Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

_____

Emily Aidan Berthiaume   Thursday April 10, 2014

Pitch Shift Correction of Song Patterning Changes Induced by Delayed Auditory Feedback in Bengalese Finches

by

Emily Aidan Berthiaume

Samuel J. Sober, PhD
Adviser

Neuroscience and Behavioral Biology

Samuel J. Sober, PhD

Adviser

Donna Maney, PhD

Committee Member

Susan Tamasi, PhD

Committee Member

2014

Pitch Shift Correction of Song Patterning Changes Induced by Delayed Auditory Feedback in Bengalese Finches

By

Emily Aidan Berthiaume

Samuel J. Sober, PhD

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Neuroscience and Behavioral Biology

2014

Abstract

Pitch Shift Correction of Song Patterning Changes Induced by Delayed Auditory Feedback in
Bengalese Finches
By Emily Aidan Berthiaume

The mechanism that causes human stuttering remains ambiguous, even with a considerable
amount of research examining potential components. One of the investigated components is a
potential delay in which a stutterer hears their own vocalizations. A postponement in receiving
recently produced vocal utterances via the auditory system is known as delayed auditory
feedback (DAF.) Structural changes in adult birdsong were induced by a DAF sensory
perturbation. Using miniature headphones on Bengalese finches, the probabilities of transition
to different song renditions after a branch point, a place in the song where there may be more
than one following syllable, were observed to change with DAF application. For birds with song
comprised of multiple motifs, motif repetition increased during DAF application. This increase
in motif repetition occurred, however, only when the gap between the end of the branch point
syllable and the beginning of the following syllable was small. When the gap between these
syllables increased to the point of delayed feedback expiration, the motif repetition returned to
a probability similar to that of baseline recordings. As the bird waited to continue the song, he
was less likely to repeat a motif. This reduction in motif repetition by singing slower is similar to
the reduction in syllable repetition when human stutterers are trained to speak more slowly.
Given this comparable therapy, the motif repetition behavior induced by DAF may be analogous
to vocal repetitions in human stuttering. The findings of this study suggest that DAF application
using miniature headphones to Bengalese finches could be used as an animal model of
stuttering. In this study, we examined the potential alleviation of motif repetition by applying a
large pitch shift. If proven effective in reducing motif repetition, the pitch shift could potentially
have clinical application for stutterers to reduce abnormal syllable repetitions. The results of
this study show that the probability of repeating a motif increases during DAF application, and
suggest that a large pitch shift may reduce the increase in repeated motifs induced by DAF.

Pitch Shift Correction of Song Patterning Changes Induced by Delayed Auditory Feedback in Bengalese Finches

By

Emily Aidan Berthiaume

Samuel J. Sober, PhD

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Neuroscience and Behavioral Biology

2014

Acknowledgements

Upon reaching this section of my thesis, I was at a complete loss for words. I can't even begin to thank Dr. Samuel Sober for giving me the opportunity to work in his lab. Not only has it been a second home over the past year and half, but it has also been a center of my academic and personal growth. Working here has been the highlight of my academic career at Emory. This experience has introduced me to the field of research, and this endeavor has been one of the greatest gifts I've ever received.

To Lukas Hoffmann: Thank you so much for running my headphones experiments, for teaching me to code, and for fixing my erroneous coding lines when my efforts went awry. Learning to code has been the most difficult challenge I've faced in my entire academic career. Throughout my entire learning process, you were patient and helped me through with such composure. I can't wait to read your next published paper, and see where your talents take you in the coming years.

To Conor Kelly: Thank you so much for fitting the birds with headphones, and monitoring the technical side of my experiments. You should win the award for dedicated lab member of the year given your efforts to keep the birds taken care of. Even on holidays and Atlanta citywide shut downs for snow, the lab could always count on you to make sure experiments were running smoothly. I wish you the best of luck in your future pursuits, especially for when you take your MCAT and apply to medical school. You will be an incredible doctor, and you are an incredible scientist already.

To David Nicholson: Thank you so much for helping me deduce the solutions to problems that emerged in my code, and for working on analysis programs for my project. Additionally, thank you for making me laugh at midnight on weekend evenings in lab as we talked about everything from breakfast tacos to Jaws 1, 2, 3, and 4. If there is one thing I will take away from getting to know you, it is to question everything. That is an understatement; after working with you, I could question my certainty of being a human on this planet. Your passion and care for your work is what makes you such a great scientist, and your dedication will pay off in the near future.

To MacKenzie Wyatt: Thank you so much for helping me sort through data and labeling birdsong recordings. It has been a pleasure to come into lab and see your smile. You have been an amazing student (although, it is really an unfamiliar experience to think of myself as a teacher.) You have an extraordinarily bright future ahead, and I look forward to completing this project with you!

In conclusion, thank you to you all. I wish the best of luck for you in the coming years. It has been such a pleasure to learn from and work with your incredible minds. I can't think of a better way to end this but with my favorite quote: "great moments are born from great opportunity" *~Herb Brooks*. You all gave me a great opportunity, and I have had an endless amount of great moments in the time I've spent here.

Table of Contents

List of Figures

Introduction

Sensory feedback is important to maintaining the accuracy of behavior. During vocal behaviors, the brain uses auditory feedback to make adjustments. For example, children that are born deaf typically cannot develop spoken language, and often compensate by learning a signing system instead (Stokoe 1980; Petitto 1993; Doupe and Kuhl 1999). Adults that experience hearing loss are afflicted with gradual speech degradation (Waldstein 1990; Cowie and Douglas-Cowie 1992; Doupe and Kuhl 1999). Similarly, disruption of auditory feedback in songbirds via deafening can cause degradation of song syllable structure (Nordeen and Nordeen 2004; Heaton et al. 2010). The structure in song syllables of deafened zebra finches begins deteriorating 6-8 weeks after the birds can no longer hear themselves (Nordeen and Nordeen 2004). Deafening adult budgerigars alters the acoustic structure of their songs (Heaton et al. 2010). Because both humans and songbirds dependent on vocalizations for communication, the ability to hear oneself is of great value.

Stuttering, a speech disorder characterized by involuntary sound repetition, can negatively impact vocal communication ability and mental health. A commonly observed "stuttering-like disfluency" is the reiteration of a syllable within a word, known as partial word repetition (Bloodstein and Grossman 1981; Ambrose and Yairi 1999; Chon et al. 2012). This pattern is observed in children's speech more frequently than in speech of adults (Andrews and Harris 1964; Bloodstein 1995; Yairi and Ambrose 1999). This age-dependent difference is likely due to spontaneous recovery of normal fluency as children outgrow their stuttering before reaching adulthood (Bryngelson 1938; Andrews and Harris 1964; Yairi and Ambrose 1999). Unfortunately for the estimated 1% of adults with persistent stuttering, personal satisfaction with life can be severely affected (Bloodstein 1995; Yairi and Ambrose 1999; Craig et al. 2009). Stuttering is often associated with secondary psychiatric disorders; it can impact quality of

life by affecting emotional and mental health functioning, and can hinder social interaction experiences such as talking on the telephone (Stein et al. 1996; Craig et al. 2009).

The cause of human stuttering is still being debated, and there is no established cure (Bloodstein 1995). Behavioral speech therapies are among the most widely used treatments, and seem to have a positive effect (Herder et al. 2006). Nevertheless, these treatment options require dedication, practice, and time in order to correct the speech disfluencies. Additionally, long-term results show a majority of people can maintain satisfactory fluency 1-2 years after an intensive behavioral program, but a subset of people who undergo treatment do not maintain adequate fluency rates when compared with non-stutterers (Boberg and Kully 1994). Without fully understanding the mechanism causing the speech disfluencies, attempting to create more a more effective treatment may be infeasible. Possible mechanisms for stuttering include motor irregularities and hemispheric lateralization abnormalities, but neither has been confirmed as the cause (Freeman and Ushijima 1978; Braun et al. 1997; Watkins et al. 2008). In addition to these potential mechanisms, a sensory perturbation has been found to produce stuttering-like speech abnormalities when applied to human subjects with normal fluency patterns (Fairbanks 1955; Chon et al. 2012). This sensory alteration, delayed auditory feedback, may cause these disfluencies because the brain receives the sensory information later than normal (Yates 1963; Code C 2008). Because these sensory feedback perturbations have been found to cause vocal disfluencies like those observed in human stuttering, delayed auditory feedback could be used in animal models for testing new therapeutic techniques. In this study, we will test whether the application of delayed auditory feedback alters vocal sequencing in Bengalese finches, a species that has been used to model vocal communication in humans (Doupe and Kuhl 1999).

The mechanisms that humans use to produce speech and birds use to produce song are similar, and the singing behavior of songbirds may be compared to human speech behavior for better

understanding of human speech abnormalities (Doupe and Kuhl 1999). The way in which humans learn

to produce speech is similar to the way in which songbirds learn to produce their songs (Marler 1970;

Kuhl 1989; Doupe and Kuhl 1999). Beginning when a human is born, there is a period of sensory

acquisition characterized by listening to adult speech (Doupe and Kuhl 1999). Children grow up to speak

the same language and dialect as the adult figures in their lives (Doupe and Kuhl 1999). In songbirds,

when a male chick hatches, an adult male bird "tutors" him during a period of auditory sensory

acquisition (Brainard and Doupe 2002). Songbirds, even when exposed only to the song of another

species of songbird, learn their songs from the adult birds that are around them (Thorpe 1961; Marler

1970; Doupe and Kuhl 1999). As the young humans and songbirds mature, they practice vocalizing while

continuing to listen to adult articulation until their utterances reach a stable and consistent output that

reflects their early learning experience (Doupe and Kuhl 1999). In this way, vocalizations are culturally

transmitted from one generation to the next in both humans and songbirds (Marler and Tamura 1962;

Slater 1986; Doupe and Kuhl 1999). Along with these similarities, both human speech and birdsong are

highly stereotyped and stable throughout adulthood (Sober and Brainard 2009).

Human speech and birdsong are both maintained throughout adulthood by active error

correction using auditory feedback (Okanoya and Yamaguchi 1997; Houde and Jordan 1998; Sober and

Brainard 2009). Altering auditory feedback with a pitch shift causes an adjustment in fundamental

frequency of human speech in the direction opposite that of the induced perturbation (Burnett et al.

1998). Similarly, altering auditory feedback in songbirds can result in changes to their stereotyped songs

(Okanoya and Yamaguchi 1997; Sober and Brainard 2009). Recent work has shown that an applied pitch

shift causes birds to make a compensatory shift in syllable frequency in the opposite direction of the

feedback shift (Sober and Brainard 2009). Previous studies have demonstrated that song syntax and

phonology changed when speakers were pointed at zebra finches to play back their songs at a delayed

rate while the birds were singing (Cynx and von Rad 2001). The song of a Bengalese finch differs from

that of a zebra finch; certain syllables in Bengalese finch song can be followed by one of several different syllables, as opposed to a highly fixed order of syllables throughout the entire song pattern in zebra finches (Okanoya 2006). These parts of Bengalese finch song can be considered "branch points;" places where there may be more than one following syllable (Figure 1) (Okanoya 2006). Branch points in the song can be at the end of a "motif," a part of a song comprised of the same syllables (Wada 2012). Certain motifs can be repeated, and branch points may be used to test for the likelihood of an alteration in the repetition probability in response to an induced auditory feedback delay.
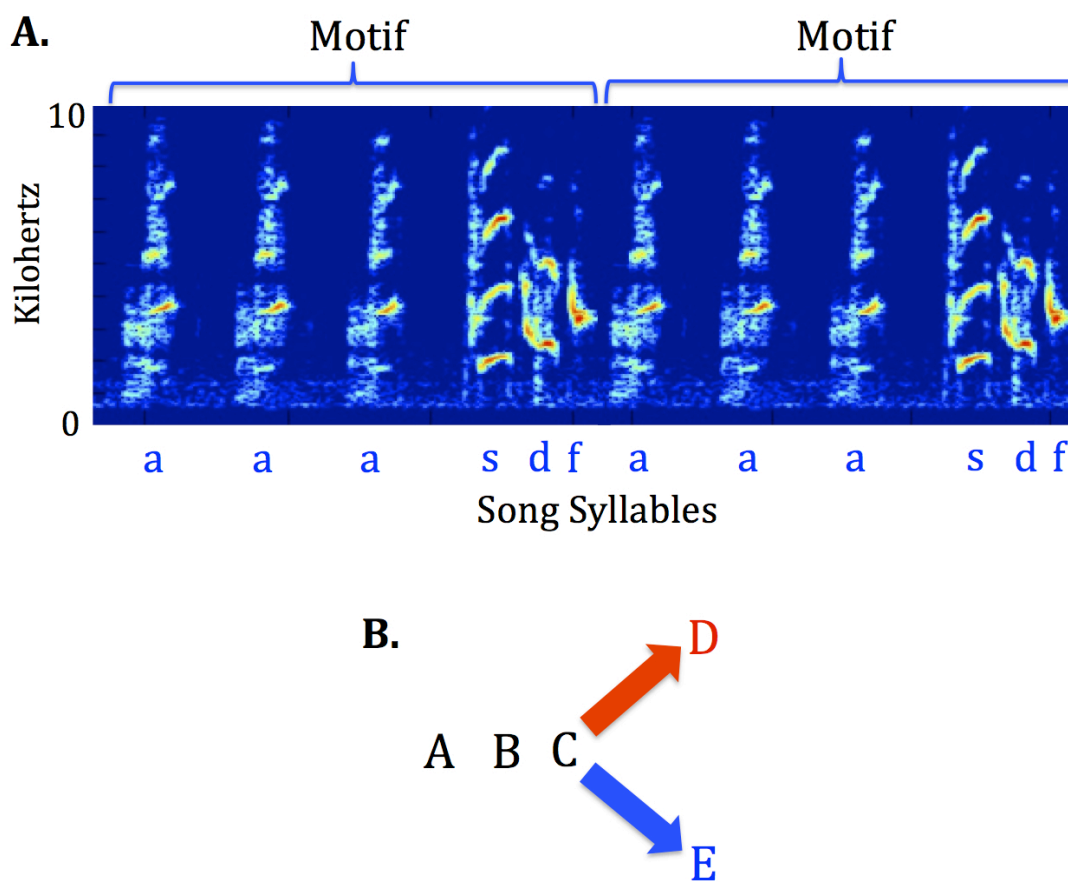

Figure 1: Depiction of song terms. A. A song spectrogram depicting single syllables (individual elements of song that have emphasized frequencies). Syllables can be visually compared for similarities, and can be arranged into motifs (distinct parts of song). **B.** A diagram of a branch point, where syllable 'C' can transition to either syllable 'D' or syllable 'E'.

For our experiment, the use of a large pitch shift may reduce the effects of delayed auditory feedback because the birds will rely less on the altered feedback with the addition of a pitch shift. In humans and songbirds, the use of auditory feedback for behavioral maintenance can change based on the size of an applied pitch shift. Humans respond to small applied shifts in pitch by adjusting their voice fundamental frequency in the opposite direction of the stimulus, which essentially corrects for the shift (Burnett et al. 1998). When the pitch shift is large, however, the person does not compensate for the perturbation by adjusting their voice fundamental frequency in the opposite direction as much (Burnett et al. 1998). Likewise, songbirds will compensate less for larger pitch shifts than smaller pitch shifts (Sober and Brainard 2012). The response to the amount of an applied shifted pitch appears to be similar in humans and songbirds. In addition, shifting the pitch of the auditory feedback that a stutterer receives may reduce speech disfluencies (Natke et al. 2001). In this study, we will test whether a pitch shift can reduce the changes in song sequence induced by delayed auditory feedback.

We hypothesize that accuracy of auditory feedback is important for the maintenance of the song sequence patterning. In this study, we tested this hypothesis by quantifying the effects of delayed auditory feedback on the patterns of vocal sequences in Bengalese finches. We predicted that delaying auditory feedback would increase sequence repetition. In addition to testing the importance of accurate timing of auditory feedback, we hypothesize that the use of online feedback for sequence maintenance depends on the acoustic similarity between the expected auditory feedback and actual auditory feedback (Sober and Brainard 2012). To test this hypothesis, we recorded song while applying a large pitch shift concurrently with delayed feedback. With the additional application of a large pitch shift, we predicted that the bird would produce sequences similar to those observed in absence of auditory perturbation.

Methods

Subjects

Four adult male Bengalese finches (*Lonchura striata* var. *domestica*) over the age of 125 days served as the experimental subjects in this study. Each bird was individually housed in an isolation chamber throughout the experiment. Food and water were provided *ad libitum* and the light/dark cycle was maintained at 14 hours/10 hours with light starting at 7:00am and ending at 9:00pm. All song recorded for experimental analysis was undirected, with no female present in the sound chamber. The care procedures used for the animals were approved by the Emory University Institutional Animal Care and Use Committee (IACUC).

Auditory Playback Manipulation

Methods of manipulation of auditory feedback were the same as those described by Hoffmann et al. (2012). Miniature lightweight headphones were attached to each bird's head, covering the ear holes on the left and right side. Two microphones were used to record the bird's song for subsequent playback during delay trials. A microphone located at the top of the cage recorded the bird's song and any other prolonged or loud noise made inside the sound chamber. The sound recorded through this microphone was processed and played back through miniature speakers housed inside the headphones. The processing equipment allowed for delayed and pitch shifted versions of the bird's song to be played back through the miniature headphone speakers. An additional microphone was used inside the miniature speakers in order to monitor the playback produced from the sound processing equipment. When the bird sang, the song was delayed and/or shifted, played back to the bird through the headphones as he sang, and the song was recorded for experimental analysis. The delay time of the playback was measured in milliseconds, and the pitch shift was measured in cents. Cents are a measurement of differences in pitch, with 100 cents representing a one-semitone pitch difference.

To make a recording of the bird's song, the amplitude threshold was set on the recording equipment so that introductory song notes crossed the threshold, and a song file was created. Approximately three seconds before the sound that crossed the threshold frequency and three seconds after the last crossing of the threshold frequency were included in a recorded song file. Files were sorted to ensure the data sets were comprised only of song files.

Birdsong Labeling Techniques

A bird with normal feedback sings a highly stereotyped song that is comprised of unique syllables that are similar throughout song motifs (Sober and Brainard 2009). Each syllable, defined as a single element in the bird's song, was labeled by visual inspection of the spectrograms in Matlab computer software (Sober and Brainard 2009; Wada 2012). Each individual syllable was defined by its onset and its offset, determined by crossings of the threshold amplitude.

Testing the Range of Delay for Behavioral Response

To identify the range of delay that may elicit changes in song structure, bird 1 was tested on delay parameters in 25 milliseconds increments between the values of 25 and 200 milliseconds. These parameters were selected on the basis of published reports showing that delays between 50 and 250 milliseconds produced syntax abnormalities in zebra finches (Cynx and von Rad 2001). The delay parameters were randomized and presented in the following order: 100, 50, 25, 75, 125, 150, 0, 200, 175 milliseconds of delay. Each delay parameter was tested for a period of 5-10 hours. The first 40 song files recorded at a given delay parameter were labeled and used for analysis. In this bird's song, we found a branch point (Figure 2A) that could be followed by two different sequences of syllables. We compared the probability of transition of those sequences during normal auditory feedback with the probability of the same transitions when feedback was delayed. To test for significant changes in the probability of transitioning to each sequence, we used a z-test for proportions with a Bonferroni

correction. The alpha level of significance was set to 0.00556 for this statistical testing. Subsequent experiments were designed using 175 millisecond delay based on an anecdotal observation of a syllable repetition at the end of the song. All statistical analysis was done in Mathworks' Matlab.

Testing the Temporal Effect of Extended Delay

To test for changes in song sequencing during extended delayed auditory feedback, auditory feedback was delayed for bird 2 at 175 milliseconds delay for twenty days of recording. After 20 days, the delay was removed and the bird remained with headphones on with no delay. 170 days later, the bird's song was recorded to test whether his song returned to baseline patterning. The days that the bird receives normal auditory feedback following experimental perturbations are considered "washout" days. The first 60 song files from each testing day were labeled and used for statistical analysis. This bird's song was comprised of two different motifs.

We measured the probability of transition from motif 1 to motif 2 (1→2) and the probability of repeating motif 1 (1→1) (Figure 4A). To test for significant changes in the probability of motif repetition, we used a z-test for proportions with a Bonferroni correction by comparing the probabilities of transition on each day of delay and post-experiment recording to the baseline data recordings. Additionally, we measured the time between the offset of the final syllable of motif 1 and the onset of the first syllable of motif 2. To analyze the effect of delay on the width of the gap between song syllables, a Kolmogorov-Smirnov test with Bonferroni correction was used to compare the mean of the gap length between the syllables during delay days to the mean of the gap length between the syllables during baseline.

Testing the Effect of a Pitch Shift on Delayed Feedback Motif Repeat

Shifting the pitch of a stutterer's auditory feedback may reduce speech disfluencies (Natke et al. 2001). If delayed auditory feedback induces an increased probability of motif repetition in songbirds, a pitch shift may reduce the repetitions. Bird 3 was tested with 9 days of 175 milliseconds delayed feedback to induce the increased probability of motif repetition, followed by 8 days of delayed feedback with a 300 cent positive pitch shift to reduce the probability of motif repetition induced by the delayed feedback, and then 9 days of 0 milliseconds delay and 0 cent pitch shift to see if the song patterning returns to baseline. The song recordings used for analysis were the first 51 made on a given day. On some of the days, however, fewer than 51 song files were recorded because the bird was not a frequent singer. This bird sang less than the desired sample size on 6 of the delay days, 6 of the delay with shift days, and 1 of the washout days. To analyze this bird's song, we measured the probability of transition from motif 1 to motif 2 (1→2) and the probability of repeat of motif 1 (1→1) on each day of the experiment. We compared the probabilities of transition from each day of recording to the respective probabilities of transition from the baseline data sample. The baseline sample was comprised of 51 songs from 3 different baseline recording days with at least 51 song recordings. To measure statistical significance, a z-test for proportions with a Bonferroni correction compared the probability of a transition on an experimental auditory manipulation day to the respective transition of that probability from the baseline recording analysis data.

To control for direction of pitch shift, bird 4 was tested on 8 days of continuous 175 milliseconds delayed feedback, then 8 days of 175 milliseconds feedback with a 300 cent negative pitch shift, and washed out with 0 milliseconds delay and 0 cent pitch shift for 9 days. The first 51 song files recorded on a given day were labeled and used for statistical analysis. To analyze this bird's song, we measured the probability of transition from motif 1 to motif 2 (1→2), the probability of repeat of motif 1 (1→1), and the probability of transition from motif 1 to motif 3 (1→3) on each day of the experiment. We compared the probabilities of each type of transition on each day of experimental perturbation to the respective

probabilities of transition during baseline. The baseline sample was comprised of 51 songs from 3

different baseline recording days that had at least 51 songs. To measure statistical significance, a z-test

for proportions with a Bonferroni correction was used to test for the effect of the auditory manipulation

by comparing the probabilities of transition on baseline days to each of the experimental perturbation

days.

Results

25-175 millisecond Delay Parameters Induce a Change in Transition Probabilities at a Branch Point

For bird 1, delayed auditory feedback delivered through miniature headphones altered syllable transition probabilities. In a pilot study performed to measure the range of delay that would induce changes in song patterns, delay parameters at 25 millisecond intervals between 25 and 200 milliseconds were randomized and tested for a period of 5-10 hours each. Changes in this bird's song patterning were apparent in the probability of transition at a branch point in the middle of the song (Figure 2A). In the dominant transition (occurring in 83% of the cases in the baseline), the bird sang syllable 'f' to syllable 'k' (Figure 2B). In the minor transition (occurring in 17% of the cases in the baseline), the bird sang syllable 'f' followed by syllable 'z' and then syllable 's' (Figure 2C).
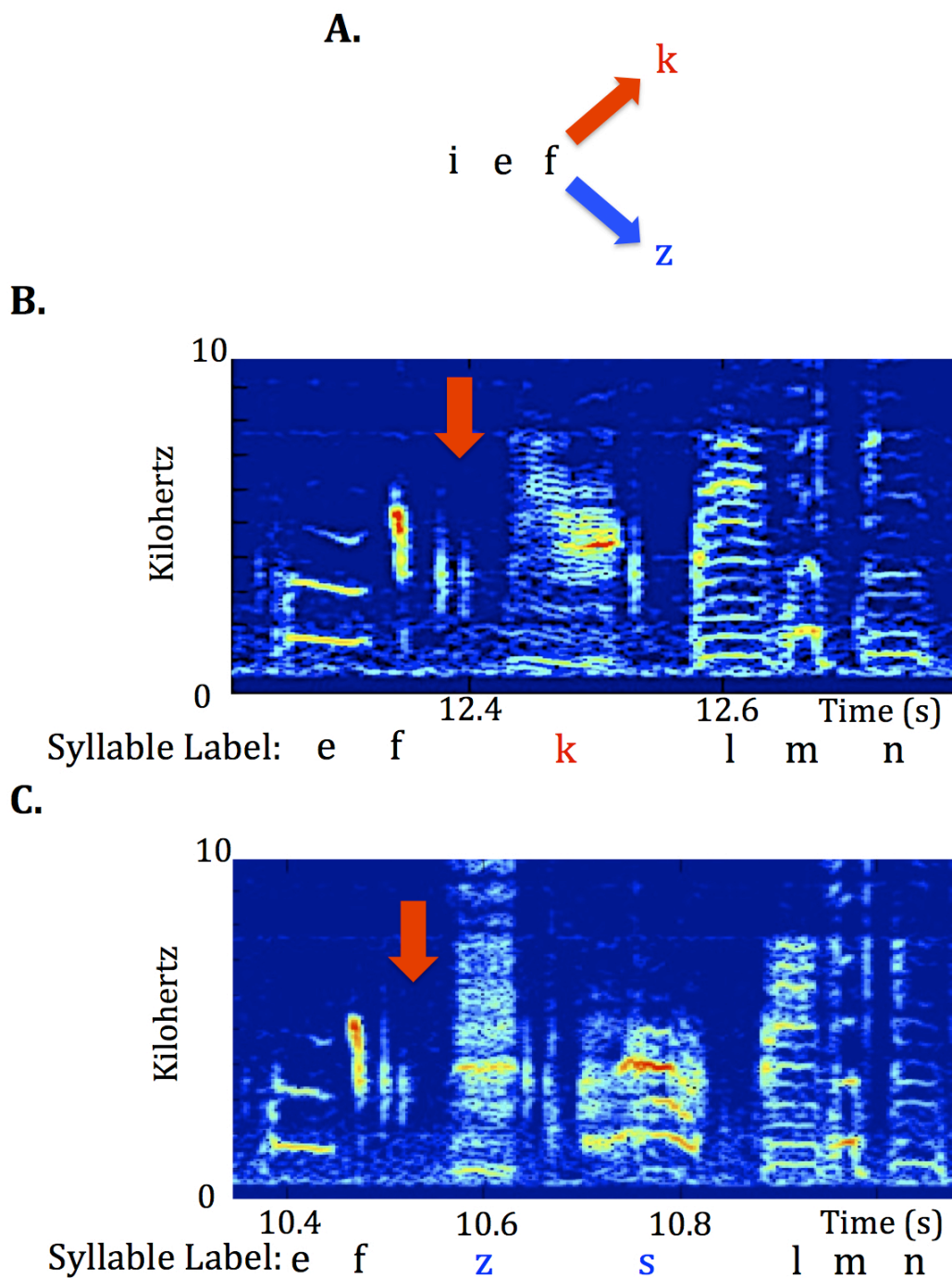
Figure 2: Depiction of two transitions at a branch point. A. Syllable 'f' represents a branch point, after which one of two syllables, 'k' or 'z' may follow. The red arrow denotes the dominant transition, to 'k', and the blue arrow denotes the minor transition, to 'z'. **B.** A song spectrogram of the dominant transition ('f'→'k') at the branch point. **C.** A song spectrogram of the minor transition ('f'→'z') at the branch point. The red arrows in B and C denote the song branch point.

Significant changes in transition probability were observed at 25, 50, 75, 125, and 150 millisecond delay parameters (Figure 3). The probability of transition to 'k', the dominant song transition, significantly decreased during application of the five delay parameters, whereas the probability of transition to the minor song transition increased. The 175 millisecond delay parameter appeared to follow the same trend as the other five significant points. The p-value of the 175 millisecond delay parameter proportion of the dominant transition when tested against baseline was 0.0165. It is worth noting that, while the p-value of the 175 millisecond delay parameter when compared to baseline data was not smaller than alpha, the p-value is still very low and follows the trend of the delay parameters with significant probabilities of transition compared to baseline.
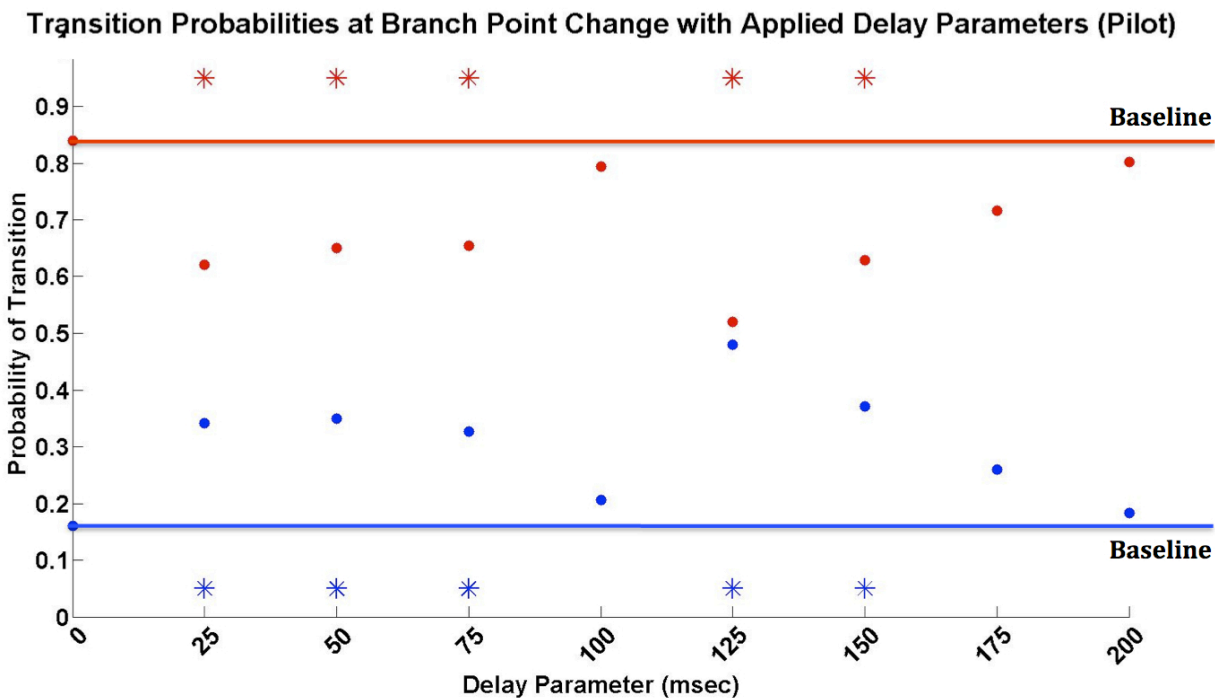
Figure 3: For bird 1 (song shown in Figure 2), the probability of transition to the dominant syllable at the branch point decreases, while the probability of transition to the minor syllable increases with 25-175 milliseconds of applied delayed auditory feedback. The red dots depict the probability of transition to the dominant song rendition and the blue dots depict the probability of transition to the minor song rendition at each of the delay parameters. The red and blue asterisks denote a significant difference between the probability of transition to syllable 'k' and to syllable 'z', respectively, to baseline transition probabilities.

The order of presentation may have affected the results shown in Figure 3. This experiment was a pilot study that served to test the range of delay that would evoke song patterning changes. If the bird's song is delayed too far beyond the range of expected auditory feedback, the bird may disregard the auditory feedback and not respond to the change (Sober and Brainard 2009). To ensure experimental validity of this study, the delay parameters were randomized into the following order of presentation: 100, 50, 25, 75, 125, 150, 0, 200, 175 millisecond delay. The 100 millisecond and 200 millisecond delay parameters immediately followed the bird experiencing no delay, with the 100 millisecond delay at the start of the experiment and the 200 millisecond delay following the baseline recording. Changes in probability of transition may have taken more than the allotted recording hours to appear.

Although effects on motif 1 repetition at the 175 millisecond delay parameter could not be detected, song patterning changes were anecdotally observed. At the end of select recorded songs, it appeared as though the bird repeated the last syllable. On the basis of this observation, subsequent experiments were designed using a 175 millisecond delay parameter. This delay elicited changes in song structure in subsequent experiments, as described below.

Delayed Auditory Feedback Increases Motif Repetition at Branch Points, but Probability of Repetition Reverts to Baseline when Accompanied by an Increase in Gap Duration

In an experiment measuring the effect of prolonged delayed auditory feedback, miniature headphones were used to delay playback of bird 2's song by 175 milliseconds for twenty consecutive days. In this bird's song, there are two distinct motifs with consistent patterning. The first motif of the song contained a variable number of 'a' syllables, followed by syllables 's' 'd' 'f'. The second motif of the song began with syllable 'j', followed by a variable number of 'k' syllables, and ending with syllable 'l'. A

branch point occurred at the end of the first motif of the song (Figure 4A), where the bird could

continue either by singing motif 2 (Figure 4B), or restart the song by singing motif 1 again (Figure 4C).
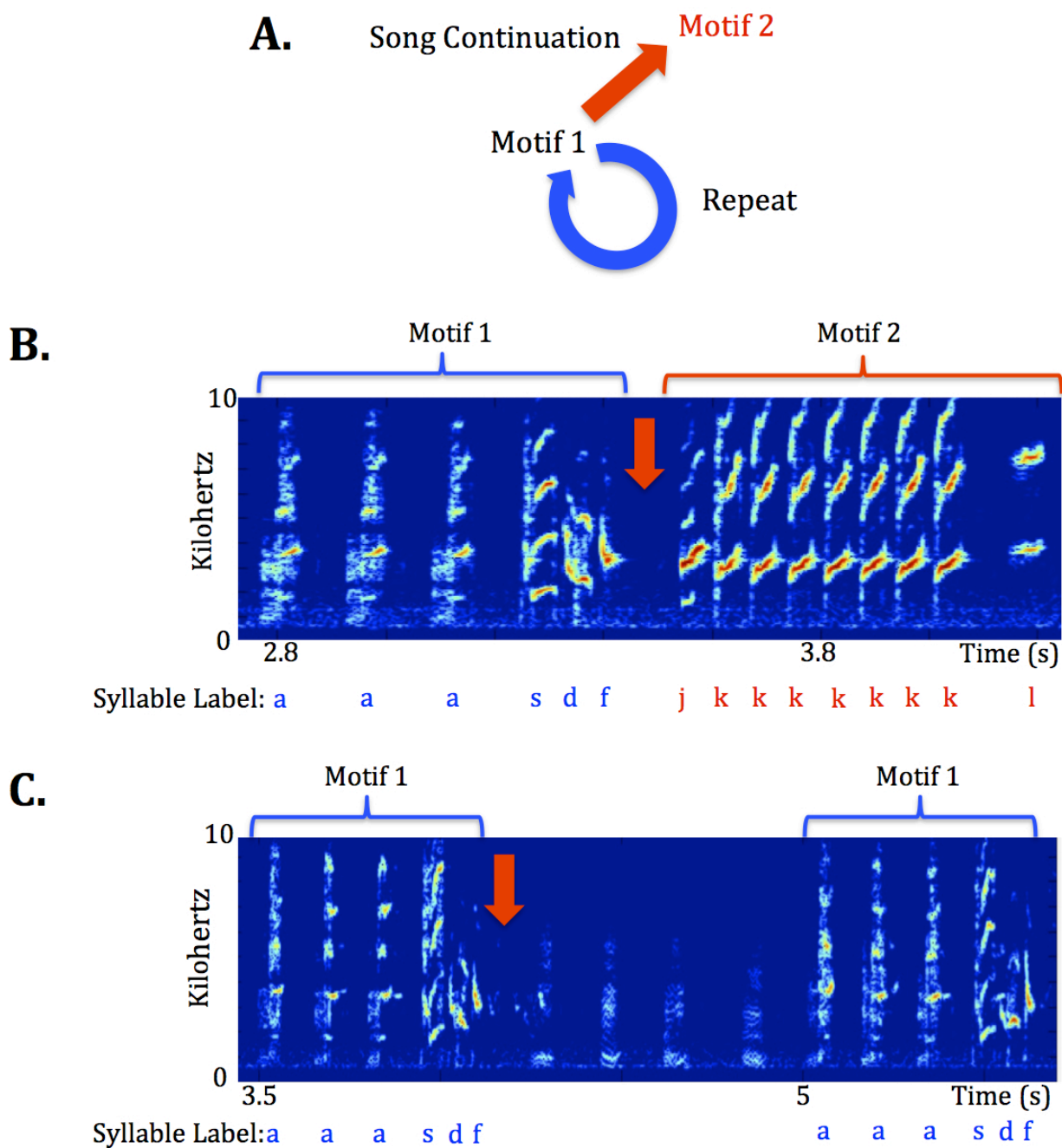
Figure 4: Diagrams of two possible transitions after bird 2 sang motif 1. A. Motif 1 could be followed by either motif 2 (song completion) or motif 1 (repeat). During baseline recording, the bird was more likely to proceed to motif 2 than to repeat motif 1. **B.** A song spectrogram of the transition from motif 1 to motif 2. The red bracket denotes motif 1 and the blue bracket denotes motif 2. **C.** A song spectrogram of a motif 1 repeat. The red arrows in B and C denote the song branch point.

Under normal auditory feedback (baseline) the bird would typically follow motif 1 with motif 2. Infrequently, the bird would instead sing motif 1 again. Beginning on the first day that the 175 millisecond delay perturbation was applied, the probability that the bird would follow motif 1 with motif 2 was significantly less than in the baseline data according to a z-test for proportions with a Bonferroni correction (Figure 5A). In other words, the probability that the bird would repeat motif 1 was significantly increased. Over time, the probability that the bird would repeat motif 1 gradually decreased, and was not significant higher than baseline on days 7-9 of delayed feedback. The probability of motif 1 repetition probability was not significantly different from baseline throughout the rest of the delayed feedback application.

Delayed auditory feedback increased not only the probability of repeating motif 1, but also the amount of time the bird waited to begin singing the second part of his song after singing motif 1. Beginning on the first day of 175 millisecond delayed feedback, there was a significant increase in the duration of the gap between syllables 'j' and 'f' (Figure 5B). This increase in gap duration was significant during the entire time that delayed auditory feedback was applied. During the washout period, when no delayed feedback was applied, there was no significant increase in gap duration.
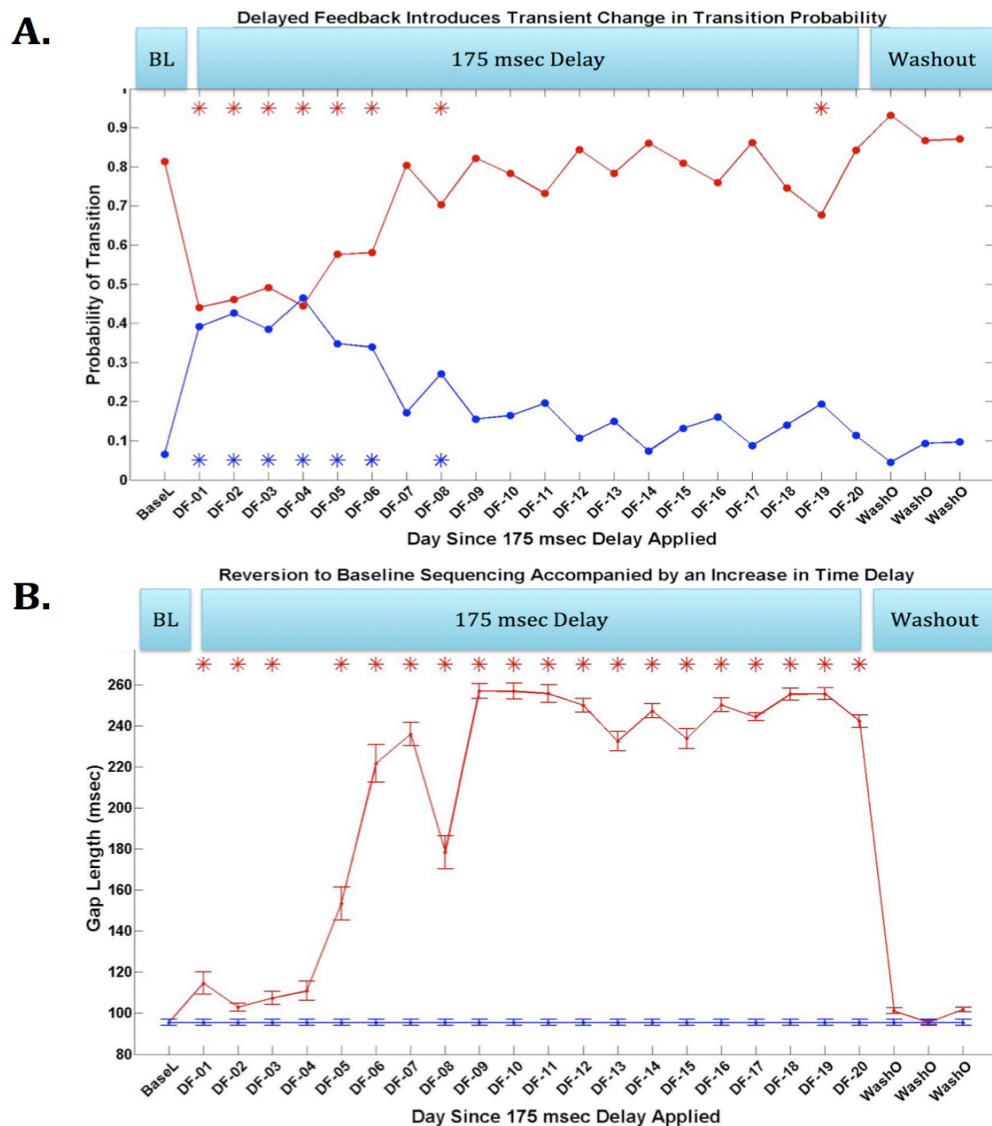
Figure 5: With delayed feedback, the probability of repeating a motif increases; when accompanied by a syllable gap increase, the probability of repeating a motif decreases for bird 2 (song shown in Figure 4). On both graphs, the experimental epochs (light blue) above the graph denote the experimental perturbation (BL = baseline, 175 msec Delay = delayed feedback, Washout = normal feedback.) **A.** The red dots denote the probability of song completion (motif 1→motif 2), and the blue dots indicate the probability of repeating motif 1 (motif 1→motif 1). The red and blue asterisks denote a significant difference between the probability of song completion and repeating motif 1, respectively. The probability of repeating motif 1 on the first 6 days of delayed feedback returned back to baseline over time. **B.** The blue line represents baseline data collected during three days of baseline recording, and the red line represents the mean duration of the gap between syllables 'f' and 'j' over the course of the experiment. The asterisks denote a significant change in the mean syllable gap duration on an individual day of recording. The gap duration increased dramatically when the probability of repeating motif 1 returned to baseline.

The significant increase in syllable gap shown in Figure 5B may explain the observed decrease in motif 1 repetition shown in Figure 5A. The bird's song consisted of two motifs; the first motif ended with syllable 'f', and the second motif began with syllable 'j'. As the average time that he delayed singing syllable 'j' increased over the first 6 days, the delayed feedback was allowed to expire. After the 8th day of 175 millisecond delayed feedback, the syllable gap was large enough that he received no delayed feedback when he began to sing the second motif of his song. His reversion to baseline probability of repeating motif 1 was likely due to waiting for the delayed feedback to end.

Changes Induced by Delayed Auditory Feedback may be reversed by a Pitch Shift

Birds 3 and 4 were used to test whether a pitch shift would reverse the increase in motif repetition induced by delayed auditory feedback. First, we collected baseline under conditions of 0 millisecond delay and 0 cent pitchshift. For bird 3, auditory manipulations began with 9 days of continuous 175 millisecond delay, followed by 8 days of continuous 175 millisecond delay with a positive pitch shift. The bird then experienced a 0 millisecond delay and 0 cent pitch shift washout period. In bird 3's song, there were two distinct motifs. The sequence of syllables 's' 'd' 'd' 'f' was designated motif 1, and the sequence of syllables 's' 'j' 'k' was designated motif 2. After singing motif 1, bird 3 could either repeat motif 1 (Figure 6B) or transition to motif 2 (Figure 6C). Syllable 'b' commonly appeared before the repetition of motif 1 (Figure 6B), but was sometimes replaced with syllable 's'.
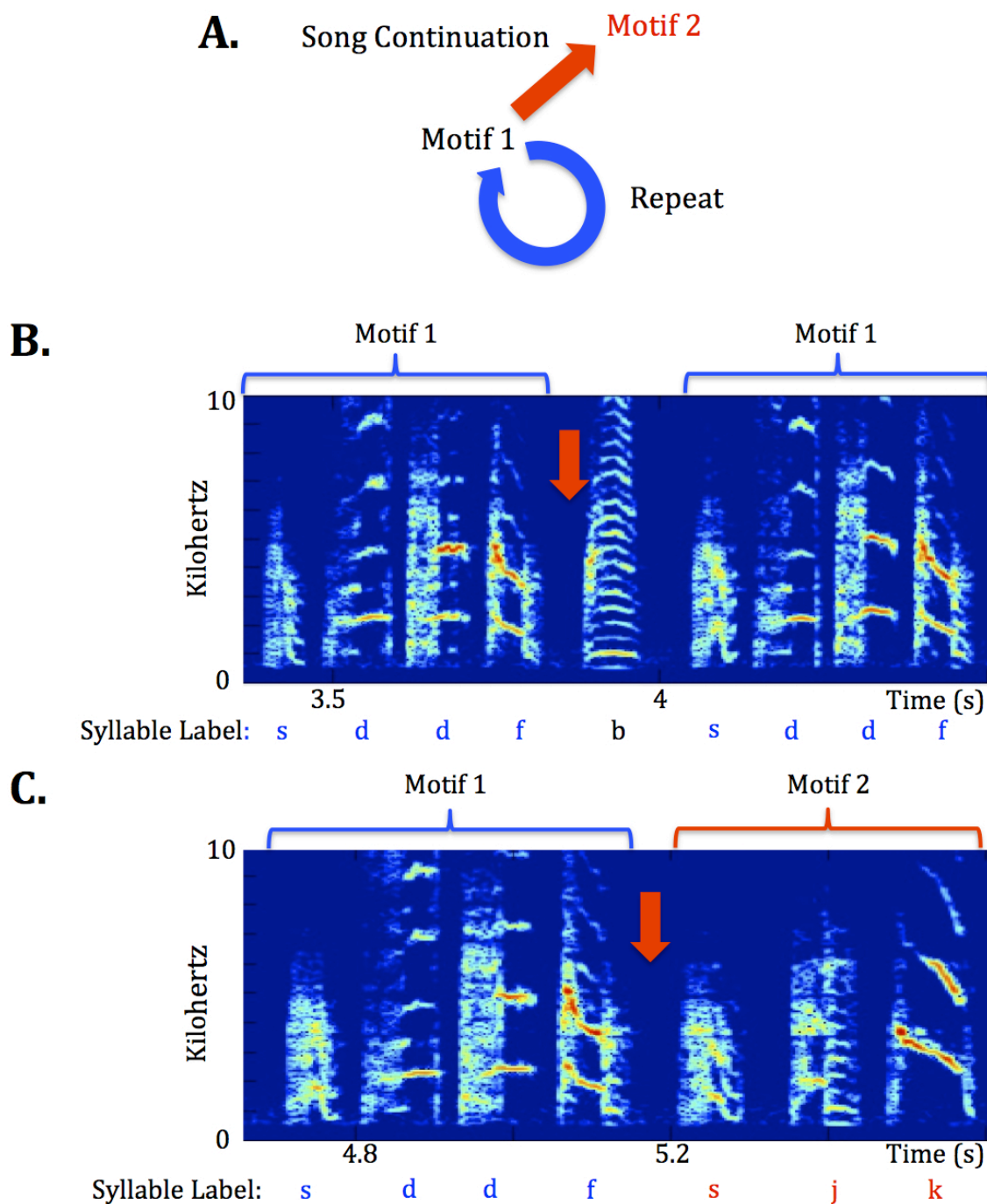
Figure 6: Depictions of two possible transitions from motif 1 for Bird 3. A. The diagram shows that motif 1 could be followed either by motif 2 (song continuation) (motif 1→motif 2) or by motif 1 (repeat) (motif 1→motif 1). During baseline recording, the probability of transitioning to motif 2 was higher. **B.** A song spectrogram depicting a repeat of motif 1. **C.** A song spectrogram depicting song continuation, where motif 1 is followed by motif 2. The red arrows in B and C denote the branch point.

During baseline recording, the more likely transition was from motif 1 to motif 2 (Figure 7). The less likely transition was a repeat of motif 1 without singing motif 2 in between. Despite a compelling trend of motif repetition on increase during the delay recording, the probability of repeating motif 1 did not change on any individual day. Although the probability of repeating motif 1 seemed to increase during delayed feedback and return to baseline during delayed feedback with pitch shift, none of these changes were significant. Our inability to detect an effect may have been due to the low numbers of song during the experiment.
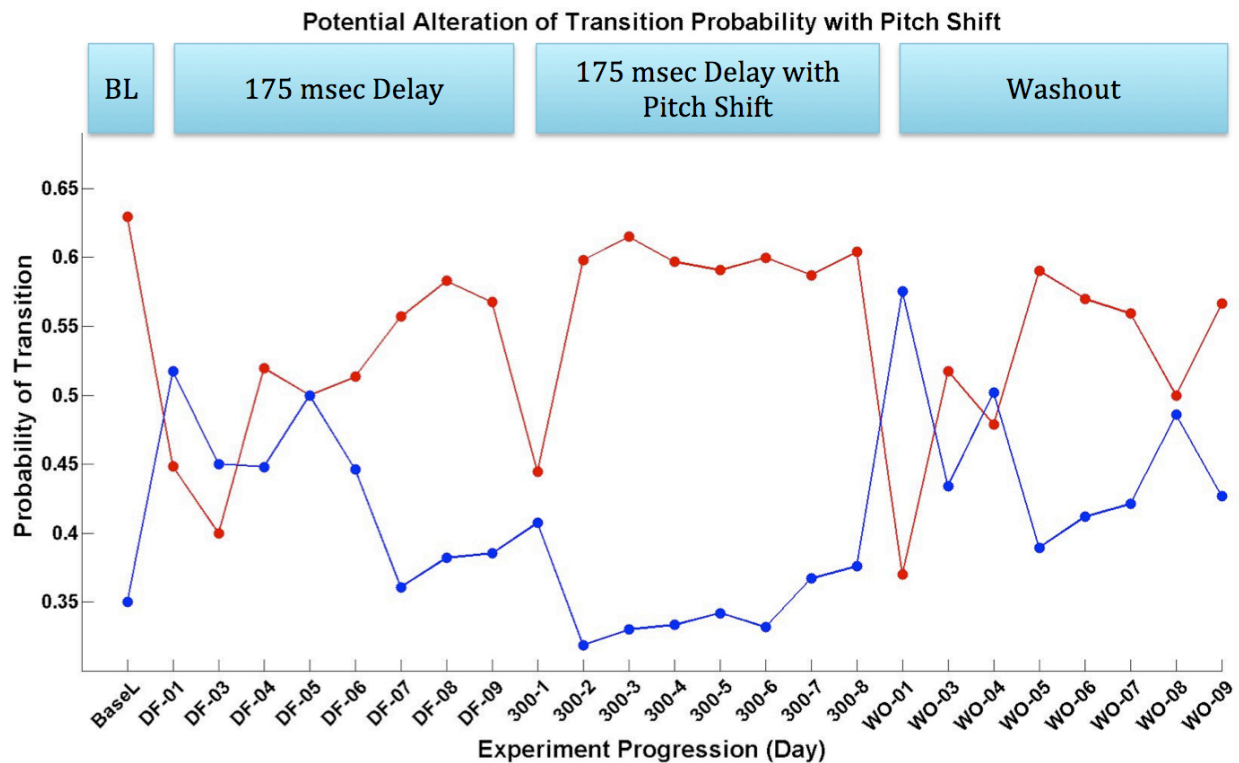
Figure 7: For bird 3 (song shown in Figure 6), the probability of repeating motif 1 appeared to increase with delayed feedback, and appeared to decrease during delayed feedback with applied pitch shift. The experimental epochs (light blue) above the graph denote the experimental perturbation (BL = baseline, 175 msec Delay = delayed feedback, 175 msec Delay with Pitch Shift = delayed feedback with pitch shift, Washout = normal feedback.) The red dots denote a song continuation (motif 1→motif 2), while the blue dots denote a motif 1 repeat (motif 1→motif 1). There were no significant effects of either manipulation.

Bird 4 was used to test whether a pitch shift could alter the impact of delayed auditory feedback on song. His song contained a branch point with 3 possible transitions from the leading motif (Figure 8A). The first motif was denoted by syllable 'j' followed by syllable 'k' (Figure 8B). The second motif was denoted by syllable 'a' followed by syllable 's', and was followed by a variable number of 's', 'd', 'a', and 'f' notes (Figure 8C). The third motif was denoted by syllables 'j' 'x' 'x' (Figure 8D). Motif 3 was not noticeably present during baseline recording or in the presence of delayed feedback, but appeared during the application of a pitch shift. First, baseline data with 0 millisecond delay and a 0 cent pitchshift were collected. Auditory manipulations began with 8 days of continuous 175 millisecond delay, followed by 8 days of continuous 175 millisecond delay with an additional negative pitch shift application. The bird then experienced a 0 millisecond delay and 0 cent pitch shift washout period.
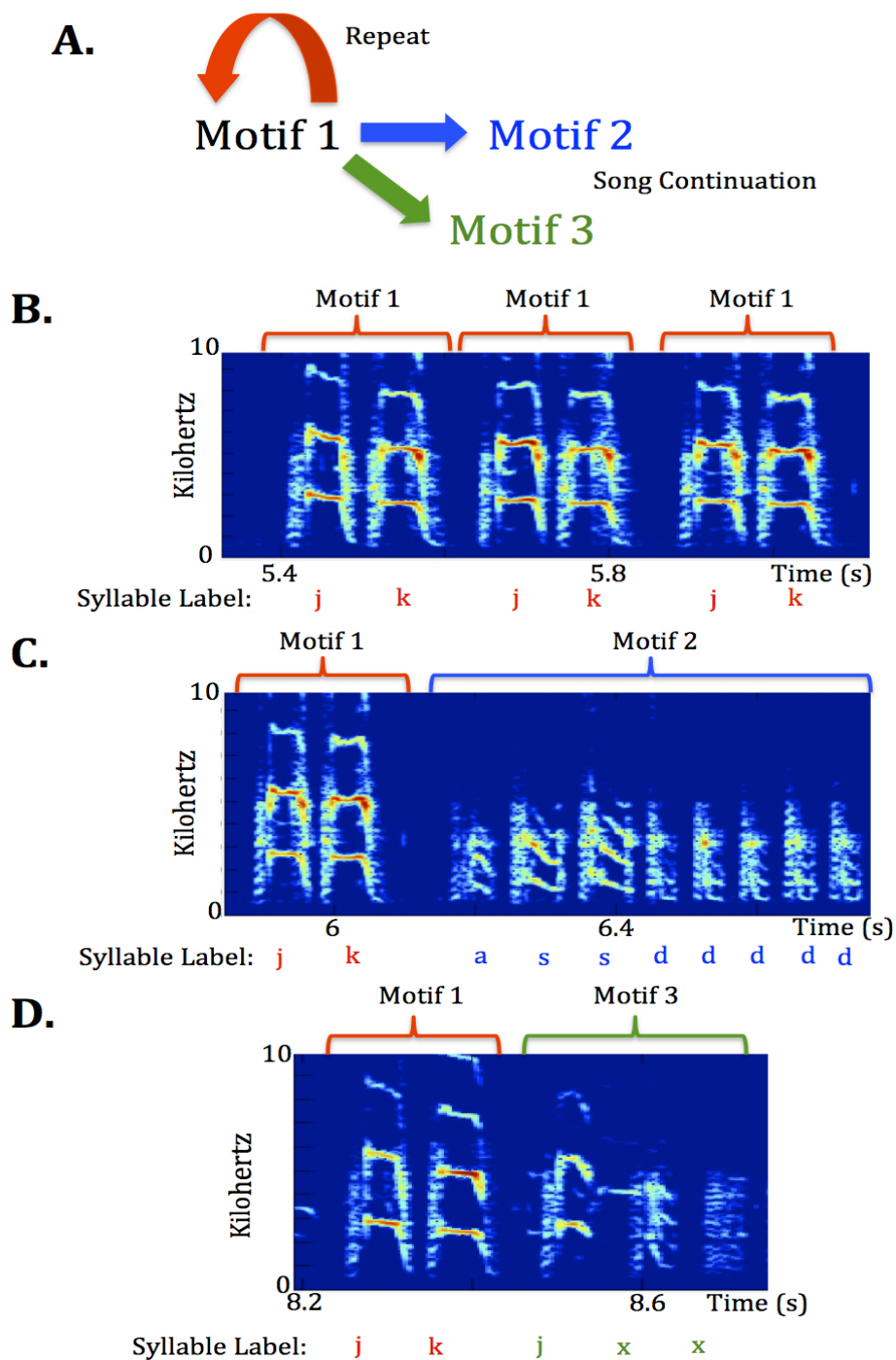
Figure 8: Depictions of three possible transitions from motif 1 for bird 4. A. The diagram shows that motif 1 could be followed either by a repeat of motif 1 (motif 1→motif 1), or by a continuation of the song with one of two different motifs (motif 1→motif 2), (motif 1→motif 3). **B.** A song spectrogram depicting a repeat of motif 1. **C.** A song spectrogram depicting song continuation, where motif 1 was followed by motif 2. **D.** A song spectrogram depicting song continuation, where motif 1 was followed by motif 3.

During baseline recordings, a repeat of motif 1 was the dominant transition (Figure 9). The probability of transition from motif 1 to motif 2 was almost as probable as repeating motif 1. The probability of transition to motif 3 was near 0. In the presence of a 175 millisecond delay, the probability of repeating motif 1 increased significantly. When the pitch shift was introduced in addition to the delayed feedback, the bird did not repeat motif 1 significantly more than during baseline recording. There was a significant increase in transitioning to motif 3 during the first few days of delay with pitch shift, and the bird sang this pattern significantly more than during baseline recording on day seven of the pitch shift.
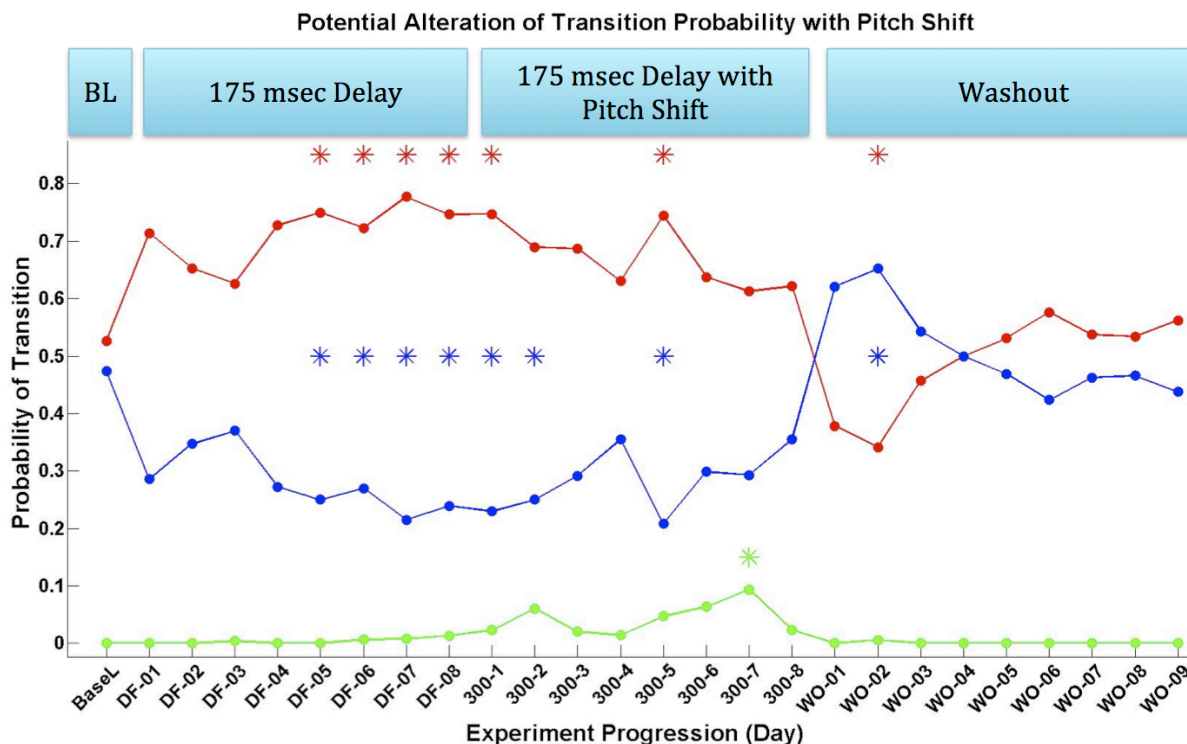
Figure 9: For bird 4 (song shown in Figure 8), the probability of repeating motif 1 increased significantly during delayed auditory feedback and appeared to decrease during the application of a negative pitch shift. The experimental epochs (light blue) above the graph denote the experimental perturbation (BL = baseline, 175 msec Delay = delayed feedback, 175 msec Delay with Pitch Shift = delayed feedback with pitch shift, Washout = normal feedback.) The red points denote the probability of a repeat of motif 1 (motif 1→motif 1), the blue points denote the probability of transition from motif 1 to motif 2 (motif 1→motif 2), and the green points denote the transition probability from motif 1 to motif 3 (motif 1→motif 3). The red asterisks, blue asterisks, and green asterisks mark days during which the repeat of motif 1, transition from motif 1 to motif 2, and transition from motif 1 to motif 3, respectively, were significantly more likely than during baseline recording according to the z-test for proportions with a Bonferroni correction.

The data from bird 2 and bird 4 show that delayed auditory feedback can increase the probability of motif repetition (Figure 5A and Figure 9). This result demonstrates the importance of auditory feedback for song sequencing; without accurate feedback, the probabilities of motif repetition can increase. Further, the application of a pitch shift in addition to delayed feedback (Figure 9) may have the potential to alleviate the repeats induced by the delayed feedback, as discussed below.

Discussion

The goals of this study were to test the effects of delayed auditory feedback, and delayed auditory feedback with a large pitch shift, on birdsong. We predicted that there would be an increased amount of motif repetition with delayed feedback, and that the increased probability of motif repetition would decrease when a large pitch shift was applied concurrently with delayed feedback. The results demonstrate that delayed auditory feedback can alter syllable transition probabilities at branch points (Figure 3). Birds repeated motifs more frequently when feedback was delayed (Figure 5A); Figure 9, "175 msec Delay"). This motif repetition can be likened to the tendency of human stutterers to spontaneously repeat a syllable while they speak. In addition, we found that delayed auditory feedback induced repetition of motifs was significantly lessened by the application of a pitch shift. Overall, our data showed that feedback timing is important for maintaining stereotyped song sequencing, and that song playback with a large pitch shift may reduce the reliance on feedback, thus reducing the detrimental impact of the inaccurate feedback.

After we tested different amounts of delay, our findings indicate that there may be a range of delay that elicits changes in song sequencing (Figure 3). The range of 25 to 175 milliseconds of delay give rise to changes in transition probabilities at a branch point. The bird 1 experiment served as a pilot to determine whether delayed feedback induced sequencing changes, and delay parameters were only tested for only 5 to 10 hours. Since the delays were presented for relatively short periods, the results may have been affected by the order in which the delay parameters were presented. There was a compelling trend with 175 milliseconds of delay, but the trend was not statistically significant in the pilot experiment. In subsequent experiments, this delay parameter caused significant changes in sequencing. Further testing is therefore necessary to determine precisely the range of delay that reliably causes sequencing changes.

An increased repetition of syllable motifs, similar to the tendency to repeat syllables in human stuttering, was observed when delayed auditory feedback was applied. When a delay was applied for twenty days (bird 2), the probability of repetition increased on the first days of recording (Figure 5A). After day 6, the probability of was not significantly higher than during baseline recording. At the same time that he appeared to be repeating motif 1 more frequently, the duration of the gap between motif 1 and motif 2 increased (Figure 5B). By day 6 of testing, the average gap between these motifs had doubled. Ranging between 220 and 260 milliseconds, this gap length was longer than the applied feedback delay. By the time the bird started singing motif 2 after he sang motif 1, he was therefore not receiving delayed feedback through the headphones. In waiting to sing the second part of the song, the bird may have been able to reduce the probability that he would repeat motif 1. As is sometimes recommended to stutterers to reduce syllable repetitions, speaking more slowly may help alleviate the number of repeat disfluencies in speech. We speculate that this bird experienced a similar effect; by singing more slowly, he was less likely to repeat himself.

Our results suggest that a large pitch shift may reduce the number of motif repetitions induced by delayed auditory feedback. Bird 4 was more likely to repeat motif 1 with delayed auditory feedback alone (Figure 9, "175 msec Delay) than when both delay and pitch shift were applied (Figure 9, "175 msec Delay with Pitch Shift"). Since this finding was observed in only one of two birds that received the pitch shift perturbation, further investigation is necessary to confirm this effect. A positive pitch shift applied to bird 3 did not induce motif repetition (Figure 7). In this case, our inability to detect an effect may have been due to the small amount of song this bird produced. Nevertheless, the significant effects of the negative shift show that pitch shift may reduce involuntary vocal repetitions (Figure 9).

For confirmation of all of the results reported in this study, analyses will continue on the collected data and further experimentation will be conducted. The average syllable gap over the course

of the entire negative pitch shift experiment (bird 4) needs to be measured. A potential explanation for the lack change in the amount of motif 1 repetition with pitch shift application when compared to baseline (Figure 9, "175 msec Delay with Pitch Shift") may be that the gap between motif 1 and subsequent motifs was increasing. This effect was observed in the experiment with delay application for twenty days (bird 2). Additionally, we would like to examine all of the gap durations over the course of the delayed feedback and delayed feedback with pitch shift experiments. This gap duration increase with delay may be limited to gaps between motifs, which may provoke additional questions about song sequencing and motif organization. In regard to further experimentation, a larger sample size of birds must be tested with delayed auditory feedback and delayed auditory feedback with pitchshift. First, the range of delay parameters that elicits sequence changes needs to be confirmed. These parameters should be tested for a few days to ensure that variation observed is neither a transient change nor a change observed by coincidence. Secondly, more birds must be tested with a pitch shift after observing changes in their song structures induced by delayed feedback. This is essential for determining the true effects of this manipulation on song sequencing. Another possible way of testing the effects of pitch shift would be to introduce pitch shifts with delayed feedback simultaneously, immediately after the bird receives no auditory feedback perturbation. We predict that the probability of repeating a motif would not be significantly different in the delayed feedback with pitch shift in comparison to normal feedback baseline recordings.

This study served to examine the effect of delayed auditory feedback on vocal sequencing in birdsong. This auditory perturbation may be similar to a part of the human stuttering mechanism. The increased probability of motif repetition seen in this study is similar to the syllable repeats that characterize human stuttering. Observing increases in repetition after the application of delayed feedback in songbirds suggests that delayed feedback alone may induce an increased tendency to repeat an utterance. Furthermore, the results of the experiment in which bird 2 was able to reduce his

tendency to repeat motif 1 by waiting for the delay to expire resembles how people who stutter can reduce speech disfluencies by speaking more slowly (Figure 5). In behavioral speech therapy, a stutterer may be taught to slow down while speaking in an attempt to reduce spontaneous syllable repetitions.

In conclusion, these results demonstrate that delayed auditory feedback can cause changes to the structure of crystalized Bengalese finch song. Delayed auditory feedback increased the probability of motif repetition, which can be compared to repetition behavior observed in human stuttering. Therefore, songbirds could potentially be used to model the human stuttering condition. Given that the mechanism that causes stuttering is still unknown, establishing an animal model to replicate this speech irregularity would useful for determining the mechanisms underlying stuttering and developing possible remedies for vocal abnormalities.

References

Ambrose NG, Yairi E (1999) Normative disfluency data for early childhood stuttering. Journal of Speech

and Language and Hearing Research 24: 895-909.

Andrews G, Harris M (1964) The syndrome of stuttering.

Brainard M, Doupe A (2002) What songbirds teach us about learning. Nature 417: 351-358.

Bloodstein O (1995) A Handbook on Stuttering. Singular Group, San Diego.

Bloodstein O, Grossman M (1981) Early stutterings, some aspects of their form and distribution. Journal

of Speech, Language, and Hearing Research 24: 298-302.

Boberg E, Kully D (1994) Long-term results of an intensive treatment program for adults and adolescents

who stutter. Journal of Speech, Language, and Hearing Research 37: 1050-1059.

Braun A, Varga M, Stager S, Schulz G, Selbie S, Maisog J, Carson R, Ludlow C (1997) Altered patterns of

cerebral activity during speech and language production in developmental stuttering. An

H2(15)O positron emission tomography study. Brain 120: 761-784.

Bryngelson B (1938) Prognosis of stuttering. Journal of Speech Disorders 3: 121-123.

Burnett T, Freedland M, Larson C, Hain T (1998) Voice F0 responses to manipulations in pitch feedback.

The Journal of the Acoustical Society of America 103: 3153-3161.

Chon H, Kraft S, Zhang J, Loucks T, Ambrose N (2012) Individual variability in delayed auditory feedback

effect on speech fluency and rate in normally fluent adults. Journal of Speech, Language, and

Hearing Research 56: 489-504.

Code C (2008) Delayed auditory feedback. Instrumental Clinical Phonetics 228.

Conture E, McCall G, Brewer D (1977) Laryngeal behavior during stuttering. Journal of Speech, Language, and Hearing Research. 20: 661-668.

Cowie R, Douglas-Cowie E (1992) Postlingually acquired deafness: speech deterioration and the wider consequences. Walter de Gruyter 62.

Craig A, Blumgard E, Tran Y (2009) The impact of stuttering on the quality of life in adults who stutter. Journal of Fluency Disorders 34: 61-71.

Cynx J, von Rad U (2001) Immediate and transitory effects of delayed auditory feedback on bird song production. Animal Behavior 62: 305-312.

Doupe A, Kuhl P (1999) Birdsong and human speech: common themes and mechanisms. Annual Review of Neuroscience 22: 567-631.

Fairbanks G (1955) Selective vocal effects of delayed auditory feedback. Journal of Speech and Hearing Disorders.

Freeman F, Ushijima T (1978) Laryngeal muscle activity during stuttering. Journal of Speech, Language, and Hearing Research 21: 538-562.

Heaton J, Dooling R, Farabaugh S (2010) Effects of deafening on the calls and warble songs of adult budgerigars (Melopsittacus undalatus.) The Journal of the Acoustical Society of America 105: 2010-2019.

Herder C, Howard C, Nye C, Vanryckeghem (2006) Effectiveness of behavioral stuttering treatment: a systematic review and meta-analysis. Contemporary Issues in Communication Science and Disorders 33: 61-73.

Hoffmann LA, Kelly CW, Nicholson DA, Sober SJ (2012). A lightweight, headphones-based system for manipulating auditory feedback in songbirds. Journal of Visualized Experiments 69.

Houde J, Jordan M (1998) Sensorimotor adaptation in speech production. Science 279: 1213-1216.

Kelly E, Martin J, Baker K, Rivera N, Bishop J, Krizizke C, Stettler D, Stealy J (1997) Academic and clinical preparation and practices of school speech-language pathologists with people who stutter. Language, Speech, and Hearing Services in Schools 28: 195-212.

Kuhl P (1995) Learning and representation in speech and language. Current Opinion in Neurobiology 4: 812-822.

Marler P (1970) A comparative approach to vocal learning: song development in white-crowned sparrows. Journal of Comparative and Physiological Psychology 71: 1.

Marler P, Tamura M (1962) Song "dialects" in three populations of white-crowned sparrow. Condor 64: 368-377

Natke U, Grosser J, Kalveram K (2001) Fluency, fundamental frequency, and speech rate under frequency-shifted auditory feedback in stuttering and nonstuttering persons. Journal of Fluency Disorders 26: 227-241.

Nordeen K, Nordeen E (2004) Auditory feedback is necessary for the maintenance of stereotyped song in adult zebra finches. Behavioral and Neural Biology 57: 58-66.

Okanoya K (2006) The bengalese finch: a window on the behavioral neurobiology of birdsong syntax. Annals of the New York Academy of Sciences 1016: 724-735.

Okanoya K, Yamaguchi A (1997) Adult bengalese finches (Lochurna striata var. domestica) require real-time auditory feedback to produce normal song syntax. Journal of Neurobiology 33: 343-356.

Pettito L (1993) On the ontogenetic requirements for early language acquisition (pp. 365-383). Springer

     Netherlands.

Sakata J, Hampton C, Brainard M (2008) Social modulation of sequence and syllable variability in adult

     birdsong. Journal of Neurophysiology 99: 1700-1711.

Slater P (1986) The cultural transmission of birdsong. Trends in Ecology and Evolution. 1: 94-97.

Sober S, Brainard M (2009) Adult birdsong is actively maintained by error correction. Nature

     Neuroscience 12: 927-931.

Sober S, Brainard M (2012) Vocal learning is constrained by the statistics of sensorimotor experience.

     Proceedings of the National Academy of Sciences 109: 21099-21103.

Sober S, Wohlgemuth M, Brainard M (2008) Central contributions to acoustic variation in birdsong.

     Journal of Neuroscience 28: 10370-10379.

Stein M, Baird A, Walker J (1996) Social phobia in adults with stuttering. The American Journal of

     Psychiatry 153: 278-280.

Stokoe W (1980) Sign language structure. Annual Review of Anthropology 9: 365-390.

Thorpe W (1961) Bird-song: the biology of vocal communication and expression in birds.

Wada H (2012) The development of birdsong. Nature Education Knowledge 3: 86.

Waldstein R (1990) Effects of postlingual deafness on speech production: implications for the role of

     auditory feedback. The Journal of the Acoustical Society of America 88: 2099-2114.

Watkins K, Smith S, Davis S, Howell P (2008) Structural and functional abnormalities of the motor system

     in developmental stuttering. Brain 131: 50-59.

Yairi E, Ambrose N (1999) Early childhood stuttering I: persistency and recovery rates. Journal of Speech,

Language, and Hearing Research 42: 1097-1112.

Yates A (1963) Delayed auditory feedback. Psychological Bulletin 60: 213.