EXPLORING PARALLELS BETWEEN LATERALIZED CONTROL OF

HUMAN LANGUAGE AND THE NEURAL CONTROL OF

BENGALESE FINCH SONG SYNTAX

by

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ABSTRACT

Human speech production and grammatical organization of language is controlled primarily by the left hemisphere of brain. Broca's area is a specialized area in the left frontal cortex that is responsible for our ability to construct grammatically correct sentences. Songbirds are studied as an animal model for understanding human language production. Our research sought to explore whether the neural control of birdsong syntax of the Bengalese finch is also lateralized; if so, the Bengalese finch would provide a good animal model to further study syntax generation in humans. To investigate this question, we recorded the birdsongs of 8 different Bengalese finches. Then, the HVC (letters used as proper name) brain region, a brain area thought to control birdsong syntax, was lesioned in either the right or the left hemisphere for each bird. Birdsong was then recorded for five months following the surgery. Song syllables were coded and analyzed to measure the syntactic structure of the song. The birdsongs were grouped into a right lesion group and a left lesion group, and they were compared based on three measures of song syntax; sequence stereotypy, sequence consistency, and sequence linearity were measured at each time point. HVC lesion initially disrupted song syntax, but song syntax recovered. There was no significant difference between the left and right lesion groups. When looking at individual time points, the right lesion group seemed to initially lose more syntactic control four days after surgery, but overall, the trends between the two groups are relatively similar. Our results suggest that the HVC control of Bengalese finch song syntax is not lateralized as it is in human speech. Our results show that HVC does play a role in the syntactic structure of birdsong, but the recovery of song syntax following brain injury suggests other areas of the brain contribute to the generation of the Bengalese finch song syntax.

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<u>INTRODUCTION</u>

Communication between individuals is crucial for social interactions, maintaining romantic relationships with others, and survival in communal species. While there are many different ways to communicate, such as with body language or kind gestures, one of the most important ways to communicate is through speech and language. Many of us may not think twice when it comes to saying something as simple as "Hey, how are you?", but the process of acquiring and maintaining language is a very complicated one that must be learned.

In order for language to be acquired, learning is critical; if a baby is not exposed to the use of speech and language during the early part of his or her life, then language will not be learned (Doupe & Kuhl, 1999). The learning must take place during a "sensitive period" early in the baby's life. After the sensitive period has terminated, communicative skill and signals are not acquired at same level (Doupe & Kuhl, 1999). There are many stages during this process to learn how to communicate. At the beginning of the sensitive period, the baby will listen to others speak to one another, but it will not attempt to imitate it just yet. Then, a baby will start to babble and make nonsensical noises. Soon, the baby will speak its first word (a highly celebrated language acquisition milestone), and, little by little, the growing toddler will begin to add more words to its repertoire. The words that the toddlers learn come from adults raising the child, the television, books, anything else generally in the toddler's environment. Eventually, the child will learn to string these words into different arrangements using learned syntactic rules in order to create sentences. This process is very dependent on imitation of adults, as well as the baby or child hearing itself practice these vocalizations. Soon, they have a waning of this auditory dependence as they have a stronger grip on language (Doupe & Kuhl, 1999).

Language is a lateralized behavior, meaning that it is controlled preferentially by one side of the brain (Secora et al., 2012). Language is largely controlled by the left hemisphere of the human brain (Doupe & Kuhl, 1999). A specific part of the human brain, Broca's area, is especially associated with speech production and other lingual functions, including understanding syntactically complex sentences and further language processing (Davis et al., 2008). Broca's area is located in the left lateral frontal lobe, and it is larger than its homologue in the right side of the brain (Keller, Crow, Foundas, Amunts, & Roberts, 2009). There have been many functional imaging studies that have shown engagement of Broca's area during a wide variety of language tasks, as well as other nonlinguistic or cognitive processes (Davis et al., 2008). Those who experience damage to the Broca's area of their brain on the left side experience loss of fluent, grammatically correct speech production (Keller et al., 2009).

In order to further learn about language among the human species, the scientific world has turned to using songbirds as an animal model for the processes of language acquisition and production. Singing in songbirds is a complex, learned behavior, and it shares many parallels with human speech (Secora et al., 2012). While some features of birdsong are not analogous to human speech, such as the lack of capacity to use language for meaning, abstraction, or flexible associations, the developmental and neural processes are similar enough between the two that songbirds are considered to be sufficient models for language acquisition in humans (Doupe & Kuhl, 1999).

One notable similarity between bird song and human speech is that they are both learned behaviors with the presence of a sensitive period at the beginning of a songbird's life. Just as human babies must be exposed to speech in the early years of their lives, songbirds must be

"tutored" by adult birds in the first weeks of their lives. Baby birds will not be able to learn bird song if they are isolated from hearing adult song during this period (Carew, 2005).

Human speech and birdsong also share parallels in the sense that the acquisition process is comprised of different stages. The first stage of birdsong development in the sensitive period is called the sensory phase, which is when the baby bird hears the adult bird song and begins to commit the song to memory, but it does not sing at this time (Carew, 2005). The next phase is the sensorimotor phase, which is comprised of two sub-phases. First, the baby bird produces a *subsong*, which includes quiet bursts of sounds at irregular intervals (Carew, 2005). This stage is comparable to a human baby's babbling phase. The next part of the sensorimotor phase is the production of a *plastic song* in which sounds are produced in more discrete clusters. There is evidence of imitation and characterization of species-specific song, but the song overall is still relatively variable (Carew, 2005). This is comparable to when a toddler begins to say words and imitate his or her parents or what he or she hears on TV, but doesn't quite have the syntactic structure of language mastered. Finally, the last phase of song production is crystallization, in which the final adult song is produced. The notes are much crisper and well-defined, and there is a sharp increase in stereotypy; the song closely approximates the model provided by the tutor (Carew, 2005; Scharff & Nottebohm 1991). After this phase in closed-ended song learners, the bird's song remains largely unchanged for the remainder of the bird's life.

Birdsong, like speech, is comprised of ordered strings of sounds separated by brief, silent intervals (Doupe & Kuhl, 1999). The most basic unit of a birdsong is a note, which is a continuous mark on a sound spectogram (Scharff & Nottebohm, 1991). A syllable is comprised of two or more notes clustered together, making a unit of sound that is separated by silent intervals on either side of the syllable (Carew, 2005). Syllables can then be grouped to form a

motif or phrase, which is typically sung in a fixed order as a unit (Doupe & Kuhl, 1999). The number of motif notes, as well as their structure, can differ between individuals; this results in unique songs that are individual for each bird. All of this can be viewed together as syntax, which is the specific timing and ordering of these notes, syllables, and phrases in a song (Doupe & Kuhl, 1999). While the syntactic structure of human language has more to do with grammar and ordering words from different grammatical classes to convey meaning, the similarity in syntax between human language and birdsong have enough parallels to allow birdsong to be used as a model for human language.

Both human and songbirds have evolved a "complex hierarchy of forebrain areas in which motor and auditory centers interact closely, and which control the lower vocal motor areas" (Doupe & Kuhl, 1999). In humans, speech production is controlled by Broca's area; in songbirds, a forebrain nucleus called HVC (letters are proper name) is essential for song production. HVC regulates the timing and sequencing of learned song (Urbano, Aston, & Cooper, 2016). Projection neurons from HVC innervate two neural circuits, one of which is important for song production, and the other which aids with song learning and acquisition (See Figure 1). Each circuit is present in both the left and right side of the brain. In the first pathway, called the Song Production Pathway, the HVC neurons project directly to the robust nuclei of the archipallium, or RA (Carew, 2005). This is the pathway responsible for the production of song. The second pathway, named the Anterior Forebrain Pathway, has projections from HVC innervating Area X, which then projects to the dorsolateral nuclei of the thalamus, or DLM. The DLM then projects to the LMAN, otherwise known as the lateral magnocellular nuclei of the anterior nidopallium, which finally projects to the RA (Carew, 2005). This is the pathway that is critical for song learning. From there, the RA on either side of the brain project ipsilaterally to

the tracheosyringeal portion of each hypoglossal nerve, which directly innervates the avian vocal organ called the syrinx (Carew, 2005).

The oscine syrinx has two functional halves. In all songbird species, the left and right sides of the syrinx make different contributions to frequency control, and the syllables of a song are produced either by just one side of the syrinx or both sides simultaneously (Secora et al., 2012). In the Bengalese finch, which is the songbird used in this study, the left side of the syrinx is specialized for the production of higher frequency sounds above 2.2 kHz to about 4 kHz, and the right half of the syrinx produces broadband noises with frequencies of 2.2 kHz or lower (Urbano et al., 2016). Each half of the syrinx has its own innervation, air supply, and labial tissue (Carew, 2005). The right syringeal muscles are innervated by the right hypoglossal nerve, and the muscles controlling the left syrinx are innervated by the left hypoglossal nerve. Recall that each hypoglossal nerve is ipsilaterally innervated by the left or right RA, with each being innervated by the ipsilateral HVC. The Bengalese finch is left-hypoglossal-dominant; in an experiment conducted by Fernando Nottebohm, it was shown that cutting the left hypoglossal nerve had much more of an effect on the birdsong than cutting the right hypoglossal nerve (Carew, 2005). This shows a clear lateralization at the level of motor control over the syrinx.

The production of song requires bilateral hemispheric control in order to both produce sound as well as inhibit sound production by either half of the syrinx (Secora et al., 2012); in other words, the left HVC and the right HVC must coordinate to produce the correct notes, syllables, and motifs that make up each bird's unique birdsong. While one side of the song motor nuclei must innervate the syrinx to produce sound during a note, the contralateral nuclei must inhibit sound production by the other half of the syrinx (Secora et al., 2012). They also must work together maintain a symmetrical respiratory motor pattern during the asymmetrical

syringeal motor pattern (Secora et al., 2012). The song motor nuclei must constantly be in communication with each other and work as a team in order to control the muscular activation so that proper birdsong can be produced.

In a paper published in 2017, a group of researchers sought to further investigate the role of HVC during Bengalese finch birdsong (Zhang, Wittenbach, Jin, & Kozhevnikov, 2017). Temperature probes were used to temporarily manipulate HVC function by cooling them either bilaterally or unilaterally. RA was also tested, but the cooling of RA had little to no effect on song syntax. Cooling HVC had many effects on birdsong. First, cooling HVC overall slowed down the tempo of the song; it had been previously discovered that HVC is important in determining moment-to-moment timings of syllables, so this was to be expected. The duration of each syllable also increased, overall increasing the duration of the birdsong. The cooling also caused the gap durations between each syllable to be even greater than the syllable stretches. In addition, it reduced the number of repetitions of longer syllables within a repeat bout (shorter syllable repeats were not affected). The transition probabilities at each branch point were also affected—this means that the syllable following a certain syllable was not as predictable. All of these experiments were conducted bilaterally, and the results show that HVC does play a role in the syntactic structure of birdsong. When unilateral cooling experiments were conducted, both hemispheres equally affected intersyllable gap duration, but the stretching of the actual syllable durations was largely due to the cooling of left HVC (Zang et al., 2017). This suggests that it might be possible that the left HVC could have more of an effect on birdsong syntax than the right HVC.

While it is known that HVC has an effect on birdsong syntax and that the two HVC nuclei work together in order to properly produce birdsong, is there any evidence of

lateralization of HVC in the Bengalese finch following permanent damage? This study seeks to further investigate whether one side of HVC has more control over birdsong syntax and more strongly affects the ordering of notes and syllables of birdsong, just as Broca's area laterally controls human speech and the production of syntactically correct sentences.

<center>METHODS</center>

Subjects. Eight adult male Bengalese finches were used in this study. Female birds were not studied because only male Bengalese finches sing.

Care. This study was conducted with approval from the Texas Christian University Institutional Animal Care and Use Committee in an animal facility, which is regularly inspected and approved by the United States Department of Agriculture. The birds were given seed and water ad libitum, and they were also fed vegetables mixed with vitamins every day. They were housed in communal cages in a room on a 14:10 light:dark cycle. All surgical procedures were carried out under isoflurane anesthesia (1-2%), and all efforts were made to minimize pain and discomfort.

Song Recording. During song recording, birds were housed in individual cages that were 31.8 x 10.5 x 25.4 cm. These cages were put into sound-attenuating boxes, of which all four sides were lined with 1" thick acoustic foam to minimize echoes in the chamber. A microphone suspended in the center of sound-attenuating box 14 cm above the cage. The acoustic data were amplified and high pass filtered (300 Hz; RME audio, Haimhausen, Germany) prior to digitization. The data were then digitized (44.1 kHz sample rate, 16 bit resolution) via an analog-to-digital converted (National Instruments, NI USB-6251, Austin, TX). It was then saved to a disk on a computer running Avisoft Recorder Software (Avisoft Bioacoustics, Berlin, Germany).

Bird vocalizations were recorded continuously for the entire duration of the bird's stay in the sound-attenuating box, but the vocalizations were only saved to the disk when the amplitude exceeded a user defined amplitude threshold. Each file was recorded as a time stamped 16 bit .wav file (22.05 kHz sample rate) directly onto the hard drive.

Song recordings were all "undirected" song, meaning that the birds were isolated from other conspecifics and were not produced as directed communication towards any other birds. Baseline song, or the "pre-song", was first recorded one to five days prior to surgery. The baseline song was used to determine initial stereotypy of each bird. After surgery, the birds were each recorded post-surgery day 4 (PSD4), post-surgery day 7 (PSD7), post-surgery month 1 (PSM1), and post-surgery month 5 (PSM5).

Lesion methods. Unilateral HVC lesion was accomplished by placing six different sites within either the left or right HVC. A row of three electrode penetrations were placed with 250 μm intervals between each lesion starting at 2.2 μm lateral to the bifurcation of the central sinus (Y0). A second row of three lesion sites were placed 100 μm anterior to Y0 with identical 250 μm lesion site intervals as the posterior sites. Electrolytic macrolesions were made by passing 100 μA for 60 seconds at each lesion site.

Histology methods. Following birdsong recording and data collection, the birds were euthanized, and their brains were extracted. The brains were stored in 4% paraformaldehyde for at least 48 hours. Then, the brains were placed in 30% sucrose, and after they sank, the brains were sectioned on a freezing microtome. Coronal sections (40 μm) were mounted on glass slides, and then stained with cresyl violet. Photomicrographs were take every 100 μm through the extent of HVC as identified by the contralateral, intact side of the brain. The sections that

displayed intact HVC were traced in Photoshop and then overlaid on the contralateral

hemisphere to quantify the percentage of HVC damaged by the lesion.

Song selection. Ten songs of equal duration were chosen for each bird from each of the

five experimental timepoints (pre-surgery, PSD4, PSD7, PSM1, PSM5). Separate song bouts

were determined by a period of silence equal to a 500 ms duration or greater. Each track

recorded was visually evaluated using spectograms generated in Avisoft-SASLabPro (Avisoft

Bioacoustics, Berlin, Germany). A spectogram image is a visual display of the frequencies that

make up the song over a function of time (Carew, 2005). Each spectogram image was then hand-

coded, as described in the next paragraph.

Song Analysis. For each bird at each recording timepoint, each unique syllable was

assigned a distinctive letter indicator; this code was used for the ten selected songs within each

time point for each bird, with each set of ten songs being coded independently of the other sets

(see Figure 2). Syllables were coded using a variety of methods, with the primary method being

visual observation. If two notes were too difficult to distinguish visually, the notes were

acoustically examined to determine if they were similar enough to be considered the same

syllable. Each spectogram was evaluated by two separate individuals to ensure consistency and

that the data were as objective as possible.

Data Analysis. The songs were grouped by each timepoint for each bird; the coding for

the ten songs were typed up into strings of letters on the computer and then were entered into a

java applet online named The Songinator (Zevin & Bottjer, 2004). The Songinator quantifies the

song linearity (SL), song consistency (SC), and song stereotypy (SS) score for each timepoint.

SL quantifies the way that notes are ordered in a song. If a song was completely linear, each

syllable would always be followed by the next one specific syllable, meaning that each note only

has one transition type. A completely linear song would result in a score of 1. SL is calculated by:

$$SL = \frac{\#different\ notes\ per\ song}{\#transition\ types\ per\ song}$$

SC, on the other hand, addresses how often a particular overall path is followed. It expresses how often a particular "main" sequence appears in a song. In the formula, the typical transition type for each note is the one that is most frequently encountered. It is measured by:

$$SC = \frac{\sum(typical\ transitions\ per\ song)}{\sum(total\ transitions\ per\ song)}$$

Last, SS is an average of SL and SC. SS takes the individual transitions between notes as well as the overall sequencing pattern into account. Complete stereotypy would yield a value of 1, while deviations from the stereotyped song create scores approaching 0. It can be calculated using the formula:

$$SS = \frac{S(linearity) + S(consistency)}{2}$$

The SL, SC, and SS scores were all computed by the Songinator applet for each timepoint for each bird. The transitional probabilities for each note were plotted into a matrix for each timepoint for each bird. All of these results were then entered into a spreadsheet, where the left HVC lesion birds were grouped and the right HVC lesion birds were grouped. A repeated

measures ANOVA was run to analyze the data, and the overall results between the two groups were then analyzed for each timepoint using SPSS (v. 22 City and State).

RESULTS

Compared to the intact hemisphere, the left-HVC lesion group had an average of 64.58% of their left HVC damaged, and the right-HVC lesion group demonstrated an average of 69.44% damage (see Figure 3).

When the spectograms were being coded, it was determined that the songs at PSM4 and PSM7 for bird BFS683 (left-HVC lesion group) were so unpredictable and unstructured that they were not able to be accurately coded; therefore, the songs were not coded. In order to fill in the missing data, data imputation was performed so that the results could be analyzed. Since the song was not able to be coded due to unpredictability, the minimum value from the SL, SC, and SS from the left lesion group were used for this individual's data for the PSM4 and the PSM7 time point.

The songs were entered into the Songinator applet, and individual SL, SC, and SS were determined for each timepoint for each bird. A repeated measures ANOVA test was run on the data to determine statistical significance. The interaction effects (left or right HVC lesion and how it interacts with recording timepoint), the between-subject effects (left HVC lesion versus right HVC lesion), and the within-subject effects (differences between each timepoint for each individual bird) were all analyzed (see Figure 4).

Song linearity (SL) quantifies the order in which notes are strung together in a song. There were no significant interaction effects observed. ($F(4,24) = 0.437$, n.s.). Additionally, when comparing the left and right HVC lesion groups, no differences were found between the

groups ($F(1,6) = 1.867$, n.s.). However, following HVC lesion, song syntax was transiently disrupted; the within-subject effects showed a significant difference in song linearity between timepoints for each bird ($F(1,4) = 3.65$, $p < 0.02$).

Song consistency (SC) is the measure of how often an overall path is followed in birdsong. When examining the interaction effects between left or right HVC lesion and the timepoints of song recording, no significant results were observed ($F(4,24) = 0.422$, n.s.). There were also no significant differences in song consistency when comparing the right versus left HVC lesion groups ($F(1,6) = 0.023$, n.s.). On the other hand, a significant difference in song consistency between different timepoints for each bird was found ($F(1,4) = 3.494$, $p < 0.022$).

The song stereotypy (SS) measure is an average of SL and SC. There was no significant interaction between left or right HVC lesion and recording timepoint ($F(4,24) = 0.42$, n.s.), and there were no significant differences in song stereotypy between the left and right HVC lesion groups ($F(1,6) = 0.023$, n.s.). On the other hand, a significant change in song stereotypy was observed across recording timepoints ($F(1,4) = 3.494$, $p < 0.022$).

For song linearity, consistency, and stereotypy, the most statistically significant differences between timepoints occurred between pre-song and PSD7 ($p < 0.05$ for each measure) and between PSD7 and PSM5 ($p < 0.05$ for each measure). The significant difference between pre-song and PSD7 demonstrate an initial loss of syntactic structure following lesion; the difference between PSD7 and PSM5 then show that syntax was recovered by the end of the experiment.

To assess whether individual birds returned to their original prelesion song syntax following recovery from the HVC lesion, a correlation data was run between prelesion song and PSM1 song, as well as prelesion song and PSM5 song. An r value of 1.0 would indicate that

birds recovered to their baseline song. For song linearity, a significant correlation was found between pre-song and PSM1 ($r(6) = 0.8476$, $p < 0.008$). However, the recovery did not persist beyond this timepoint; the pre-song and PSM5 linearity scores were no longer correlated ($r(6) = .44$, n.s.). A similar pattern was found for song consistency. The correlation between pre-song and PSM1 was marginally significant due to sample size ($r(6)=0.6412$, $p < 0.09$). However, a correlation between pre-song consistency and PSM5 song consistency was nonexistent ($r(6) = 0.0656$, n.s.). Finally, there was a significant correlation between pre-song and PSM1 song stereotypies ($r(6) = 0.78$, $p < .022$), but the recovery did not persist beyond PSM1, as pre-song and PSM5 song stereotypy scores were no longer correlated ($r(6) = 0.17$, n.s.).

DISCUSSION

The results of this study do not show that HVC control over Bengalese finch birdsong syntax is lateralized. If neural control over syntax had been lateralized, the repeated measures ANOVA would have shown a significant interaction effect between the variables. The between-subject effects were also shown to be insignificant. However, the within-subject effects showed significant differences between pre-song and PSD7, which demonstrates that birdsong syntax was transiently altered after lesioning HVC. This supports the notion that HVC does play a large role in organizing syntactic structures of Bengalese finch birdsong. While SL, SC, and SS were all initially affected by damage to HVC, the birds did eventually return back to their original levels of syntactic structure, which is shown by the significant differences found between PSD7 and PSM5 data for each of the three measures.

There are two potential hypotheses as to how the birds were able to eventually recover syntactic structure of song; the brain is a very plastic organ, and it is able to adapt to different

situations astonishingly well in order to maintain overall function. First, it is possible that the contralateral HVC took control over structuring birdsong when the lesioned HVC was damaged and not able to perform its tasks. If this did happen, any effects of lateralization would be silenced due to the contralateral HVC adopting bilateral control. Another possibility is that the ipsilateral LMAN influences the production of song syntax more than is realized. As discussed in the introduction, HVC is part of two different neural pathways involved in song learning and production. In the Song Production Pathway, HVC directly projects onto the RA; however, in the much less direct Anterior Forebrain Pathway, HVC can only innervate the RA by activating a series of other brain nuclei. In the Anterior Forebrain Pathway, the LMAN actually is the nuclei that directly projects onto the RA. When HVC was rendered useless, it is possible that the ipsilateral LMAN utilized its direct projection onto RA and directed the production of song syntax by allowing the RA to continue to do its job by means of a different pathway.

The significant linearity, consistency, and stereotypy correlations between pre-song and PSM1 show that the Bengalese finches nearly return to their baseline song one month following surgery. This indicates that the level of birdsong linearity, consistency, and stereotypy before lesioning HVC is a strong predictor as to how the bird's song will become after the recovery process following surgery. Interestingly, the correlation deteriorates for all three measures when comparing pre-song and PSM5. There are a couple of potential reasons as to why this is observed. First, it could be due to the age of the birds studied. During the process of this study, the age of each Bengalese finch was unknown. This could have definitely affected how well each bird recovered from macrolesion surgery, which is a major procedure. An older bird will simply not make the same amount of progress in five months following surgery that a younger bird would make.

Another hypothesis is based on a previous study that measured the recovery of frequency following hypoglossal nerve lesion (Urbano, Peterson, & Cooper, 2013). As discussed in the introduction, each half of the syrinx is in charge of producing different frequencies of sound, and each half is ipsilaterally innervated by the left or right hypoglossal nerve. In the study, the left and right halves of the syrinx were denervated. Right side denervation didn't have a large effect on phonology, while the denervation of the left side led to songs that were mainly composed of lower frequency note syllables. This was to be expected since the left half of the syrinx is responsible for high frequency sounds. However, interestingly enough, three of the five birds recovered their higher frequency syllables. It was hypothesized that this occurred either due to the neural task control being switched to the right hemisphere or due to respiratory muscle compensation. This can be compared to the results of our correlation analysis between pre-song and PSM1 and our hypotheses about how it could be possible that other areas of the brain compensated for the damaged HVC so that proper song production could be restored.

However, in the same study, many of the high frequency notes and syllables were lost after more time passed (Urbano et al., 2013). It was hypothesized that it was too physiologically taxing for those specific parts of the oscine body to adopt these new responsibilities in addition to the tasks that they were initially performing without any help from the left hypoglossal nerve. We can take these results and compare them to what we see with the absence of correlation between pre-song and PSM5 song syntax. Just as it is hypothesized in the frequency recovery study, it is possible that it became too tiring for the contralateral HVC or the ipsilateral LMAN to compensate for the damaged HVC. The experiment shows that acoustic recovery at one month following surgery does not match the acoustic recovery at five months following surgery, which

is not too far off from the findings in this one; there potentially may be a link between acoustic recovery and syntactic production following surgery.

Another study to consider is the experiment in which HVC was cooled (Zhang et al., 2017), as mentioned earlier in the introduction. As one may recall, the results from the unilateral cooling of HVC suggest that the left HVC could have had more of an effect on birdsong syntax than the right HVC, therefore showing lateralization. These results were different from what we found in our study; however, it may be possible to compare the results from the cooling study with data from the PSD4 timepoint in this study. The cooling effect was a transient, reversible disruption to the HVC, while the methods in this experiment permanently damaged the brain region. In both the cooling effect and PSD4 timepoint in this experiment, the brain would not have had time to adapt to the new circumstances of having one nonfunctional HVC. Without having neighboring brain regions "take over" the responsibilities for the lesioned HVC, stronger effects would indubitably be observed. However, overall, the results between the two studies are not comparable after the PSD4 timepoint due to the nature of the brain damage suffered. With the brain being as adaptable and plastic as it is, the neuroanatomical adjustments made would help explain the correlation between pre-song and PSM1 song.

There were several limitations that may have led to the outcome of this experiment. First, the study had very low statistical power; only eight birds were studied, four in each condition. Had more birds been studied, the results would have had a higher power, and the means for each unit of measure would have been more stable and not subject to as much change. Also, as mentioned earlier, the age of each bird was not known. This leads to not having a perfectly uniform response rate following surgery, which could have affected the data. Finally, the nature of the Bengalese finch birdsong surely could have had an effect on the measure of data.

Bengalese finch birdsong syntax is variable across individuals within the species, as each bird sings its own individual song and has syllables that are different from other individuals. This makes it difficult to truly assess each bird on the same level. Additionally, while the Bengalese finch does serve as a common model for birdsong, the zebra finch is primarily used when it comes to studying birdsong. Many methods that are used to study birdsong, including the Songinator applet used to calculate the data for this experiment, were created with data from zebra finch birdsong in mind. While song linearity, consistency, and stereotypy may be great measures to study zebra finch birdsong, it is possible that these measures may not be ideal when it comes to analyzing the song of the Bengalese finch. It could even be possible that they are great measures to study Bengalese finch birdsong, but they may need to be adjusted slightly in order to take the differences between species into account.

Future experiments could make slight adjustments to this study in order to fully examine the lateralization effects of syntax control by the HVC. One potential study could lesion both HVCs in order to prevent the contralateral HVC from taking on the responsibilities of the other. While this would make it difficult to study lateralization effects, it would be a great way to study the overall effect of HVC control over syntactic structure. Another study could lesion the HVC of a bird along with the ipsilateral LMAN in order to cut off all signaling to the RA in that hemisphere of the brain. This would further help us focus on HVC's lateralized effects, as opposed to having the potential of the LMAN contributing to the results. Last, perhaps having methods to better analyze Bengalese finch song specifically instead of using the same applets and formulas used for zebra finch birdsong would be beneficial in accurately describing the results of HVC lesions in Bengalese finches.

The results of this study do not show signs of lateralized control over Bengalese finch song syntax, which is different from what we see in humans with lateralized control of language . Nonetheless, Bengalese finches are still great models to study birdsong. Overall, many similarities exist between the process of language acquisition in the Bengalese finch and humans, with both species having sensitive periods early in life and having specialized areas in the brain related to speech production. Also, while HVC may not mimic Broca's area in the sense of lateralization, it is known that they do perform a lot of the same basic functions. Both brain areas are involved in the learning of speech, as well as the production of syntactically structured communication. While we may not be able to use the Bengalese finch when it comes to studying lateralized control over language, it can still serve as an animal model to study the learning, acquisition, and production of speech.

It is important to continue to research Bengalese finches, as well as other songbirds, so that we can find an animal model that closely imitates language acquisition and production in humans. If we do find an animal that has lateralized control over language production, we can use that animal to study how different types of brain damage affect speech. In humans, damage to Broca's area occurring through a stroke, an aphasia, car accidents, or generally any other method leads to the loss of fluent speech production. Since Broca's area is thought to be the main center of speech learning and production, and is only present on one side of the brain, recovery of this skill is very difficult. In Bengalese finches, many different areas of the brain are involved in song learning and production, and they are present on both sides of the brain; this could be why they were able to recover the ability to produce syntactically structured songs following surgery. However, if an animal exhibiting lateralized control over communication is discovered, then we not only can learn how damage affects this complex behavior, but we also can use these

studies to develop better therapies for the recovery of speech production following different types of brain damage. Communication is crucial to our survival and maintenance of interpersonal relationships; discovering an animal model for this important behavior would greatly benefit not only those who have lost this ability, but also the general population who use this behavior every day.
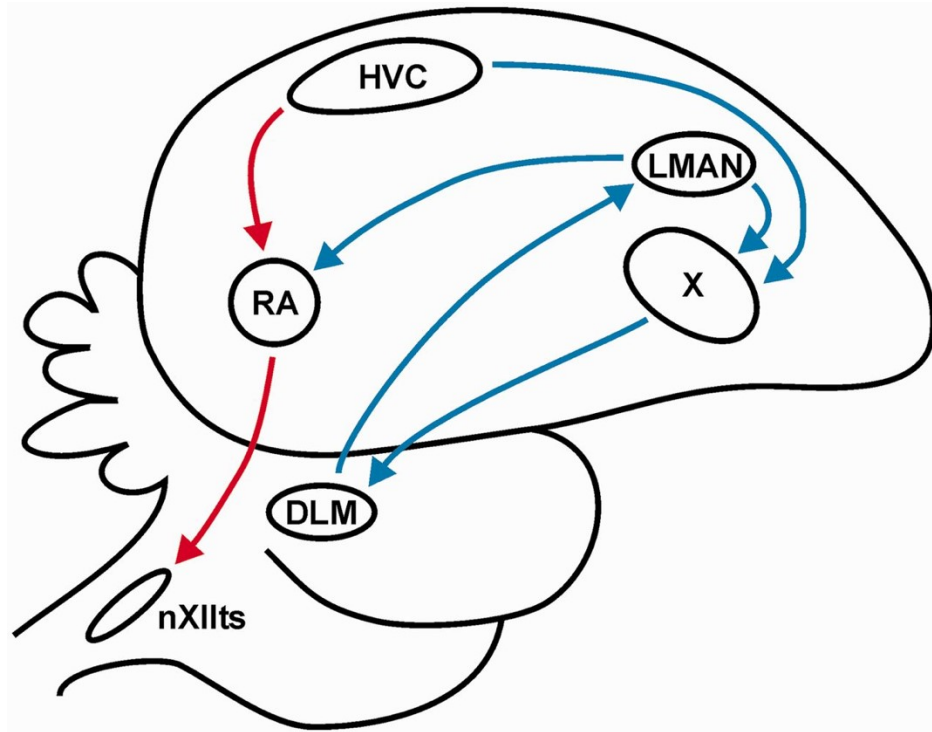
Figure 1

Figure 1. The avian song system. HVC is involved in two neural pathways used for the learning and production of birdsong. The Song Production Pathway, shown in red, is responsible for song production. In this pathway, HVC directly projects onto the RA. The Anterior Forebrain Pathway, shown in blue, is involved in the learning of birdsong. It is much less direct; the pathway starts with HVC and ends with RA, but other brain nuclei contribute in the middle of the pathway. In both pathways, innervated RA will project onto the nXIIts (the tracheosyringeal nucleus of the 12th cranial nerve), which will ipsilaterally innervate the syrinx on both halves of the body. Adapted from "Developmental Stress, Song-Learning, and Cognition," by Susan Peters, William A. Searcy, & Stephen Nowicki, Integrative & Comparative Biology, 54, p. 559. Copyright 2014 by Oxford Academic.
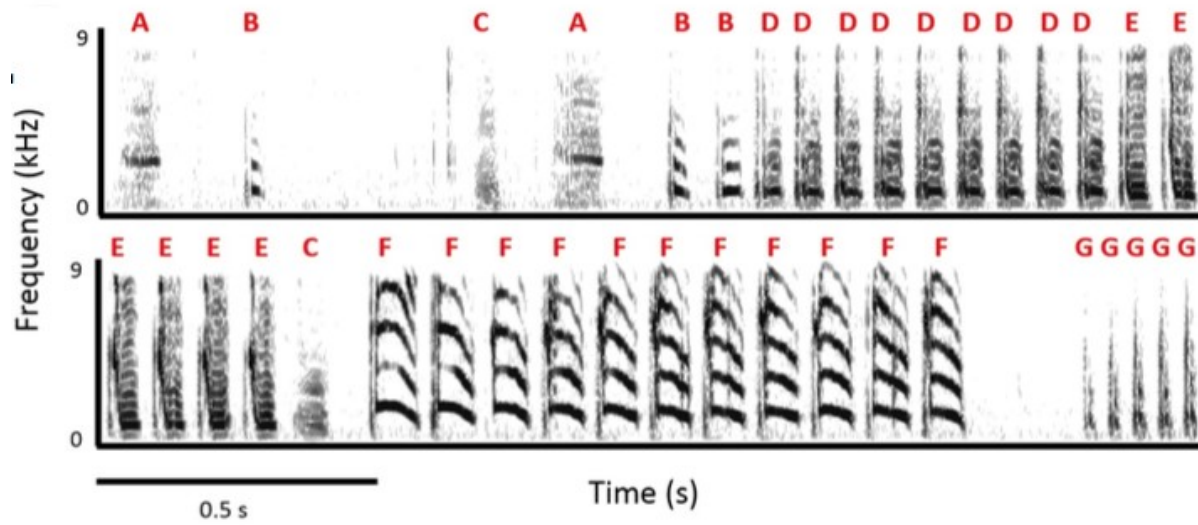
Figure 2

Figure 2. Sample song spectrogram. In a spectrogram, birdsong is visually displayed by showing the different frequencies of each syllable on the y axis and time on the x axis. To code the birdsong, each syllable is given an alphabetical letter. This code is used for the ten songs at each specific timepoint for each bird. The coded birdsong was then entered into the Songinator applet to calculate the syntax measures.
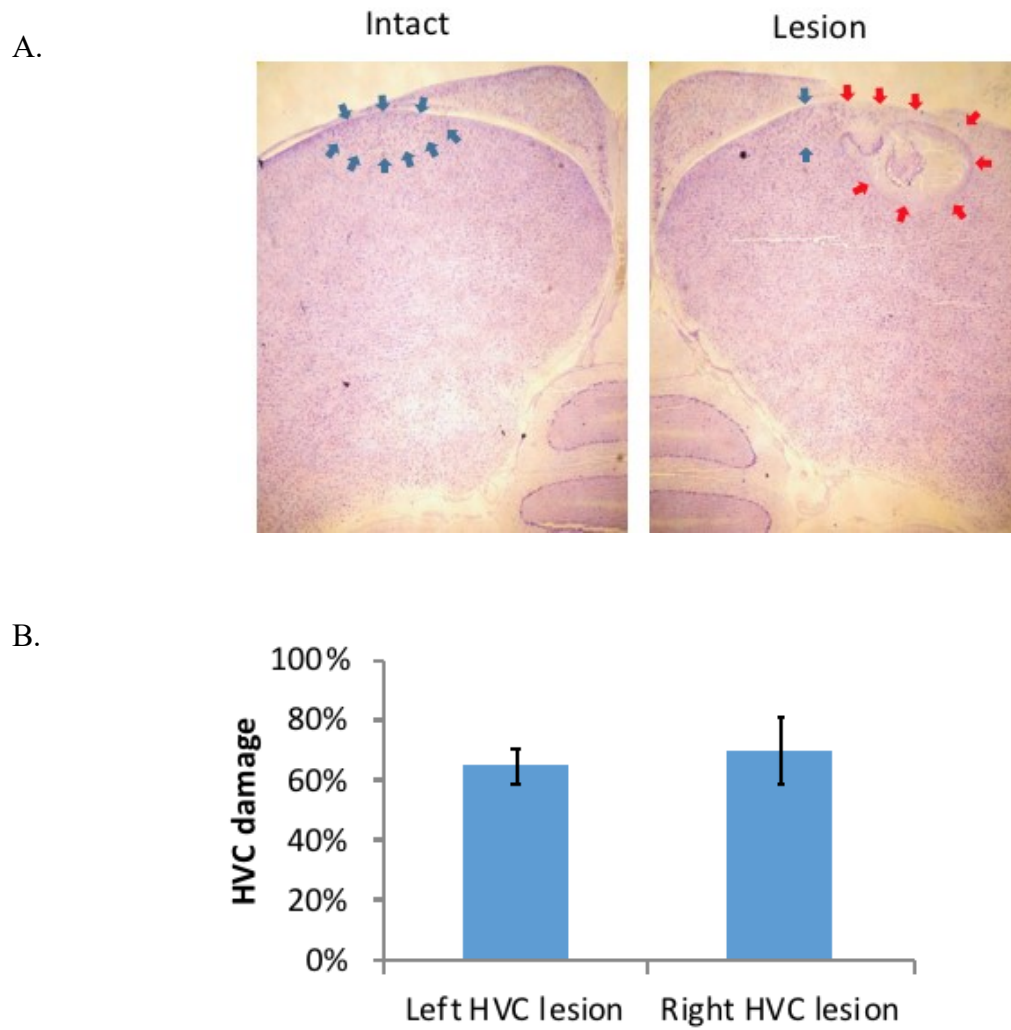
Figure 3

A.

B.

Figure 3. Histology and lesion damage. A) Coronal sections (40 μm) were made for each bird brain and were then analyzed in Photoshop to quantify the extent of HVC damage. The left photo shows a brain before lesion, and the blue arrows outline an intact HVC. The right photo shows the damaged HVC, with the red arrows outlining the extent of the damage. B) For the left HVC group, the birds endured an average of 65% damage following lesion. The right HVC group showed an average of 70% damage to their HVC nuclei.
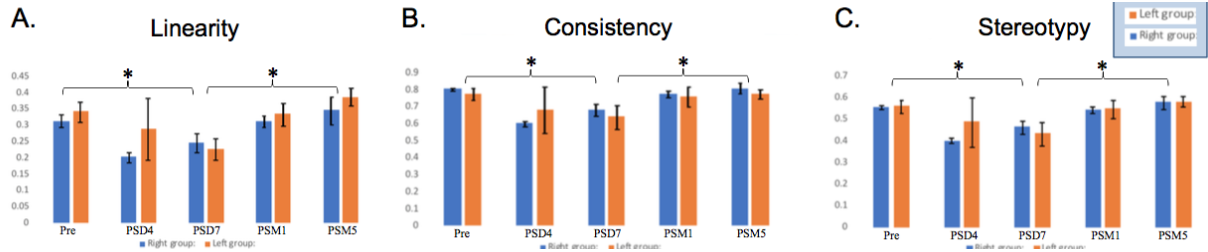
Figure 4

Figure 4. Song linearity, consistency, and stereotypy. These graphs show the averages for the left-lesion group and the right-lesion group at each time point for song linearity (A), song consistency (B), and song stereotypy (C). The interaction effects and the between-subject effects were not significant, but the within-subject effects did show some significant differences, demonstrated by the asterisks ($p < .05$) and brackets for each of the three measures. The first significant difference between pre-lesion song and PSD7 song demonstrates that song syntax was transiently disrupted. The other significant difference between PSD7 and PSM5 shows that syntax eventually recovered.
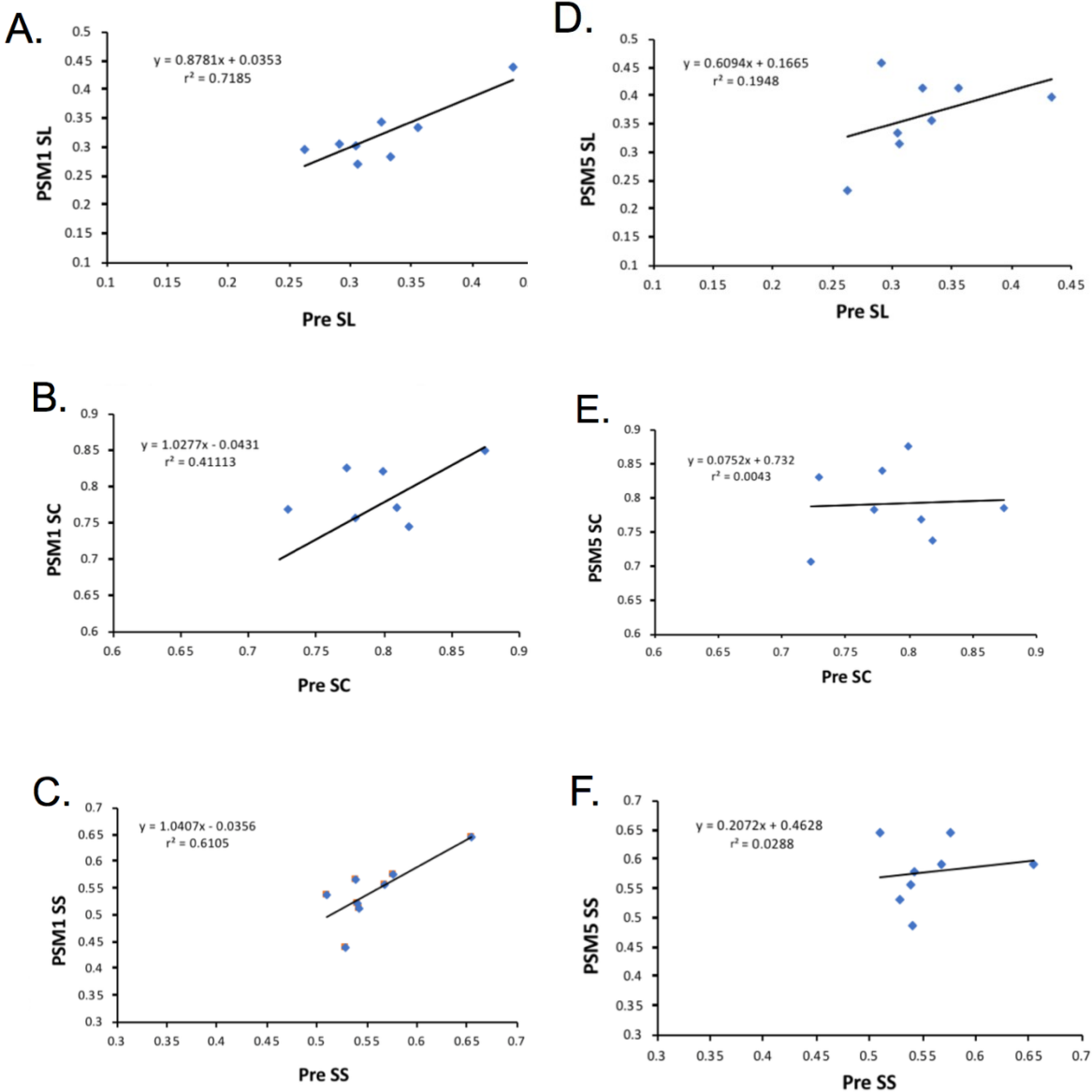
Figure 5

Figure 5. Correlation analysis for SL, SC, and SS. The correlations between pre-lesion song and PSM1 song were ran for song linearity (A), consistency (B), and stereotypy (C). The pre-lesion song and the PSM1 song were significantly correlated for all three measures. When analyzing the correlations between pre-lesion song and PSM5 song, however, there was no significant correlation found for linearity (D), consistency (E), or stereotypy (F).

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