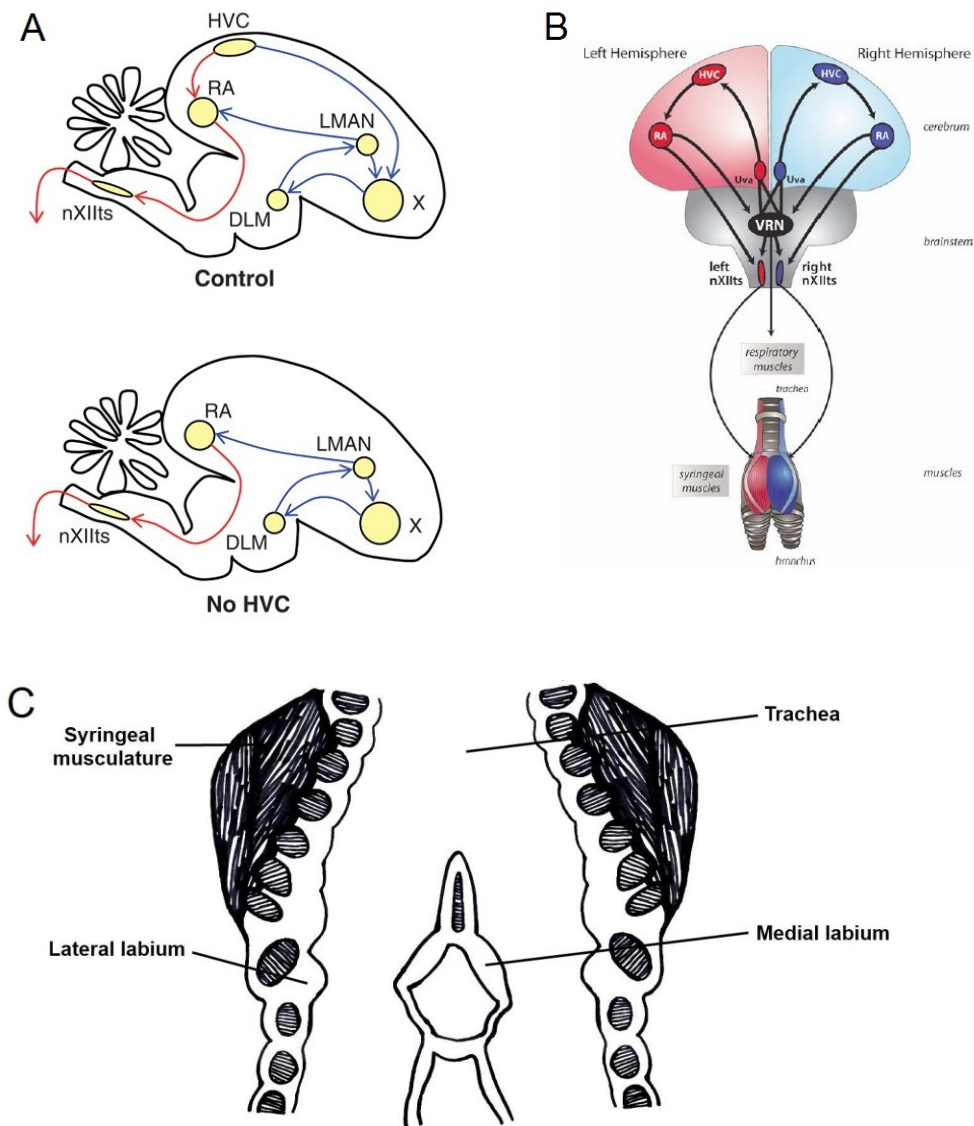
Figure 1: A. This is a map of the songbird brain detailing the nuclei of the anterior forebrain pathway (Blue) and the motor pathway (Red). The anterior forebrain pathway includes the HVC, LMAN, Area X, and DLM of the thalamus which form a loop. The motor pathway includes HVC, RA, and the tracheosyringeal portion of the hypoglossal nerve (nXIIts). The brain below it shows how these pathways would be impacted if input from the HVC is removed. B. Peripheral control. Syringeal musculature is controlled by the nXIIts from the ipsilateral hemisphere. The connections from the nXIIts to the HVC are detailed above. There is some cross over to the opposite hemisphere at the level of Uva and VRN which are below the level of the RA in the motor pathway. C. The syrinx, the avian vocal organ. This picture shows the bifurcation of the syrinx into right and left sides with two sets of vocal folds consisting of the right and left medial labia and right and left lateral labia.

generated by the right side of the syrinx (Secora et al., 2012). This greatly contrasts the closely related zebra finch which is known to use the right side of the syrinx for some higher frequency portions of the song, but for the most part lack acoustic lateralization (Cooper & Goller, 2004). The left hemispheric dominance seen in the Bengalese finch makes it an excellent model for human speech production and vocal learning. A possible explanation for this dominance is that it is due to asymmetries in the brain that allow the left hemisphere to specialize in higher frequency sound production.

The avian brain is organized into discrete, interconnected nuclei involved in different components of song learning and memory, and song production. These nuclei are broken into two separate pathways that ultimately work together to form a cohesive song: the anterior forebrain pathway which is mainly responsible for song learning and memory and the posterior forebrain pathway responsible for motor production of song. One of these nuclei, the HVC, functions as both part of the anterior forebrain pathway and as part of the motor pathway (Aronov, 2008; Okanoya, 2001). The sensory feedback loop begins when the HVC signals to area X in the basal ganglia which then sends projections to a nucleus called DLM in the thalamus. The signal then loops back to the LMAN before it is able to propagate to the motor pathway via LMAN projections to RA. The HVC is involved in the motor production of song by its direct projections to the RA which is connected to the hypoglossal nerve that innervates the musculature of the syrinx (Hahnloser, Kozhevnikov, & Fee, 2002). This makes the HVC an ideal place to begin searching for neural lateralization because it is involved in the manipulation, whether directly or indirectly, of so many components of the song.

After examining previous research, we predict that HVC controls many temporal and structural aspects of song. In zebra finches, HVC has been shown to control the timing and generation of song syllable sequence after neural stimulation (Hahnloser et al., 2002). The zebra finch also uses this timing and synchronization of syllables to determine the timing of the following song elements (Margoliash, 2013). This control of timing is proposed to be organized as a series of chain-reaction like steps. Electrical perturbation of normal HVC activity completely halted song production in the experiment Long and Fee performed on zebra finches in 2008 which provides additional support that the HVC may also be responsible for song timing and song production in the Bengalese finch. Furthermore, it has also been indicated through previous research in Bengalese finches that the HVC is responsible for the syllable sequence or syntax of the song after lesions to the HVC critically impaired proper song timing and song production (Okanoya, 2001). Since relatively few studies have examined the HVC of the Bengalese finch, we believe there is more knowledge to be gained about HVC function by continuing this research on HVC lesions and song analysis. We can determine if HVC is a possible source of some of the vocal lateralization seen in Bengalese finches by performing lesions to either the right or left HVC.

There are two approaches to HVC lesions that have been studied thus far: microlesions which partially damage the function of the nucleus and full lesions which completely obliterate all functionality. Microlesions are deemed "transient" because the effects of the lesion are temporary even though the damage to the nucleus is sustained. The term "microlesion" was coined by Johnson and Thompson when they performed lesions the HVC of zebra finches. The birds regained the ability to produce normal song

within two weeks (Johnson & Thompson, 2006). Another study conducted by Okanoya also examined the effects of unilateral full lesions to the HVC in Bengalese finches. Damage to the left HVC led to birds producing severely degraded song which remained degraded over time. Birds with right HVC lesions displayed a more transient degeneration and within a month had regained a majority of their song producing abilities (Okanoya, 2004). The study was then continued by focusing on the effects of partial ablation of the HVC on the left side. It was determined that these lesions reduced song complexity and variation seen in syllable patterning suggesting the HVC may code the organization of the syllable "phrases". Phrases are defined as groupings of syllables that appear in a consistent order (Okanoya, 2004). Given that lesions to the left and right HVC were producing different effects upon song, these results are highly promising that neural asymmetry may exist within the HVC.

We performed unilateral full HVC lesions to the right and left hemispheres to examine the temporal and syllabic effects the HVC has over song production to continue this work. We believe that these lesions are important in determining the extent of the control the HVC has over song production and timing. When examining the previous research on microlesions, there is no literature at all on the effects of microlesions in the Bengalese finch. The study that did examine partial ablation did so with varying degrees of damage to the left HVC and did not investigate right HVC lesions. Even the partial left HVC lesions were relatively few in number (n=3) (Okanoya, 2004). We would like to look at performing lesions of a consistent size and include right microlesions in our analysis.

We believe that by looking at these two different types of lesions that we will be able to form more solid conclusions about the role of the HVC in song production in Bengalese finches and thus learn more about the structure and function of the avian brain. As stated above, previous work has compared the effects of full lesions upon the left and right HVC which suggested that the role of the HVC in Bengalese finches may indeed be lateralized. We anticipate that the lateralization will manifest in the degradation of temporal and structural elements of the song. We hypothesize that the left HVC full lesions will lead to increased song degradation, especially of structural and temporal features. These degradations, we believe, will appear to less of an extent with right full lesions to the HVC. Additionally, we anticipate that microlesions of the HVC will provide further evidence of neural lateralization. We expect that left microlesions of the HVC will not lead to as much degradation as the full lesions but will still have an effect upon temporal features. We expect that right microlesions will recover the initially degraded temporal features at a faster rate.

Figure 2:

Figure 2: A Bengalese finch relaxing on a perch, the utter picture of perfection.

Subjects

We performed microlesions (right: n = 4, left: n = 4) and full lesions (right: n = 4, left: n = 4) on male Bengalese finches (Maryland Exotic Birds, Pasavenia, MD). Each bird was recorded for several days prior to the lesion and then for seven days following the lesion. The microlesion birds were then sacrificed, and their brains were extracted.

Housing

The birds were group housed, but isolated during recordings in a custom built sound attenuating chamber. They were maintained on a 14:10 hour light:dark cycle. Microphones were positioned 14 cm directly above the perch during recording in the chamber.

Surgical procedure

Birds were anesthetized for the procedure with isoflorane gas (Miller Vet Supply, St Louis, MO) set at a level of 1-2.5%; this variation was due to the birds' differing responses to the gas. Once the procedure began, the level of isoflorane was adjusted as needed to keep the subject satisfactorily sedated. Anesthetized birds were placed on a stereotax (MyNeuroLab, St. Louis, MO) and secured in place with beak and ear bars. Using a No. 11 scalpel, the skin was cut midsagitally expose the skull. The skin was held back with small sections of KimWipes and the skull was wet with a saline solution. A scalpel was used to mark just anterior and posterior to the site after determining the location of Y-0 on the top of the skull. Y-0 has been previously established as a starting point for surgical methods in the field of avian neuroscience (Thompson & Johnson, 2006). Curved forceps were used to remove the skull to expose the midsagittal sinus (Y-

0). The values for anterior/posterior and medial/lateral were reset on the stereotax once Y-0 was determined and agreed upon and marked by extending the electrode over the location. The electrode was next carried out to the particular side where the lesion was to be made and the boundaries of HVC were marked with a scalpel. Marks were made at 2.1 and 2.9 μm from Y-0. A square was then made with the scalpel by completing the anterior and posterior sides. Forceps were then used to remove the first two layers of the skull. The borders of the square are then checked by repositioning the electrode, and then the third layer was removed. The lesions are made at the proper coordinates after removing the dura.

Microlesions

Microlesions were performed using a 200 μm diameter insulated tungsten wire electrode with 1 meg impedance (FHC, Brunswick, Maine). The lesions were made starting at coordinates 2.1 μm and a depth of .6 mm for 30 s with a current of 100 μA. The next two lesions were each .4 μm lateral from the previous lesion to end at 2.9 μm lateral from Y-0 to either the right or left depending on the predetermined lesion side. (Thompson & Johnson, 2006)

Full Lesions

Full lesions were conducted as described above, but were made at 6 lesion sites with a current of 100 μA for 60 s. The electrode was moved 2.1mm laterally from the bifurcation and lesions were made at four sites 0.25mm apart. At the outer sites, the electrode was inserted to a depth of 0.6mm and middle sites were inserted deeper at 0.7mm. Then, the two middle sites were replicated 0.2mm anterior to create a second row of lesion sites. This was done to cover as much of HVC as possible.

Table 1:

	A (μm)	L (mm)	V (mm)	Impedence (μA)	Duration (s)
Microlesion	0	2.1, 2.5, 2.9	0.6	100	35
Full lesion	0	2.1, 2.35, 2.6, 2.85	0.6	100	60
	0.2	2.35, 2.6			

Table 1: Coordinates of the lesions performed where A represents anterior / posterior and L represents medial / lateral and is measured in mm.

Following the lesion, the skull was closed with dental cement (Reprosil, Milford, DE). We then removed the sections of KimWipes and sutured the scalp shut. The bird was returned to his home cage and allowed to recuperate in the sound attenuating chamber.

Recording procedure

Birds were recorded inside closed sound attenuating chambers with a Pre-Amplifier RME Intelligent Audio Solution Quadmic and sent to the recording program (Avisoft Recorder from Berlin, Germany), which recorded sound and saved selected files to a computer hard drive. Sounds that exceeded a user-determined energy threshold were saved onto the computer hard drive. Birds were recorded for 3-4 days preceding surgery, and then 7 days following the procedure.

Data analysis

Analysis of the files, which was performed using Avisoft SasLab (Berlin, Germany), began by manually sorting the recorded files and filtering the samples of song.

Song files were batch processed and filtered so that only frequencies between 350 Hz to 8500 Hz remained. These frequencies were selected because they represent the bird's own range of hearing. After this filter was applied, songs were analyzed according to lesion condition.

Microlesions

Birds that had undergone microlesions had samples of full song from each day analyzed as a whole. We looked for spectral features which may have changed following the lesions such as peak frequency, amplitude, syllable duration, and intersyllable interval. Song bouts were highlighted, measured for length, and then analyzed for these qualities. The phrase analysis was performed by selecting specific syllable "phrases" which may be defined as a sequence of 2 or more syllables that are always produced in linear order (Okanoya, 2004). A different phrase was selected for each bird. The phrases were also analyzed for spectral characteristics, and additionally we included fundamental frequency in our analysis. We included this variable because the phrases were constant for each bird and so the change could be measured across one particular grouping of syllables.

Full lesions

Full lesion subjects had their song analyzed in the same manner for similar variables such as peak frequency, amplitude, syllable duration, and intersyllable interval. We conducted uniform sectional analysis on the songs of full lesion birds. These sections will be operationalized as 2-second sections taken 2-seconds after the onset of song which were analyzed for comparison of smaller groupings of syllables against the larger unit of song. We did not measure the sections for fundamental frequency because the

song was so degraded following a full lesion that the f_0 was unable to be systematically determined.

Statistical analysis

All data were analyzed using SPSS and a mixed models ANOVA to test for significance.

<u>RESULTS</u>

Microlesions

While performing analyses on the songs that we collected, we looked for temporal and structural changes to the song and song elements. We began by investigating full song, and investigated several temporal and structural features in our analyses. When analyzing the songs, we looked at structural features of each of the syllables. The first thing we measured was amplitude (dB) which is the measured intensity of the song elements. We also measured peak frequency (kHz) which is the frequency that reached the greatest intensity in each syllable. When looking at temporal aspects of the song, we examined duration (s) of the phrase itself and the inter-syllable interval, or ISI (s) which is the time between each syllable. There were significant changes following the microlesions, but we could not determine where these effects took place because there were no significant interactions between days or between left and right lesions. This led us to investigate "phrases", or groups of syllables that frequently are grouped together in the same order. We examined the same temporal and structural features as above, but also included fundamental frequency (kHz); this is the very first frequency reached or the first harmonic within each syllable.

Figure 3:
A. BFW55 LHVCml Pre Surgery:

1 s

BFW55 LHVCml Post Surgery Day 1:

B. BFW27 RHVCml Pre Surgery:

BFW27 RHVCml Post Surgery Day 1:
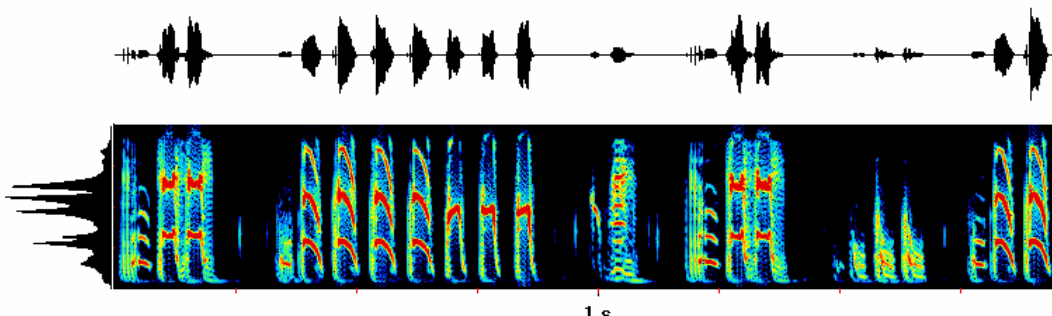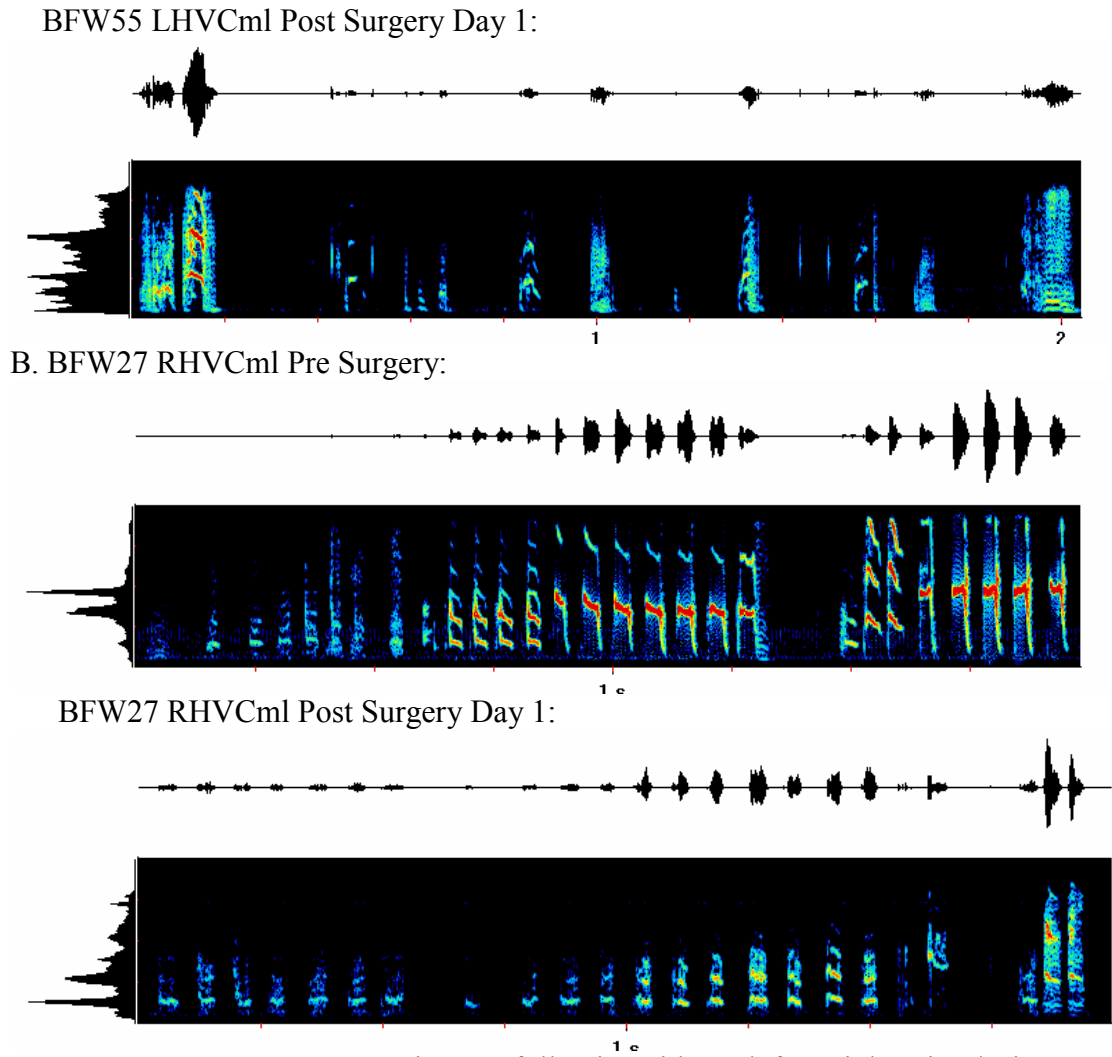
Figure 3: A. & B. Spectogram images following either a left or right microlesion, respectively. The Y axis represents frequency (kHz) and the X axis displays time (s). Above the spectrogram image is an oscillogram trace illustrating the amplitude modulation of the syllables. To the left of each spectrogram is the mean power spectrum which displays the average of the acoustic energy in the frequency domain. These spectrograms act as a visual representation of the song degradation; there are visible changes to the song structure.

When we examined full song, we found that song bout length ($F_{(3, 18)} = 6.211$, $p = 0.004*$), syllable duration ($F_{(3, 18)} = 3.926$, $p = 0.026*$), and amplitude ($F_{(3, 18)} = 5.212$, $p = 0.009*$) all exhibit significant changes following surgery for both right and left microlesions. Peak frequency approaches significance following a
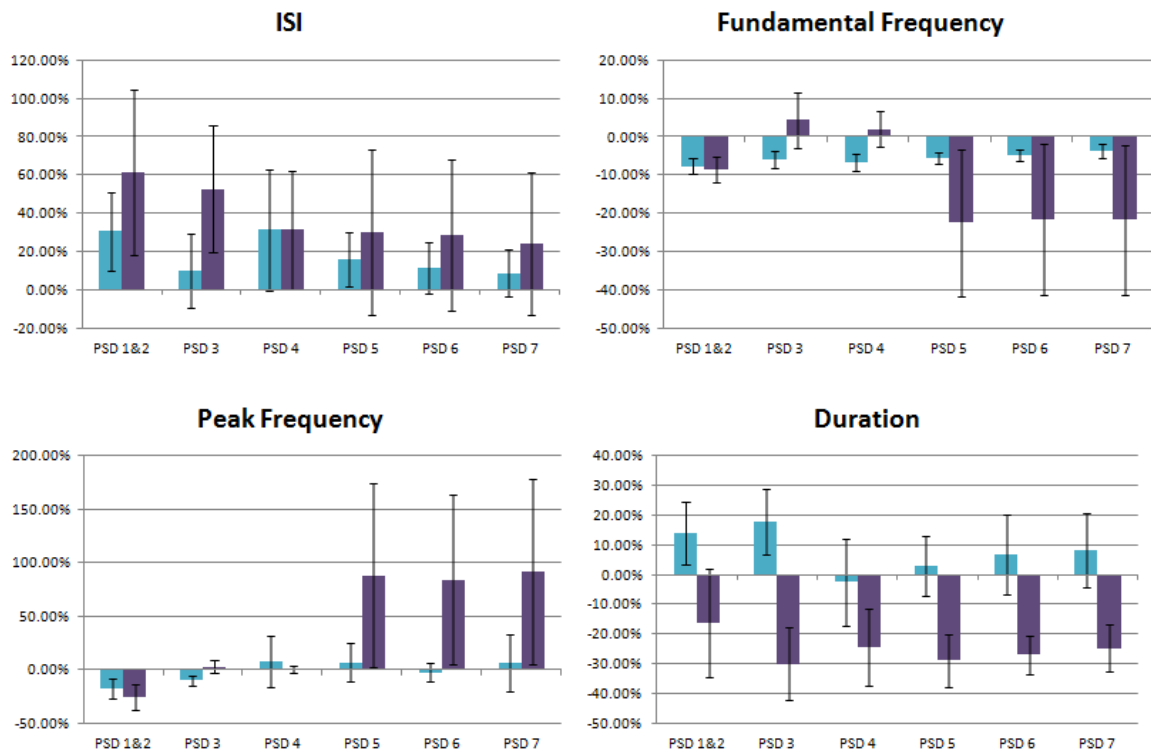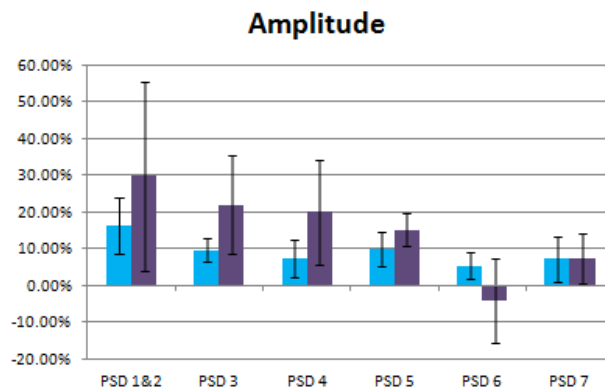
Figure 4:
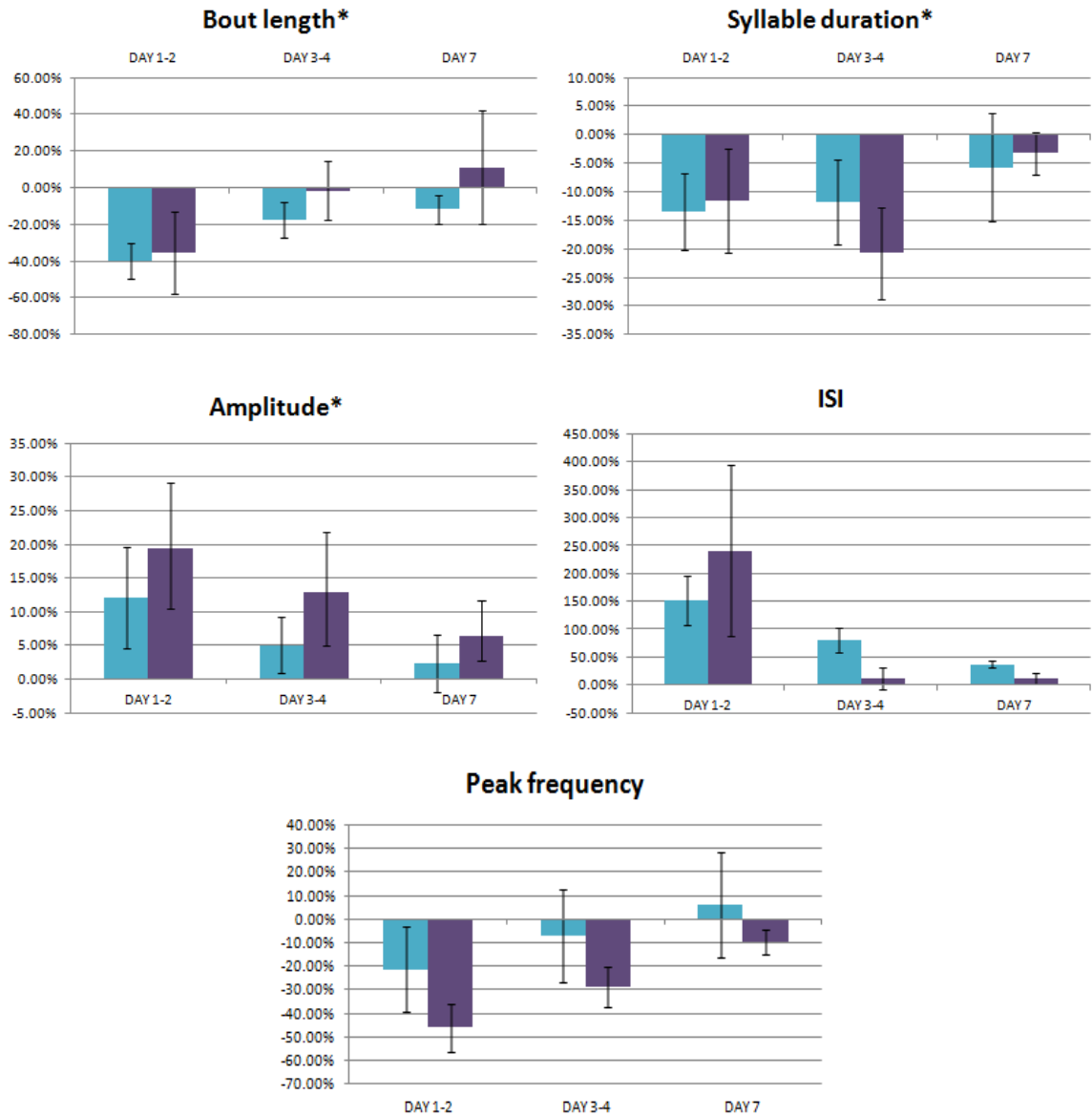
Figure 4: These are the graphs of full song analyses with the days 1-2 and 3-4 compiled. Left microlesions are indicated by the turquoise bars and right microlesions are indicated by the purple bars. Song bout length and duration show significant effects and each shorten following the lesion and then return to baseline by day 7. The same can be said for Amplitude which becomes quieter following the lesion and shows the same trend of recovery. Peak frequency does not show significance, but the graph shows that there is a decline in peak frequency that is recovered by day 7. ISI becomes longer following the lesion and also recovers by day 7.

microlesion ($F_{(1.351, 8.109)} = 4.620$, $p = .056$), but barely misses the mark. The effects upon the ISI ($F_{(1.033, 6.196)} = 3.908$, $p = 0.093$) were not significant. We discovered this after running a mixed model ANOVA; however, there were no interactions between groups, meaning the effects due to left microlesions are not significantly different from right microlesions.

We ran a mixed model ANOVA on all of the data collected from the phrase analyses. What we discovered after running the ANOVAs is that none of the song structural elements or temporal features within a phrase change significantly following microlesions to the left or right hemisphere. Duration ($F_{(1.773, 10.635)} = 0.393$, $p = 0.660$), ISI ($F_{(2.339, 14.037)} = 2.345$, $p = 0.127$), peak frequency ($F_{(2.202, 13.215)} = 2.826$, $p = .091$), and peak amplitude ($F_{(6, 36)} = 1.870$, $p = .113$) all did not display any significant effects. There is also no statistical significance between groups.
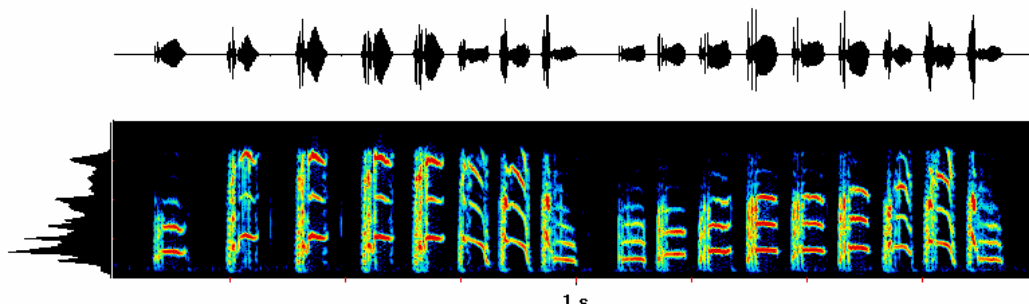
Figure 5:

Figure 5: These are the graphs of percent change following phrase analysis. Left microlesions are indicated by the turquoise bars and right microlesions are indicated by the purple bars. None of these graphs show a significant trend reflecting the results of the ANOVA in the table above. There is a slight trend towards recovery in all cases, but the effects of the lesion are not significant upon these phrases. Duration appears to be the only lateralized feature over all, but this effect is not significant.
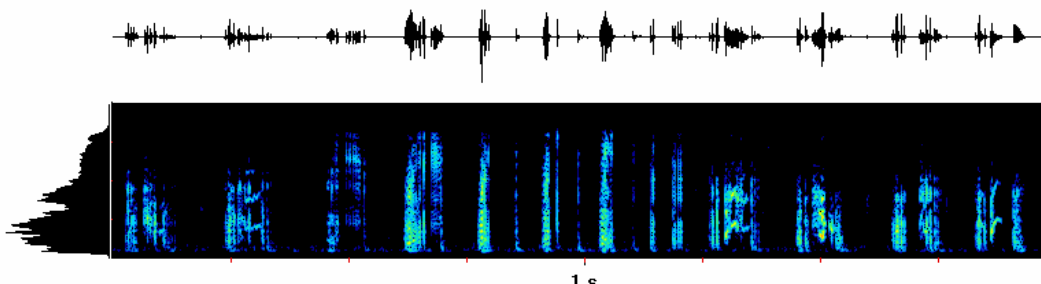
Full lesions

The following spectral and temporal features were measured: peak amplitude (mean), duration, peak frequency (mean), and inter-syllable interval, to examine the effect of a right or left HVC full-lesion on song production.
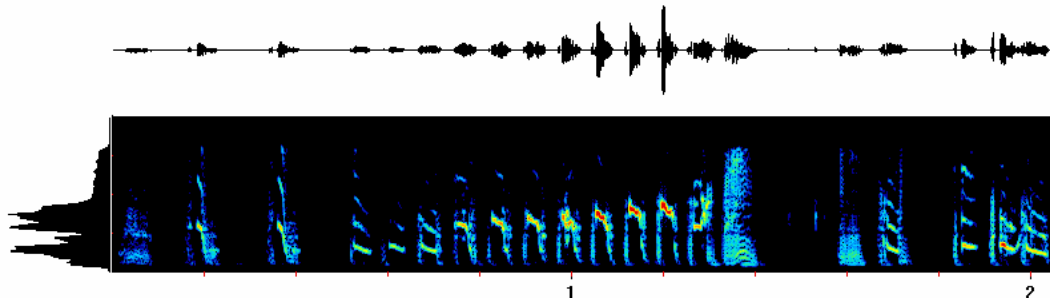
Figure 6:
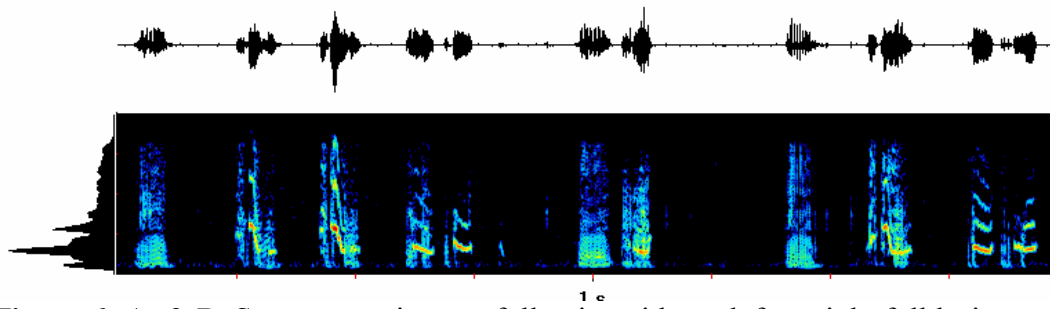A. BFW4 LHVCfl Pre Surgery:

BFW4 LHVCfl Post Surgery Day 3:

B. BFW45 RHVCfl Pre Surgery:

BFW45 RHVCfl Post Surgery Day 1:

Figure 6: A. & B. Spectogram images following either a left or right full lesion, respectively. The Y axis represents time (s) and the X axis represents frequency (kHz). Above the spectrogram image is an oscillogram trace illustrating the amplitude modulation of the syllables. To the left of each spectrogram is the mean power spectrum which displays the concentration of acoustic energy. These spectrograms act as a visual representation of the song degradation; there are visible changes to the song structure.

Peak amplitude (mean) decreased following both right and left lesions (RHVC: $19.96 \pm 2.30\%$; LHVC: $31.12 \pm 5.72\%$). The peak amplitude was measured over the next four days and differences were analyzed with a mixed-measure ANOVA. We found that a change in peak amplitude (mean) was affected by the side of the lesion ($F(1.492, 30) = 114.978$, $p = 0.000$).

Duration decreased following both right and left lesions (RHVC:$-27.31 \pm 7.10\%$; LHVC: $-34.68 \pm 17.40\%$) Duration was measured over the next four days and differences were analyzed with a mixed-measure ANOVA. We found that a change in duration was not affected by the side of the lesion ($F(1.552, 9.314) = 3.229$, $p = 0.094$).

Inter-syllable interval increase following both right and left lesions (RHVC: 136.78± 57.36; LHVC: 141.97± 40.43%). Inter-syllable interval was measured over the next four days and differences were analyzed with a mixed-measure ANOVA. We found that a change in inter-syllable interval was affected by the side of the lesion $(F(5, 30) = 13.150, p = 0.000)$.
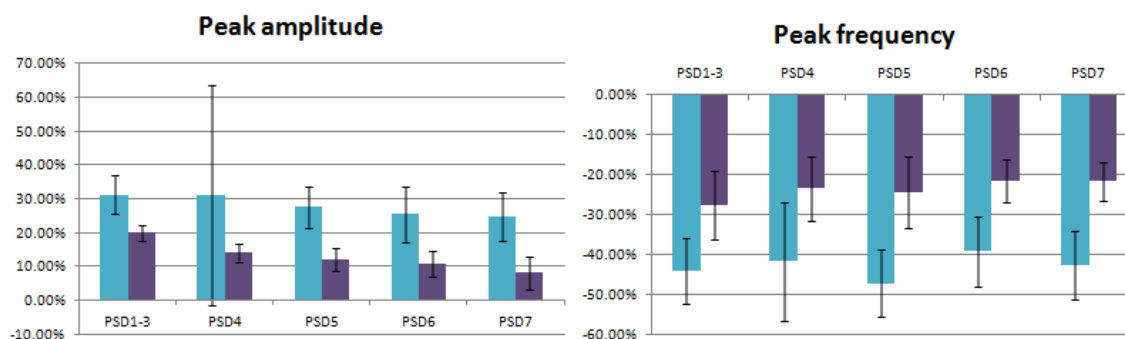
Peak frequency (mean) decreased following both right and left lesions (RHVC: -27.65± 8.60%; LHVC: -44.23± 8.27%). Peak frequency (mean) was measured over the next four days and differences were analyzed with a mixed-measure ANOVA. We found that a change in peak frequency was affected by the side of the lesion $(F(5, 30) = 19.332, p = 0.000)$, main effect $(F(5, 30) = 2.341, p = 0.066)$.

Figure 7:
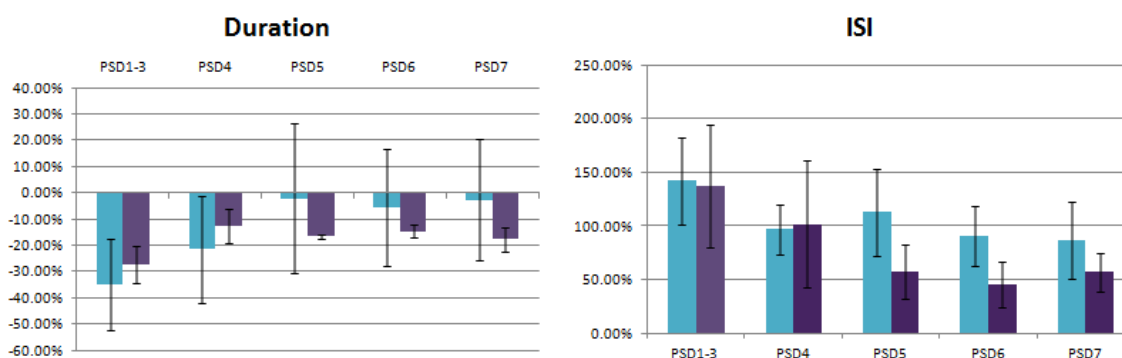
Figure 7: Left microlesions are indicated by the turquoise bars and right microlesions are indicated by the purple bars. Percent Change in Duration. Duration, or length of song syllable, was reduced in Bengalese finches irrespective of the side of the HVC full-lesion, irrespective of the side of the HVC full-lesion. Error bars indicate SEM. Percent Change in ISI. Inter-syllable Interval, ISI, is the distance between syllables. After right or left HVC full-lesions there was an increase in ISI compared to pre-lesion song. Error bars indicate SEM.

Figure 8:

Figure 8: Left microlesions are indicated by the turquoise bars and right microlesions are indicated by the purple bars. Percent Change in Peak Frequency (mean). Following either a right or left HVC full-lesion there was a decrease in peak frequency. These results show that damage to left HVC decreases peak frequency at least twice as much as damage to right HVC. This data illustrates that peak frequency is a lateralized component of song. Even though overall duration was reduced in both right and left lesions, the recovery of vocalization exhibited lateralization. On days 5-7 there is lateralized recovery, with left HVC full-lesions recovering more of their song. Error bars indicate SEM. Percent change in Peak Amplitude (mean). Following either a right or left HVC full-lesion there was a decrease in mean peak amplitude. This general decrease in mean peak amplitude, regardless of the side of the lesion, illustrates an absence of lateralization for that feature of song. Error bars indicate SEM.

DISCUSSION

Overall, our data suggest that the premotor nucleus HVC contributes less to lateralized frequency production than what has been observed following lateralized denervation of the vocal organ. Our data do provide further evidence of the importance of the HVC in the timing of song elements and syntax, and that activity in left and right contribute equally to these functions. The results from full HVC lesions and microlesions complement each other and provide support for these functions.

Following left or right HVC microlesions, the changes in temporal features of song were the most significant effects observed. The syllable duration and bout length were both shortened following HVC lesion, but then returned to their original lengths within 7 days. This indicates that there is rapid neural compensation. These data are consistent with the hypothesis that HVC controls song timing. Activity in the intact hemisphere either recovers or the contralateral hemisphere assumes greater task control. Other acoustic features, such as song amplitude and frequency, were also affected. Amplitude of the song decreased, so the song overall was softer immediately following a microlesion. The frequency alteration was slight and approached significance, but still followed a pattern of transient disruption followed by recovery to pre-surgery levels. The findings support the past findings of the temporal and structural control the HVC has over song production in songbirds. These temporal and structural effects following microlesions are slight, and we believe that this is due in part to the fact that stereotyped phrases were largely unchanged by the lesion. Phrases are frequently repeated and the syllables included in a phrase almost always come in the same order and grouping. Because these phrases are so highly controlled, they are highly conserved

following modest damage to HVC. This would explain why they were even present on post-surgery day 1 when other syllables were absent and why the effects that appeared in the analysis of full song were minimal. These data did not support our hypothesis that there would be neural lateralization or asymmetry in HVC. Instead, these data illustrate the asymmetric control of song timing. Because the respiratory system controls temporal features of song, and there is bilateral neural innervation of the respiratory muscles, these data are consistent with the view that HVC is essential for executing and directing the rhythm of the bird's song.

Structural and temporal elements of song were degraded following full-lesions to both the right and left HVC. The features that were analyzed all exhibited immediate post-lesion effects. Duration, peak amplitude, and peak frequency all decreased, whereas inter-syllable interval increased. These results are consistent with what would be expected immediately after such a lesion. These changes were observed regardless of the hemisphere that was damaged, showing that HVC is important in both the timing and syntax of song. It takes both hemispheres contributing equally to achieve the balance needed to express song. Neither hemisphere is more involved in generating the timing and amplitude of song syllables. These results show the importance of bilateral, synchronized neural control underlying song production, similar to the results following HVC microlesions. However, even though both sides showed a decrease in peak frequency, a full lesion to the left side showed a decrease at least twice as much as damage to right HVC immediately post-surgery. While the data were not significant, the effect of the lesion on peak frequency supports previous research that indicated that the left hemisphere is responsible for the production of higher frequencies. These findings

are consistent with the lateralized frequency control at the level of the syrinx (Secora et al., 2012).

Micro and full lesions to the HVC resulted in degradation of many of the structural and temporal features that characterize Bengalese finch song. The absence of lateralization illustrates the importance of bilateral, synchronized neural control underlying song production. There may be several factors contributing to this lack of lateralization, but the most intriguing is the idea of interhemispheric switching. What may be reducing the capacity of the HVC to develop asymmetrically is the necessity for synchronization when producing song. Both sound sources within the syrinx must communicate to produce the lower and higher frequency components in harmony with one another much as two singers must work together when singing a duet. One model suggests that this switching takes place at lower levels of the motor pathway to synchronize breathing and song production (Schmidt, 2008). It is believed that the signals cross to the other hemisphere in order to coordinate breathing with phonation and allow two sides of the syrinx to work synergistically. Unilateral stimulation of the HVC in zebra finches resulted in song distortion which was corrected after 20 ms providing support for interhemispheric communication (Wang, Herbst, Keller, & Hahnloser, 2008). This interhemispheric connection is necessary for motor function and the extent of communication is what prevents hemispheric asymmetries. If song elements cross to the other hemisphere, there is less of a chance that one hemisphere would develop more than the other (Serrien, 2006).

This opens the door to a realm of possibilities for future research and exploration of the neural networks within the Bengalese finch. If there is little to no lateralization at

the level of the HVC, then the source must be found elsewhere. A possible next step would be to examine other nuclei or to study which aspects of song trigger interhemispheric switching. Either way, there is the promise of discovering the identity of the source of neural lateralization in the Bengalese finch and improving our understanding of such a majestic creature.

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ABSTRACT

Song learning in oscine birds shares many similarities with vocal imitation underlying the development of human speech and language. The Bengalese finch (*Lonchura striata domestica*) is one of few species with left hemispheric dominance over song production and discrimination. This parallels the left hemispheric dominance seen with human speech production and perception. It has been argued that lateralized neural control facilitates the vocal learning that underlies human speech. Given the lateralized control of song production, the Bengalese finch is a useful model for exploring avian parallels with human vocal learning. We sought to determine whether unilateral brain lesions would differentially contribute to the degree of song degradation and re-acquisition. The HVC is a premotor nucleus that is critical for control of timing and sequencing of syllables, similar to premotor cortical contributions to speech production in humans. We explored lesions to the left or right HVC of two different sizes: microlesions and full lesions. Microlesions were performed to transiently disrupt song production and enable quantification of song recovery whereas full lesions would induce more long term damage and to determine whether the side of the lesion will affect the rate of song recovery. Damage to the left or right HVC resulted in equivalent changes in song structure for all measured spectral and temporal parameters regardless of lesion size. Furthermore, the side of the microlesion did not affect rate of recovery of song production. Although the final motor commands to the syrinx are highly lateralized to control particular frequency ranges of song syllables, the premotor control of song production is not lateralized. These data illustrate the importance of bilateral, synchronized neural control underlying song production.