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MacKenzie Wyatt April 15, 2016

Pitch Shift Partially Restores Song Sequencing Changes Induced by Delayed Auditory Feedback in Bengalese Finches

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An abstract of
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Abstract

Pitch Shift Partially Restores Song Sequencing Changes Induced by Delayed Auditory Feedback in Bengalese Finches

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Songbirds learn their vocalizations in a similar way that humans learn how to speak. Auditory feedback plays an important role in bird's maintenance of its stable rendition of song (crystallized song) and human speech. This feedback gives the bird important information to correct for errors in the song. The timing and pitch of auditory feedback influence how birds correct for these errors. However, large changes in pitch constrain error correction. Characteristics of auditory feedback also affect human speech. Because delayed auditory feedback induces repetitive vocal behavior in fluent speakers, one model proposes that delayed auditory processing underlies one mechanism of stuttering. A large pitch shift can partially restore normal speech to dysfluent speakers. Despite the connection to timing and pitch of auditory feedback in repetitive motor behavior, no study has investigated the influence of repetitive song aspects in songbirds. We hypothesized that delayed auditory feedback would induce song sequencing changes and that a concurrent large pitch shift would partially ameliorate these induced changes by delayed auditory findings. Consistent with our hypothesis, we found that delayed auditory feedback induces robust changes in repeated syllable distributions and transition probabilities at divergent branchpoints. A large pitch shift significantly reduced induced changes by delayed auditory feedback. Although not significant, a large pitch shift concurrent with delayed auditory feedback appeared to induce smaller magnitude of changes compared to delayed auditory feedback. These findings together support our hypothesis and fill a gap in the literature by examining the combination of auditory feedback characteristics on birdsong sequencing.

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Introduction

Although many animal species vocalize, few learn their vocal behavior like humans do. While humans are predisposed to speech, acquiring this complex vocal behavior also requires hearing adults and practicing the vocalizations (Doupe and Kuhl 1999). Bats, dolphins, some elephant species, and songbirds do not have purely innate vocalizations, and learn them from older animals of the same species (Marler 1970). Because songbirds have an isolated brain area specifically for singing, they serve as a good model for human speech and help us see how the brain corrects errors to perform complex motor behaviors like singing (Brainard and Doupe 2002). This study examines the effect of sensory feedback on motor behavior specifically to auditory feedback (how the birds hear their song) on vocal output.

This research study involves Bengalese finches (*Lonchura striata* var. *domestica*). The male birds of this species learn songs from a male tutor (Brainard and Doupe 2002). While they are young, these birds learn their songs. This time frame is a critical period where the birds are most sensitive to learning and are most sensitive to exposure of their tutor's song (Bolhuis et al. 2010). They attempt to copy the syllables of their tutor's songs through a trial-and-error learning experience. By 90 days post-hatch, their song becomes stable in a crystallized form with little variation in sequence, tempo, or pitch (Konishi 2004). This stable rendition of the bird's song is known as the crystallized song.

Songs consist of individual notes known as syllables which are separated by $\geq$5 ms of silence (Okanoya and Yamaguchi 1997; James and Sakata 2014). Examples of a bird song and the terms are shown in a spectrogram (Figure 1a). A syllable can follow itself, which is known as a repeated syllable (Figure 1b). Syllables have typical patterns, which comprise motifs. These

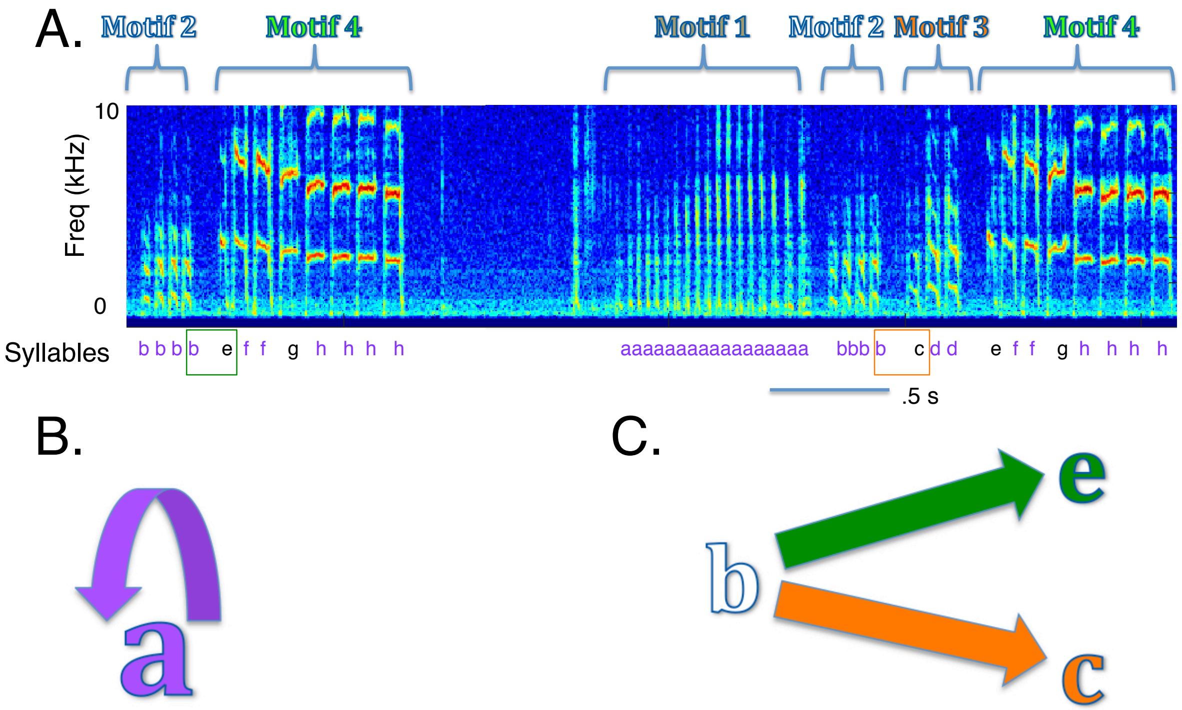


Figure 1. Description of bird song terms. **a.** Bird spectrogram. A bird's song is comprised of individual notes called "syllables." These notes are grouped in a stereotypical fashion in units called "motifs." Motifs are separated by >5 ms. Repeated syllables are highlighted in purple. Branchpoints are boxed in green and orange. **b.** Repeated Syllables Description. A repeated syllable is a syllable that is followed by itself one or more time. **c.** Branchpoint Description. Branchpoints are syllables in a song that can be followed by two or more different syllables. One branchpoint in this spectrogram is labeled with green and orange boxes where syllable "b" can be followed by syllable "e" or "c" respectively. When a syllable follows itself one or more times, it is known as repeated syllables. Repeated syllable labels are purple.

motifs typically follow an ordered sequence. When a syllable can be followed by more than one syllable, it is known as a branchpoint (Figure 1c), and these typically occur at the end of a motif (Okanoya 2004; Wada 2012).

Auditory stimuli of the tutor's song are a crucial element for vocal production. For example, isolated birds without access to a tutor develop abnormal songs with phonologically atypical notes (Morrison and Nottebohm 1993). Birds must hear the song of an adult male to model their songs after during their learning period. It was thought that once birds learned the crystallized song, the rendition remained the same without variation regardless of other factors. However, scientists later discovered that auditory feedback also plays a role in song maintenance. Auditory feedback is the signal that birds receive when they hear their songs in real time as they sing. Deafened birds lack auditory feedback because they cannot process auditory stimuli. Prior literature has shown that deafened birds cannot maintain crystallized songs, which demonstrated that sensory feedback is important to vocal production (Lombardino and Nottebohm 2000; Okanoya and Yamaguchi 1997; Wooley and Rubel 1997; Nordeen and Nordeen 1992).

Human speech parallels birdsong because they are both acquired and influenced by auditory stimuli (Brainard and Doupe 2002; Marler 1970). Feral children who grow up in isolation have irregular language development without normal vocalizations (Bettelheim 1959). Even when exposed to speech later in life, these children have difficulties with speech production due to the presence of a critical period like songbirds. Both humans and birds must be exposed to vocalizations from a tutor in order to develop speech and song normally (Newport et al. 2001). Like birdsong, the absence of auditory feedback also causes humans to produce irregular speech. Deaf humans produce irregular speech, due to a lack of a reproducible template to emulate (McGarr 1983).

Researchers later determined that altered auditory feedback, not just the absence of auditory feedback, impairs the birds' crystallized song (Leonardo and Konishi 1999). By altering the timing of auditory feedback and altering the playback of certain song syllables, researchers demonstrated that delayed auditory feedback (DAF) changes song production in real-time (Sakata and Brainard 2006). After being subjected to DAF, birds' crystallized songs degrade in a variety of ways, including omissions and novel sounds (Cynx and von Rad 2001). Besides timing, pitch was also found to play a role in maintaining crystallized song within birds. Sober and Brainard (2009) showed that the brain compensates for perceived error by shifting pitch in the opposite direction. For example, if a bird's syllable is selectively manipulated in real time with a pitch lower than the expected auditory feedback, it will compensate by singing higher in order to minimize error. The converse also applies, so when a bird's syllable is pitch shifted to a higher note, the bird will correct this perceived error by singing a lower note.

Birds do not always change their song when their auditory feedback is altered. Error correction is constrained to auditory feedback that is similar to expected acoustic output. For example, for large pitch shifts of 300 cents (or 3 semitones), birds do not correct for the change (Sober and Brainard 2012). The overlap between expected and observed auditory feedback is minimal, and thus the birds do not correct for this behavior. Additionally, older birds that have a more stable and crystallized song generally correct less for pitch shifts than younger birds who have a wider variety of song production (Kelly and Sober 2014). The magnitude of the pitch shift induces varying levels of error correction in birds of different ages, demonstrating that birds account for some auditory feedback more than others.

The effects of timing and frequency on sensory feedback in humans have been studied as well. Delayed auditory feedback influences speech for fluent and non-fluent speakers. In fluent speakers, delayed auditory feedback causes speech perturbations and can alter timing and phoneme production (Fairbanks 1955). Delayed auditory feedback also causes an increase in repetition of consonants and words in people who produce speech normally (Chase 1958; Yates 1963). One model of stuttering proposes that stutterers have impaired auditory feedback processing, specifically in the properties of timing and rhythmic pacemaking (Büchel and Sommer 2004). Delayed auditory feedback is used to treat some dysfluent speakers for stuttering (Kalinowski et al. 1996; Ryan and Van Kirk 1974). Pitch shifted auditory feedback also impacts speech and causes an increase in speech fluency in some stutterers (Kalinowski et al. 1993; Natke et al. 2000).

The overlap between stuttering and delayed auditory feedback led us to investigate repetition and sequencing. Prior studies have examined how repeat distributions change with social context (Sakata and Brainard 2008) and in the absence of auditory feedback when birds are deafened (Wittenbach et al. 2015). However, no studies have examined the influence of delayed auditory feedback on repeat distributions specifically in songbirds, despite the implications of delayed auditory feedback on human behavior such as stuttering, both of which involve the repetition of motor sequences. Other studies have shown that the deafening of birds and humans induces more drastic changes that confound the findings of Wittenbach et al. (2015), whereas delayed auditory feedback has a subdued but pronounced effect (Brainard and

Doupe 2000; Konishi 2004). Sequence repetition occurs not only at the individual syllable level, but also with syllables in relation to one another. Syllables are ordered in typical sequences known as motifs, which follow a general order. Motifs can repeat as well, and thus we investigate the change of motif sequencing in this study. Transition probabilities in branchpoints develop with age and social context (James and Sakata 2015) and can be manipulated to change through experimental paradigms such as white noise experiments (Warren et al. 2012). Sakata and Brainard (2006) found that they were actively maintained in real-time and delayed auditory feedback induces changes in transition probabilities. Our study examines whether a large pitch shift could restore the normal transition probabilities and repeat syllable caused by the delayed auditory feedback.

It is important to examine the characteristics of auditory feedback that brains find salient when correcting for errors. Although the effects of alterations in timing and pitch on auditory feedback have been investigated in songbirds, a gap in the literature exists when considering both factors together. As stated previously, delayed auditory processing is one model for stuttering in humans. In humans, a large pitch shift has been shown to improve speech fluency in stuttering. Delayed auditory feedback could serve to model this scenario in songbirds. As has been demonstrated, alterations in auditory feedback do not always generate a behavioral modification. If the delayed auditory processing model is accurate, stutterers treated with frequency altered auditory feedback experience a combination of altered feedback in timing and pitch. Thus, it is important to examine the intersection of these two factors (timing and pitch) on auditory feedback.

We hypothesized that delayed auditory feedback will induce changes in song sequence, specifically to repeat distributions and transition probabilities. We also hypothesized that a large pitch shift of 300 cents would partially ameliorate the changes induced by delayed auditory feedback. Our study investigates the effects of delayed auditory feedback concurrent with a large pitch shift in an effort to observe whether characteristics of altered auditory feedback can generalize. By applying a large concurrent pitch shift with a delay, we predicted the birds will have less song sequence changes compared to only delay conditions, as the expected auditory feedback will overlap less with the observed feedback. Consistent with our hypothesis, we find that delayed auditory feedback induces robust song sequence changes at both the syllable and motif level. The changes in repeat syllables induced by delayed auditory feedback were reduced significantly reduced with a large pitch shift supporting our hypothesis. Although not significant, there was a trend that transition probabilities change more with delayed auditory feedback only compared to a delay concurrent with a pitch shift.

Materials and Methods

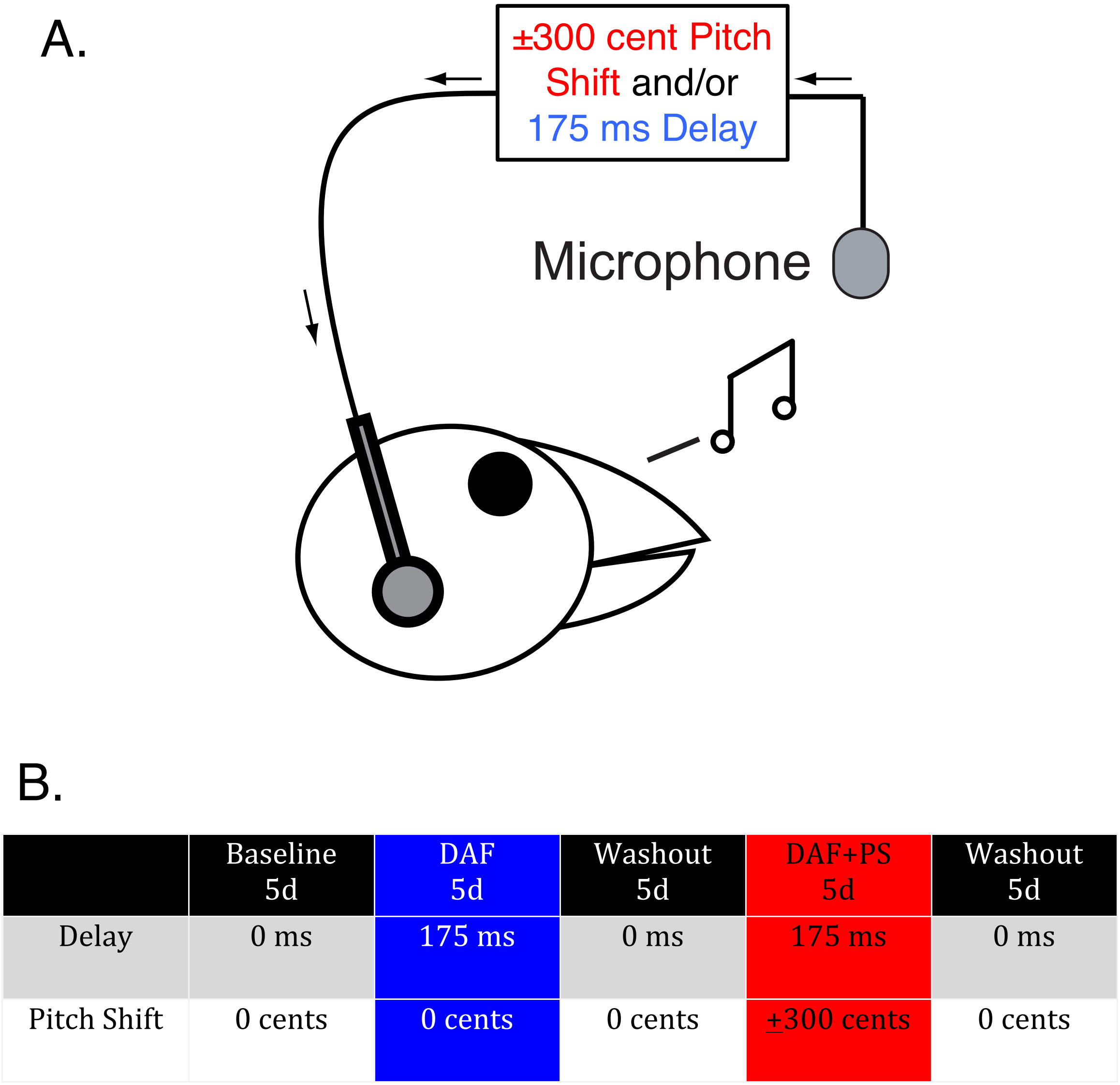
Subjects and maintenance.

Four adult male Bengalese finches (*Lonchura striata* var. *domestica*) were used as the experimental subjects. During the experiments, birds were housed individually in an isolated sound-attenuating chamber. The light/dark cycle was maintained for 14 h:10 h, with lights on beginning at 7 AM and ending at 9 PM. All procedures were approved by Emory University Institutional Animal Care and Use Committee.

Experimental procedure.

Online, real-time perturbations of the pitch and timing of auditory feedback were used. Miniature, lightweight headphones were custom-built and attached to each bird's head as previously described in the protocol by Hoffman et al. (2012). Sound processing hardware altered the auditory feedback, relaying the signal <10 ms to the birds via the headphone speakers during non-delayed conditions (Figure 2a). Pitch shifts were relative to the current vocal output. The headphones shielded the birds' airborne vocalizations, allowing the altered feedback to replace the natural version.

Once the birds habituated to the headphones, they were subjected to a series of alterations in auditory feedbacks (Figure 2b). Birds sang during a baseline period (5 d) with zero pitch shift or delay. After the baseline period, the birds' auditory output was altered with delayed auditory feedback ("DAF block") for five days. The birds heard their natural vocalizations at 175 ms delay relative to output. After the altered auditory feedback block, birds were subjected to a "washout" period (5 d) of singing with zero pitch shift or delay. The birds were then subjected to a delayed auditory feedback and pitch shift block ("DAF+PS"). During



	Baseline 5d	DAF 5d	Washout 5d	DAF+PS 5d	Washout 5d
Delay	0 ms	175 ms	0 ms	175 ms	0 ms
Pitch Shift	0 cents	0 cents	0 cents	±300 cents	0 cents

Figure 2. Technique for altering auditory feedback. **a.** The experimental paradigm. Song is collected and altered through a processor by either a delay or a delay and pitch shift. This altered auditory feedback is relayed in real time to birds while they sing. **b.** Schedule of experimental conditions. This is the experimental blocks presented to one bird during the experiment and shows how timing and pitch are altered. Blocks of conditions were presented in various orders to counterbalance learning effects. For example, in some instances DAF+PS was presented after Baseline, and DAF was presented after Washout.

the DAF+PS block, delayed feedback at 175 ms was concurrently pitch shifted ±300 cents (up or down three semitones). After this experimental condition, the birds underwent 5 d with zero shift or delay with the headphones apparatus in another “washout” period. The order of presentation was varied within birds to counterbalance for any learning order effects. For example, in some birds, the DAF+PS block immediately followed the baseline period, and DAF immediately followed the washout period. For all analysis, the “baseline” condition immediately preceded the experimental condition, so washout served as a baseline for each bird for either DAF or DAF+PS.

Measuring song syntax features.

Songs from 8 am to 12 pm were analyzed, with no more than sixty songs per day included in the analysis. Syllables were segmented using an amplitude threshold. Upon manual visual inspection of song spectograms, syllable labels were assigned with arbitrary letters (for examples a-j) based on their acoustic structure and sequential context. Overlapping letters between birds did not indicate similar features in notes.

Birds will repeat singing certain syllables a variable number of times. These are known as “repeats,” and examples are highlighted in purple (Figure 1a and Figure 1b). The number of times a syllable repeated in a single motif was measured. For example, a sequence could include syllable “dd” where syllable “d” is repeated twice. The number of times a syllable was sung in a single iteration was measured to produce repeat distributions. To determine whether repeat distributions were statistically significant from one another, a Kolmogorov-Smirnov test with a Bonferroni correction was used to compare repeat distributions from the altered auditory feedback conditions with the repeat distributions from the “baseline” period immediately preceding it.

In a divergent branchpoint, a single syllable can be followed by multiple different syllables, illustrated with the green and orange boxes (Figure 1a and Figure 1c). Branchpoints comprise a key part of syllable sequencing, and are actively maintained (Warren et al., 2012). The probability that a bird would transition to various syllables within a branchpoint was measured and reported as percentages. To test statistical significance, a z-test for proportions with a Bonferroni correction was used to compare probabilities from altered auditory feedback conditions to the baseline condition immediately preceding it.

Group Analysis.

The difference in repeat averages from the baseline to DAF or DAF+PS was calculated across all birds’ syllables. The Wilcoxon signed-rank test was used to determine whether the change from baseline to DAF was significantly different from the changes induced by the DAF+PS.

The difference in transition probabilities from the baseline to DAF or DAF+PS was calculated across all birds’ branchpoints. The Wilcoxon signed-rank test was used to determine whether the change from baseline to DAF was significantly different from the changes induced by the DAF+PS.

For group data, only the last three days of each condition was analyzed because sequence changes had a learning period. Washout periods didn’t always return to normal for a few days, so this accounts for the learning period. Lasting effects from the prior experimental condition was disregarded. Results weren’t altered significantly compared to analyzing all five days of the condition.

Results

Our findings confirmed that delayed auditory feedback induces robust changes in Bengalese finch song sequencing as found in prior studies. The divergent branchpoint transition probabilities changed significantly when auditory feedback was delayed as found in the prior study by Sakata and Brainard (2005). Additionally, the delayed auditory feedback induced significant changes in the repeat distributions, which had not been examined before. We tested the hypothesis that a large pitch shift would reduce the changes induced by delayed auditory feedback. We predicted that the DAF would induce larger changes than DAF+PS because it is more similar to bird's expected vocal output.

Syllable Repeats

Repeated syllables were also analyzed across birds. In bird 4, syllable "i" repeats (Figure 3a). In this particular spectrogram, syllable "i" repeats ten times. We measured the number of times syllable "i" was repeated during each condition. During baseline, the repeat distribution was centered on 8 with an average of 8.33 (Figure 3b). During the DAF condition, the repeat distribution shifts to the left and is centered around 7 with an average of 6.64. These distributions were significantly different (p-value<0.05, KS test with a Bonferonni correction). The baseline average for DAF+PS was 8.61 with the DAF+PS distribution shifting to the left with an average of 7.71. Although the baseline distribution was significantly different to the DAF+PS repeat distributions (p-value<0.05, KS test with a Bonferonni correction), the magnitude of the average syllable repeat change appears lower for the DAF+PS compared to DAF.

We analyzed repeat averages across birds (Figure 4). Figure 6a depicts the raw repeat averages in the bird's baseline condition compared to the DAF and the DAF+PS. The baseline

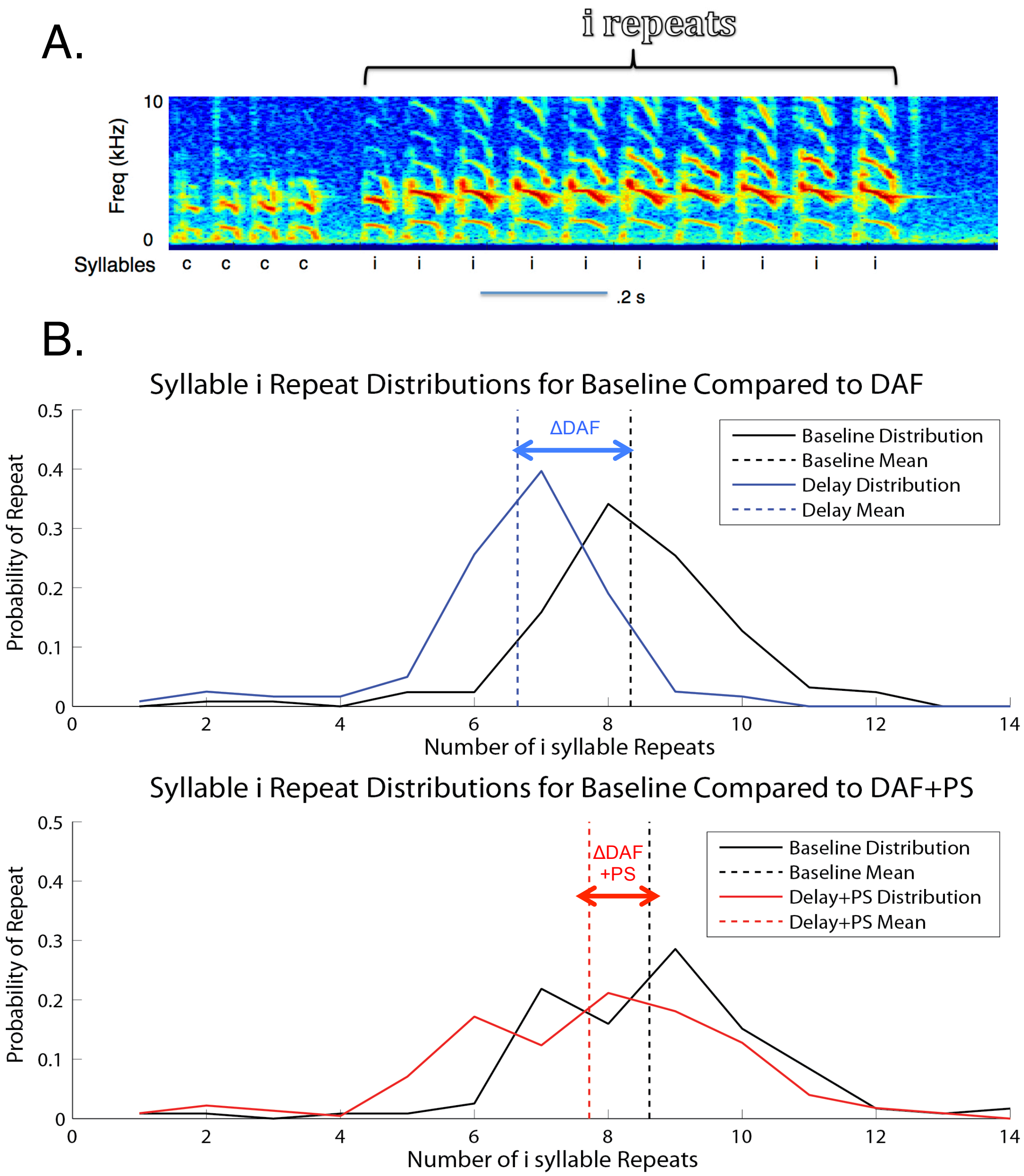


Figure 3 Example of pitch shift partially ameliorating DAF-Induced changes in repeat distributions in Bird 4. **a**. Description of bird's song. Syllable "i" is repeated a variable number of times in a single motif. We quantified the number of repeated "i" syllables within each experimental condition to create a repeat distribution. In this particular song sequence, "i" is repeated ten times. **b**. This compares the repeat syllable distribution of each baseline compared to DAF and DAF+PS, indicated by the blue and red lines respectively. The means are indicated by the dotted lines. Both repeat distributions in DAF and DAF+PS change significantly compared to baseline (KS test). During the DAF+PS condition, the induced changes (red double arrowed line) appear lower than the changes induced by DAF (blue double arrowed line.

condition is the 0 delay and 0 shift period immediately preceding each condition. For both DAF and DAF+PS, half of the birds' washout period is considered the baseline period. We compared the raw differences in transition probabilities for baseline to DAF versus baseline to DAF+PS (Figure 4b). Qualitatively, there is a greater spread for changes in transition probabilities induced by DAF compared to DAF+PS. We plotted the absolute magnitude of the repeat average change in DAF+PS versus DAF condition (Figure 4c). This change was calculated by finding the difference in repeat averages from baseline to DAF and DAF+PS. The magnitudes of the induced changes by DAF were significantly greater than the magnitudes of DAF+PS induced changes with a Wilcoxon Sign-Rank test (p-value<0.05, n=20).

Divergent Branchpoints

Across four birds, songs were analyzed for transition probabilities in divergent branchpoints (where syllables can be followed by one or more syllable). One sample of a transition probability is shown (Figure 5). In bird 1, syllable "b" can be followed by syllable "c", marking a new motif to motif 2, or by syllable "a", marking a repeated motif transition back to motif 1 (Figure 5a). During baseline, motif 2 almost always followed motif 1 (Figure 5b). However, during the DAF condition, the transition of syllable "b" to syllable "a" significantly increases whereas the transition of syllable "b" to syllable "c" significantly decreases (p-value<0.05, z-test for proportions with a Bonferroni correction) (Figure 5b). The transition probabilities return to almost normal during washout, and then there is a smaller change during the DAF+PS condition (Figure 3b). This change was significantly different from washout (p-

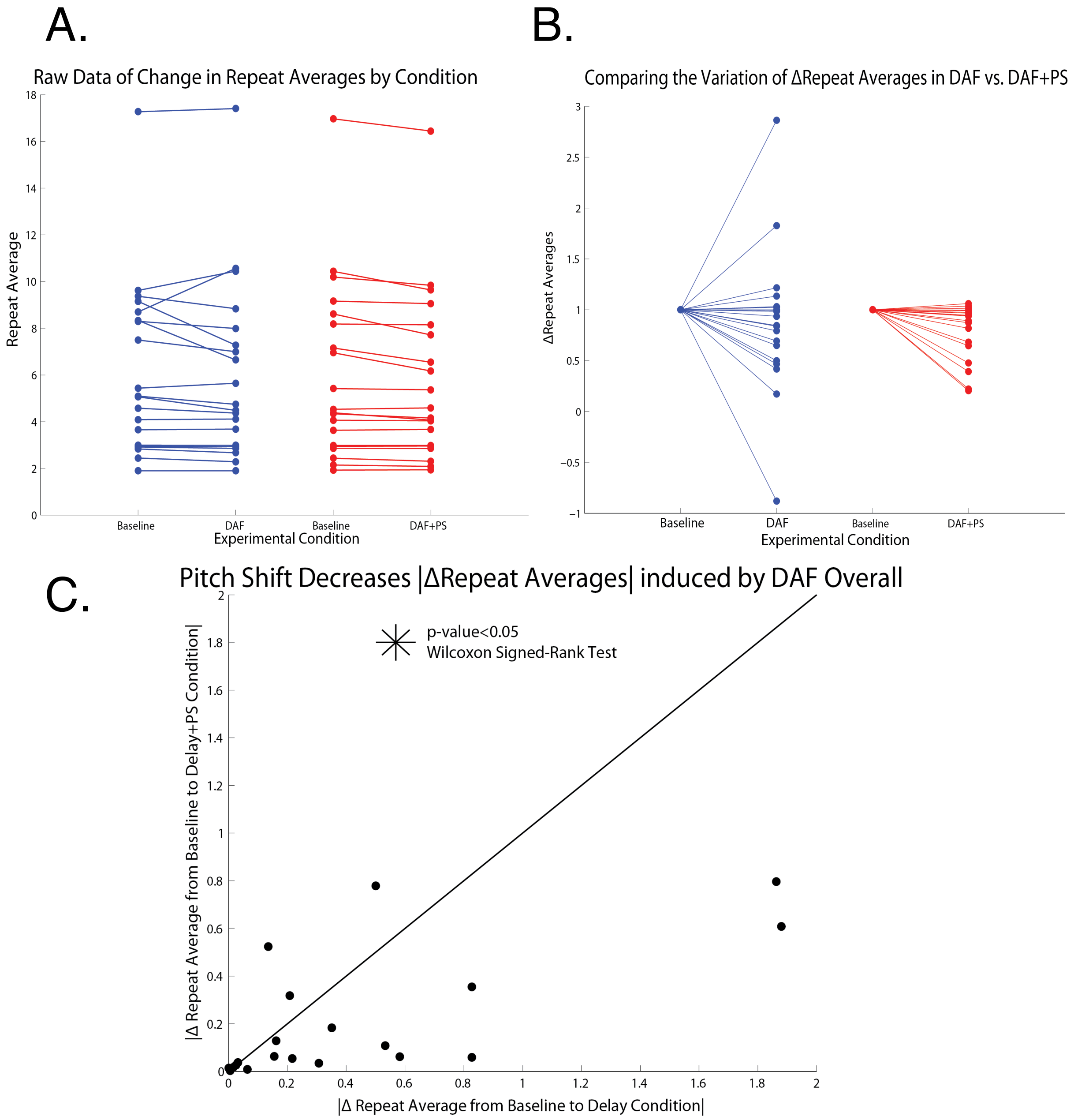


Figure 4. Group results for repeat averages across all birds. **a.** Raw data for the repeat averages during experimental conditions. The repeat average for each condition is shown. **b**. This figure depicts the change from baseline transitions to DAF (blue lines) and DAF+PS (red lines) with the baseline averages. A steeper the slope indicates a greater a magnitude of change. The DAF condition appears to have a trend of greater change in transition probabilities, as shown in a greater spread of lines. **c.** This plots the absolute change (difference) from baseline to DAF+PS versus the absolute change from baseline to DAF in transition probabilities. The induced changes are significantly greater for DAF (p-value<0.05, Wilcoxon Signed-Rank Test, n=20) than DAF+PS changes.

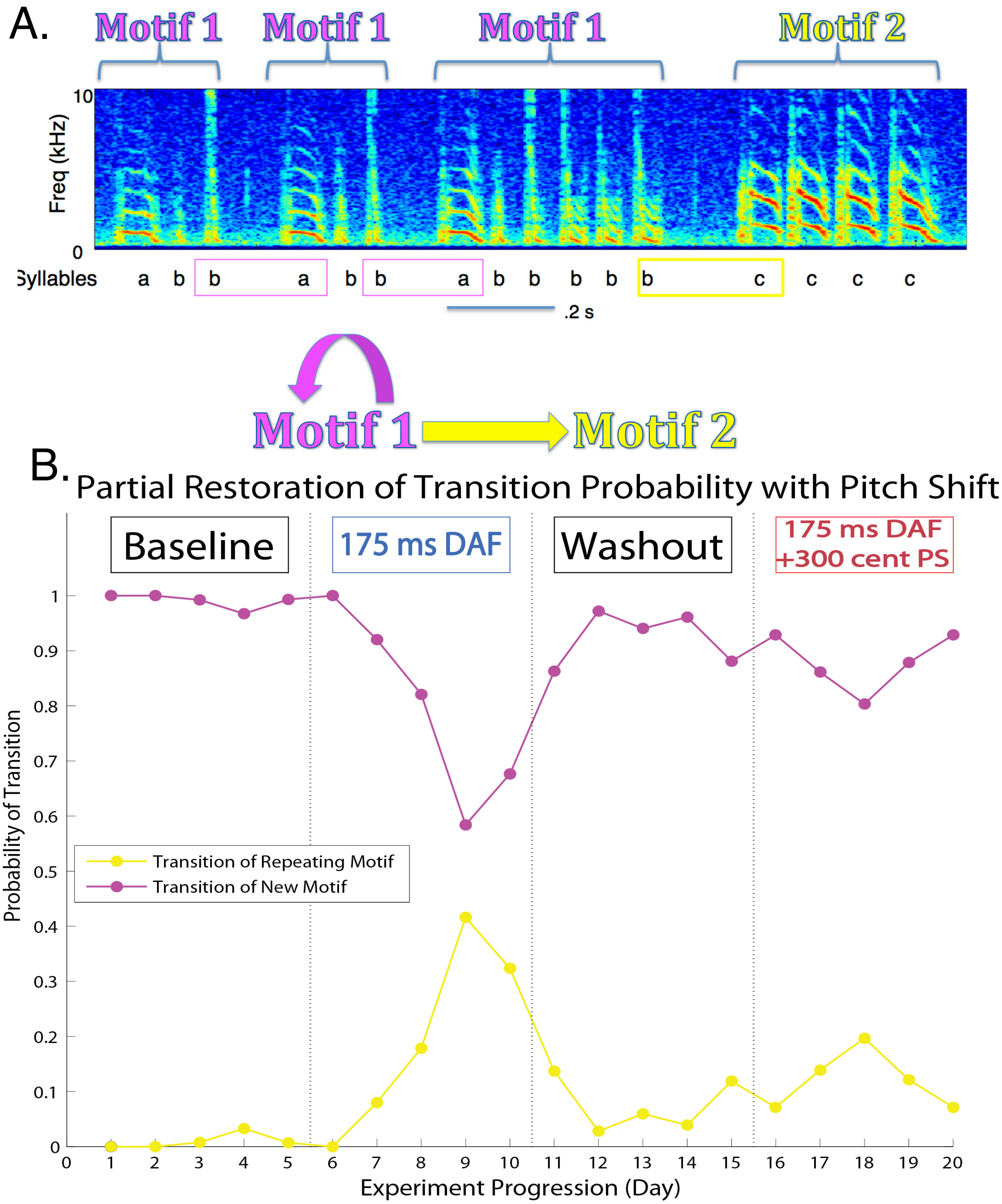


Figure 5. Example of pitch shift partially ameliorating DAF-Induced changes in transition probabilities in Bird 1. **a**. Description of bird's song. Motif 1 (comprised of syllables "abbb" where "b" can repeat a variable of number of times) can be followed by a new motif (Motif 2) or the same motif (indicated by the magenta and yellow arrows, respectively). **b**. During the DAF condition, the probability of transitioning to the new motif after motif 1 significantly decreased (magenta line) whereas the probability of repeating the motif significantly increased (yellow line). During the DAF+PS condition, the induced changes are lower.

value<0.05, z-test for proportions with a Bonferroni correction) but the change is qualitatively smaller than the DAF induced changes.

We analyzed divergent branchpoints across birds (Figure 6). Figure 6a depicts the raw transition probabilities of the bird's baseline probabilities compared to the DAF and the DAF+PS. The baseline condition is the 0 delay and 0 shift period immediately preceding each condition. For both DAF and DAF+PS, half of the birds' washout period is considered the baseline period. We compared the raw differences in transition probabilities for baseline to DAF versus baseline to DAF+PS (Figure 6b). Qualitatively, there is a greater spread for changes in transition probabilities induced by DAF compared to DAF+PS. We plotted the absolute magnitude of the change in DAF+PS versus DAF condition (Figure 6c). The magnitudes of the induced changes by DAF compared to DAF+PS were not significantly different, but there is a trend that the probability changes are lower for DAF compared to DAF+PS (p-value=0.0884, n=23, Wilcoxon Sign-Rank test).

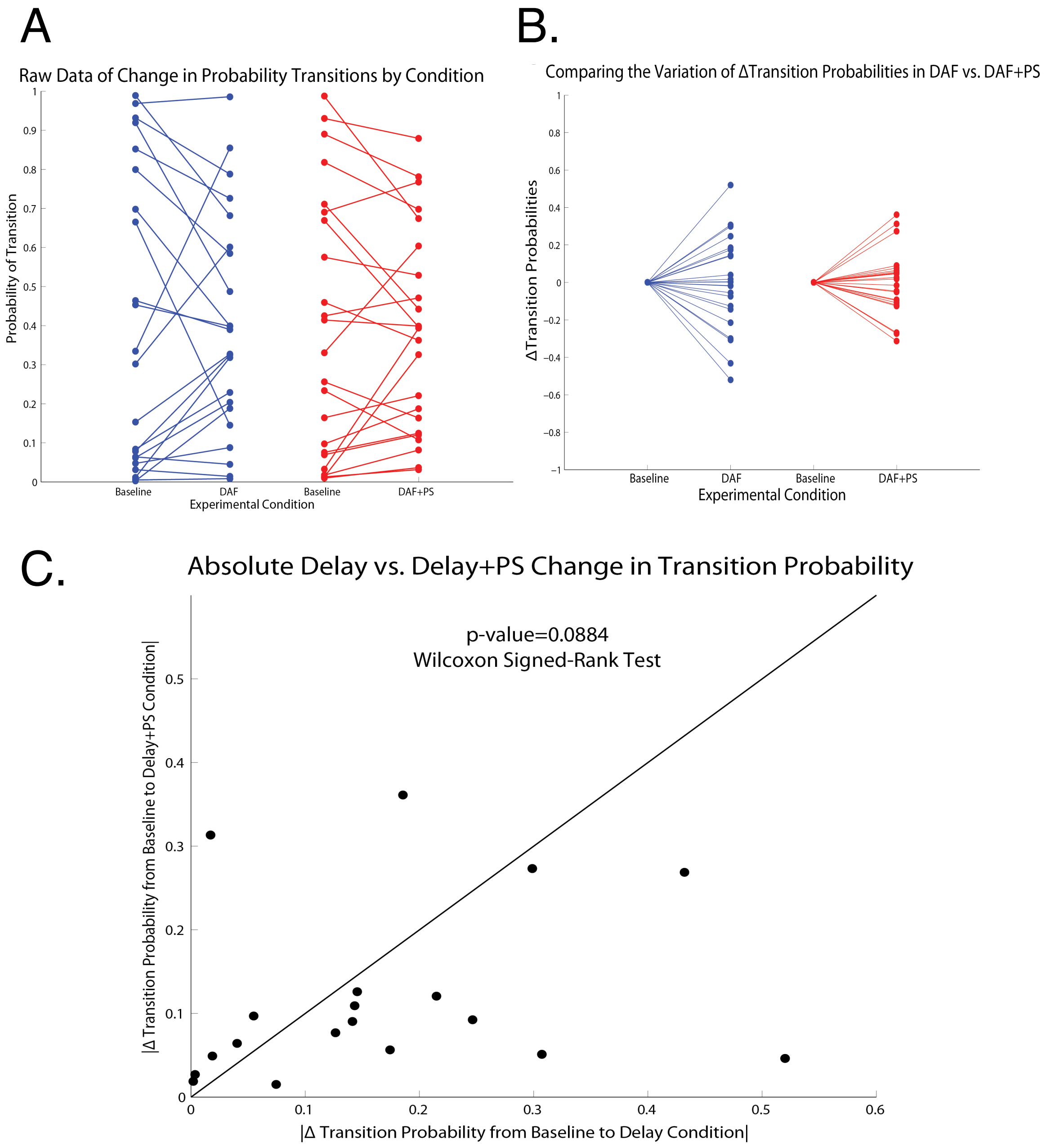


Figure 6. Group results for divergent branchpoints across all birds. **a.** Raw data for the transition probabilities during experimental conditions. The transition probability for each condition is shown. **b**. This figure depicts the change from baseline transitions to DAF (blue lines) and DAF+PS (red lines) with the baseline probabilities. A steeper the slope indicates a greater a magnitude of change. The DAF condition appears to have a trend of greater change in transition probabilities, as shown in a greater spread of lines. **c.** This plots the absolute change (difference) from baseline to DAF+PS versus the absolute change from baseline to DAF in transition probabilities. Although not significant (p=.0884, Wilcoxon Signed-Rank Test, n=23), it does appear that the induced change in transition probabilities is greater with DAF than DAF+PS.

Discussion

Delayed auditory feedback induced robust sequence changes in the stereotypical adult crystallized bird songs. This is consistent with prior studies that have examined the degradation of songs due to altered auditory feedback. We tested the hypothesis that a large pitch shift would reduce the changes induced by delayed auditory feedback. We predicted that a pitch shift could restore normal song sequencing induced by delayed auditory feedback because there is less overlap between expected and observed auditory feedback for DAF+PS compared to DAF alone.

We examined the effect of DAF and DAF+PS on repeat distributions of repeated syllables in bird song. DAF induced significant changes on repeat distributions (Figure 3). We could not predict whether repeat distributions would shift to the left or right to decrease or increase repeat averages. Pitch shifts significantly partially reduce the DAF-induced repeat distribution changes as the magnitude of repeat averages decreases from DAF+PS compared to DAF (Figure 4c).

Our findings found that delayed auditory feedback caused robust transition probability changes, consistent with prior studies like Sakata and Brainard (2006). Transition probabilities changed significantly from baseline to delay conditions (Figure 5). One example of a pitch shift partially ameliorating the effects of DAF can be seen in Figure 5. It could not be predicted whether transition probabilities would decrease or increase (Figure 4a). Although the magnitude of change induced by DAF was not significantly different from the magnitude of change induced by DAF+PS (Figure 6c), there was a trend that DAF induced greater differences in transition probabilities within birds (Figure 6b).

Our findings that a large pitch shift can partially restore normal sequencing in repeat distributions parallel the human literature results in which frequency-altered feedback improves speech fluency in stutterers (Kalinowski et al. 1993). Our study is significant in being the first to specifically examine the characteristics of auditory feedback on the repeat distributions of song sequencing of repeat distributions.

Our findings highlight the importance of the characteristics of auditory feedback on vocal behavior. DAF induced a significantly larger magnitude of changes in repeat averages compared to DAF+PS consistent with our hypothesis. Although not significant, there was a trend for a larger magnitude of DAF-induced changes on transition probabilities compared to DAF+PS-induced changes. The differences in song sequencing changes between DAF and DAF+PS could be due to the salience that the auditory feedback carries. With a greater convergence of expected and observed sensory feedback, auditory feedback may be more valuable to the bird, carrying more information. As the similarity decreases, the brain may find that the information of the auditory feedback is unreliable and inaccurate causing less behavioral modifications.

Birds have other forms of gauging vocal output, such as bone conduction and muscle proprioception. Bone conduction is the transmission of sound from the inner ear bones through the skull bones, whereas muscle proprioception is feedback from sensory receptors within muscles. When an organism produces a behavior, it is thought that the brain produces an internal signal to be sent to the body, known as a motor command. The bird internalizes an efference copy of this outward signal. The bird’s brain may compare other forms of feedback to the motor template creates. When the motor command is so distant from the auditory feedback the bird receives, the bird may rely more on other forms of feedback such as bone conduction and proprioception from vocal muscles rather than auditory feedback.

Although the headphone apparatus does not eliminate bone conduction and proprioception, our findings support conclusions in prior literature that songbirds rely on auditory feedback. Bone conduction remains constant in both baseline and altered auditory feedback conditions, yet there are still robust song sequence changes. Our study examines the effects of DAF and DAF+PS within the same bird, so individual variation in reliance on auditory feedback is controlled.

Our results have implications in the human field. Songbirds are used as an animal model for human speech, and our results parallel the human findings that a large pitch shift can reduce vocalization changes induced by DAF. This supports the use of Bengalese finches as a stuttering model.

Future studies should examine the underlying mechanism that causes a difference in DAF+PS changes between repeat distributions and branchpoint transition probabilities. A large pitch shift may decrease the bird's attention to vocal repetition but not global sequencing. Not every divergent branchpoint included a repetition of a motif, which may cause the auditory feedback to be more salient despite the large pitch shift. Understanding these mechanisms in songbirds are also beneficial due to the potential of neuronal recording. Bengalese finches have a separate brain system for song that is analogous to regions of the human brain involved in processing human speech. Researchers could record from neurons during playbacks of repeated syllables to determine which areas control these parts of song sequencing and how timing affects the firing patterns.

In conclusion, our study shows that delayed auditory feedback impacts song sequencing at the syllable and motif level in Bengalese finches. Delayed auditory feedback induces significant changes in divergent branchpoint transition probabilities and repeat distributions. A large pitch shift concurrent with delayed auditory feedback ameliorates some DAF-induced changes in repeated syllables. DAF induces a greater magnitude of changes in repeat averages. Although not statistically significant, there is a trend that DAF induces greater changes in transition probabilities. These findings together support our hypothesis that a large pitch shift could partially restore changes in normal song sequencing induced by delayed auditory feedback.

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