STEREOTYPY AND VARIABILITY PREDICT INTERRUPTIBILITY OF ZEBRA FINCH SONG

by

Rima Abram

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Project Approved:

Supervising Professor: Brenton Cooper, Ph.D.

Department of Psychology

Gary Boehm, Ph.D.

Department of Psychology

Jean-Luc Montchamp, Ph.D.

Department of Chemistry

ABSTRACT

Motor preparation deficits are a feature of many neurological disorders in humans. Developing animal models to study deficits in motor preparation is essential to explore the potential therapeutic treatment avenues. In male zebra finches (*Taeniopygia guttata*), song is composed of a fixed syllable sequence and is preceded by a series of introductory notes. It is thought that introductory notes may be a preparatory motor program, but this has not been experimentally tested. We aimed to differentiate preparatory motor periods from song execution by interrupting female-directed song and song produced in isolation (undirected song) with white noise playback. The amplitude and duration of the white noise were systematically varied along with when the playback occurred during song. We hypothesized that interruption events would occur more frequently during the preparatory phase of song production. Song respiratory motor gestures were measured in six birds experiencing the noise disruption events. During both directed and undirected song, playback disrupted the typical syllable progression seen in undirected song without playback (control condition). Interruption events occurred at the highest frequency during introductory notes compared to other points in the song motif. Higher frequencies of interruption also occurred with higher amplitudes or longer durations of white noise. Ascertaining patterns of stereotypy and variability in zebra finch vocalizations can elucidate where in song motor program preparation leads to execution. The results suggest that introductory notes are a form of motor planning for the execution of the song motor program. These data illustrate that the zebra finch song is an excellent model system for exploring how motor planning deficits manifest in language and communication disorders.

INTRODUCTION

The study of language is essential to understanding human and nonhuman animal communication and the cognitive processes enabled by descriptive and analytical language (Pervlovsky, 2009). Motor preparation is necessary for many of our everyday actions, from limb movements to speech production. Human speech is a learned behavior that involves preparatory motor actions that result in the production of vocalizations. Preparation for speech involves laryngeal, respiratory, and articulatory components (including the pharynx, lips, jaw, and tongue) controlled by premotor and motor neurons. The neural regions that underly these components include the primary motor cortex, prefrontal and premotor cortical areas, and regions of somatosensory cortex (Jürgens, 2002; Simonyan & Horwitz, 2011).

Human speech involves not only the voluntary initiation of vocalizations but also the attribution of syntax and meaning to various combinations of speech sounds. Therefore, both cognitive and physical preparation are necessary for volitional speech (McArdle, 2009). The laryngeal motor cortex (LMC) plays a vital role in the motor coordination of human vocal control (Simonyan & Horowitz, 2011). Damage to the LMC results in the loss of volitional speech production in humans and nonhuman primates (Kumar et al., 2016). By contrast, non-volitional vocalizations, such as crying or laughing, were unaffected by LMC deficits. This demonstrates the uniquely sophisticated control that underlies human communication.

Many neurological conditions feature deficits in preparatory neural pathways. When motor planning is inhibited in disorders such as speech apraxia, aphasia, or stuttering, individuals are unable to execute typical speech patterns. In children who stutter, for instance, deficiencies were noted in the left premotor cortex compared to their typically developing peers (Chow, 2023; Trupe, 2013). Similarly, individuals with autism spectrum disorder (ASD) or developmental coordination disorder have difficulty with physical movements that include speech (van Swieten, 2010). Thus, exploring potential therapeutic avenues for these disorders necessitates a thorough understanding of vocal motor preparation and the underlying neural mechanisms.

Songbirds are a widely used animal model to study the neural and peripheral mechanisms of human speech due to their vocal learning capabilities, analogous neural structures, and known respiratory and neural precursors of song (Colquitt et al., 2021; Doupe & Kuhl, 1999; Jarvis, 2007; Jarvis, 2019; Riede & Goller, 2010). Zebra finches (*Taeniopygia guttata*) are a species of songbird that are conducive to research due to their song stereotypy (Cynx, 1990; Méndez et al., 2022). The males of the species learn to produce song in various contexts, including spontaneously, directed at females of the species, or directed at other males as a form of aggression (Byers & Kroodsma, 2009). During development, male zebra finches mimic the songs of older male tutors and fine-tune their vocalizations based on auditory feedback; in adulthood, song becomes crystallized in a specific pattern after 80-90 days that persists across the lifespan and continues to be refined by auditory input (Aamodt, 1999; Jarvis, 2007; Lombardino & Nottebohm, 2000; Pytte et al., 2007). The importance of auditory feedback to song syntax is evident in the fact that song elements in zebra finches deafened early in song development deteriorate much more quickly than in birds deafened after song crystallization. This refinement of vocalization during song development compares to the way human infants learn to speak by mimicking their parents over time. Despite the evolutionary origins of the avian dorsal ventricular ridge (DVR), responsible for vocal learning, being different from those of the neocortex in humans, their structural similarities indicate the potential for songbirds in speech research (Colquitt et al., 2021).

Structural and neural correlates exist between songbirds and humans that make zebra finches highly representative animal models for the study of human speech (Doupe & Kuhl, 1999; Jarvis, 2007; Riede & Goller, 2010). The syrinx in songbirds has similarities to the larynx in humans; both are primary vocal organs with visco-elastic properties that regulate airflow to produce sound of varying frequencies and amplitudes. In the brain, damage to the nuclei HVC (letters used as proper name) and RA (robust nucleus of arcopallium) in songbirds leads to similar deficits as damage to the left motor cortex in humans. Song syntax disruption is caused by Area X lesion, which is analogous to damage to the anterior striatum in humans, causing disruptions in learning new motifs in songbirds. In songbirds, these regions, along with the syringeal muscles, are active during internalized vocalization (e.g., song replay during sleep) as well as voiced song. Similarly, humans utilize spoken-language pathways to produce speech when reading or conceptualizing language (Jarvis, 2019). These findings illustrate the similarities in the premotor and motor pathways of birds and humans.

The inherent stereotypy of zebra finch song can be used to distinguish preparatory versus execution periods during song performance. Zebra finches sing a pattern of introductory notes followed by song syllables in a typical order, usually with specific syllables repeated as chunks (Williams & Staples, 1992). Several factors must be controlled to accurately determine changes in stereotypy at various points during song. Songs directed at a potential mate are more stereotyped and have a higher tempo than spontaneous song or during male-male aggression and vary much less across distance and time (Byers & Kroodsma, 2009; Cooper & Goller, 2006). These differences might indicate that motivational state plays a role in birdsong stereotypy.

The introductory notes have been hypothesized to have preparatory motor function, but this concept has not yet been experimentally tested (Kalra et al., 2021; Rajan, 2018).

Introductory notes appear to grow closer temporally and increase in stereotypy as they approach the first song syllable (Rajan & Doupe, 2013). This pattern of motor refinement suggests that introductory notes are preparing for singing; this study aims to extend these results by disrupting the motor program at various points within introductory notes as well as song to distinguish the motor preparation from execution.

The ability to interrupt song performance with an external stimulus is an indicator of stereotypy. The most variable periods in zebra finch song performance are the beginning of a song bout, especially the introductory notes, and the conclusion of the song performance. We hypothesize that once a motor action has begun, its susceptibility to disruption of the song motif (interruptibility) will be lower than that of preparatory periods. Therefore, the interruptibility with white noise during a song bout would be lower during the song syllables than introductory notes. We also hypothesize that white noise of lower amplitudes and shorter durations would produce lower interruptibility and that song in the absence of a female (undirected song) would be more interruptible across a song bout than female-directed song.

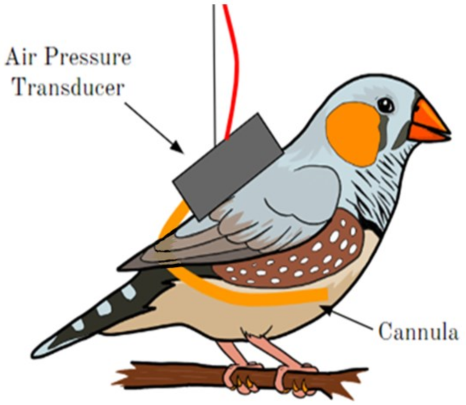
METHODS

We analyzed recordings from 6 zebra finches, ZFP72, ZFP78, ZFR108, ZFW3, ZFW6, and ZFG435. The birds came from laboratory-bred populations with an age range of 22.4 weeks to 73.4 weeks, as measured at the time of the start of directed recordings with white noise playback. The birds were housed in individual cages throughout their respective experiment trials with exposure to 14 hours of light followed by 10 hours of darkness daily.

Respiratory events were recorded for each bird using the surgical insertion of a pressure cannula into the anterior thoracic air sac. Prior to the cannula insertion, each bird underwent an acclimation period wearing the pressure transducer and supported by a counterweighted balance arm that offset the weight of the pressure transducer and associated wiring. Sedation by isoflurane (1-2%) was used for the duration of the surgical procedure. As depicted in Figure 1, the cannula was connected to a pressure transducer that monitored respiratory pressure constantly, and a microphone was placed above the cage. The birds were allowed to acclimate to the insertion of the cannula until they began song spontaneously before starting experiment trials.

Figure 1

Pressure recording setup in a zebra finch.



Semi-random trials were conducted for each bird, varying the amplitude and duration of white noise played during directed or undirected song. Playback was triggered when 75-150 ms of song was within an experimenter-determined RMS (root mean square)

$$RMS = \sqrt{\frac{1}{n}\sum_{i} x_i^2}$$

range that matched the RMS values of the individual bird’s song. The RMS range was measured during three successive equal interval time windows. Our goal was to identify periods of sound, silence, and sound, corresponding to the generation of 2 successive introductory notes and the silence between them. To identify song syllables, we used RMS values with 2 successive time windows with a longer window duration (225-600 ms); we categorized song as containing 2 periods of time with repeated RMS values in the range of the typical song syllable recordings. Any RMS values above or below this predetermined song range were excluded from the playback criteria to minimize playbacks during lower amplitude calls or higher amplitude contact calls.

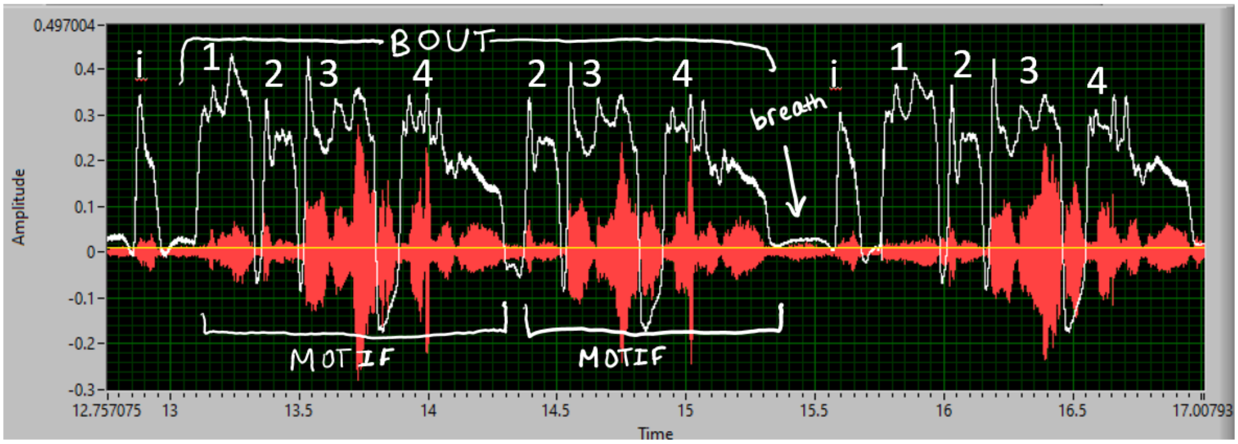
The parameters we utilized for data digitation were a 22kHz sample rate for .wav file collection and a 16-bit sample rate. For air pressure, the data were low pass filtered (2kHz) and amplified 100x. The microphone recording was high pass filtered (300 Hz) prior to digitization. Data were recorded using Avisoft Recorder software and saved whenever a user-defined threshold trigger event occurred. We visually estimated 2.5x quiet respiration to set the trigger threshold for data acquisition, and data were recorded for 5 s before and after each trigger event with minimally 10 s data files. The trigger detected several events extraneous to song, including defecation, hopping or other vigorous movements, and various calls.

Trials with playback were conducted for 60 minutes per session, or the session was terminated earlier if 60 playbacks occurred within a session. The playback sessions were administered one to two times per day, on different days, and at varying times of day. For the trials with playback, directed and undirected song were recorded for ZFP72, ZFP78, ZFW3, and ZFW6, and directed song only ZFR108. Directed song trials involved the placement of a female bird's cage in proximity for the duration of the trial. Separate channels were recorded for respiratory pressure, microphone recording, and playback.

Song segments were isolated for analysis using various custom-written programs in LabVIEW (version 2018). We defined a song motif as a stereotyped string of syllables and a song bout as one or more motifs. A new song bout was defined as the end of the bird's motif followed by a respiratory cycle and/or one or more introductory notes before the onset of the subsequent song bout. Figure 2 depicts the distinction between a song motif and a song bout. The microphone trace is shown as red, and the pressure recording is white. Syllables are numbered consecutively, with i representing introductory notes.

Figure 2

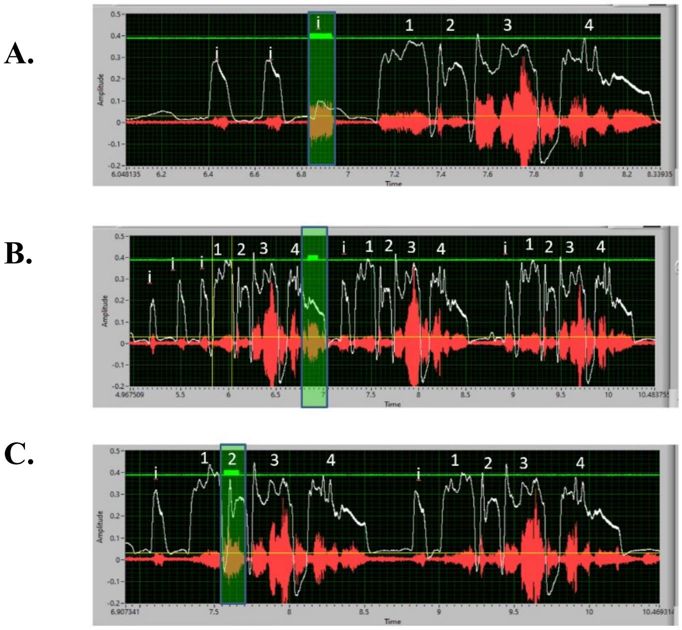
Zebra finch song motif vs. bout.



The frequencies of interruption, continuation, or termination following playback were analyzed for both directed and undirected trials with playback. We defined interruption as the deviation of a syllable from its typical form, visually approximated, or the lengthening of silence between introductory notes preceding song onset. We defined continuation as the beginning of a new motif within a song bout following playback. We defined termination as the conclusion of a song bout on a completed syllable, followed by a breath and/or one or more introductory notes. Figure 3 depicts the distinction between interruption, termination, and continuation. In addition to recordings with playback during song, for each bird, 100 bouts of undirected song without playback were isolated in a semi-random manner from recordings at different days and times to determine baseline frequencies of interruption, termination, and continuation.

Figure 3

Interruption, termination, and continuation in zebra finch song.



In addition to the microphone trace and pressure recording, the playback trigger for white noise is shown in Figure 3 in green. Syllables are numbered consecutively, with i representing introductory notes. In **A)**, interruption on an introductory note occurs during playback, changing the syllable air pressure amplitude and temporal modulation. In **B)**, the bird terminates after playback on syllable 4; in **C)**, the bird continues singing after playback on syllable 2.

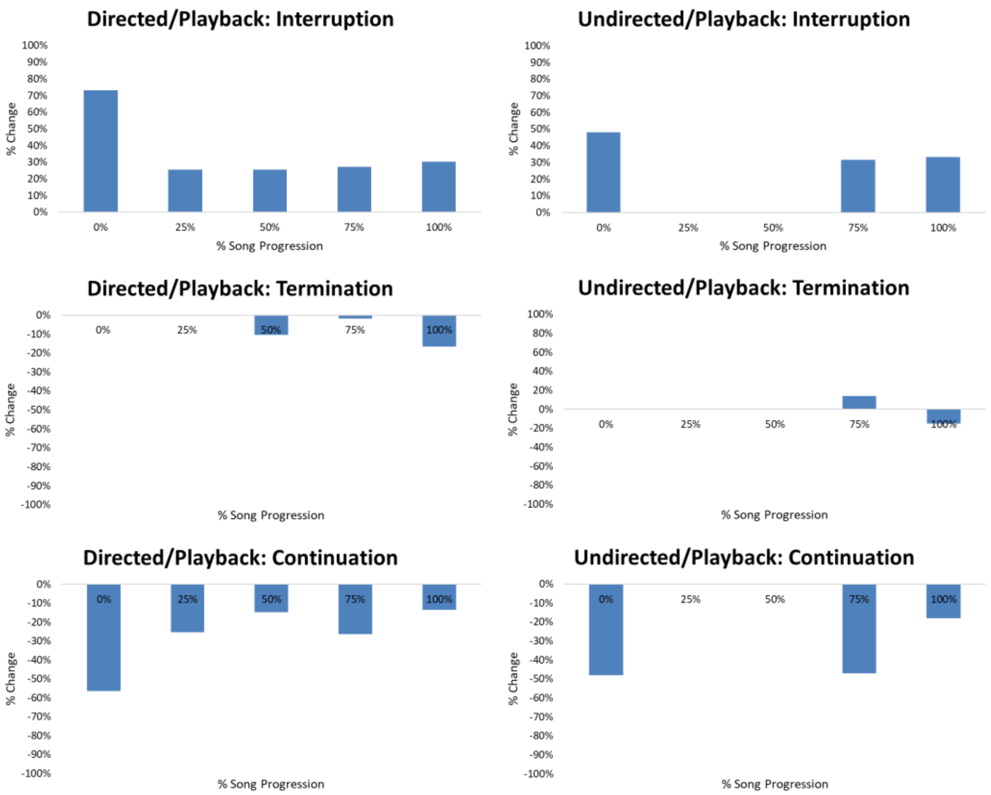
RESULTS

The purpose of our first analysis was to determine which points in the song are the most susceptible to interruption. If our hypothesis were correct that introductory notes are the preparatory periods of song execution, then introductory notes would exhibit higher rates of disruption of the song motif compared to baseline. We compared interruption, termination, and continuation as a function of song progression with and without white noise playback. To give a larger sample size of playbacks for the experimental results, white noise playback for this condition aggregated the results from all amplitude and duration trials used in this study. Differences between the amplitude and duration of white noise playback are further elaborated in the second analysis. Figure 4 indicates the average percent change from baseline frequencies of interruption (**A**, **D**), termination (**B**, **E**), and continuation (**C**, **F**) at different points during song for all six birds in the study. Syllables within the song motif were approximated as 0%, 25%, 50%, 75%, or 100% of song progression. Introductory notes are 0%; the song syllables are binned into the remaining percentiles.

A) and **D)** demonstrate increased frequencies of interruption following playback during introductory notes compared to the following song syllables. There is no apparent change in the rates of termination, shown in **B)** and **E)**. The rates of continuation, shown in **C)** and **F)**, during introductory notes are visibly lower than baseline because of song interruption events.

Figure 4

Percent Change from Baseline of Interruption, Termination, and Continuation

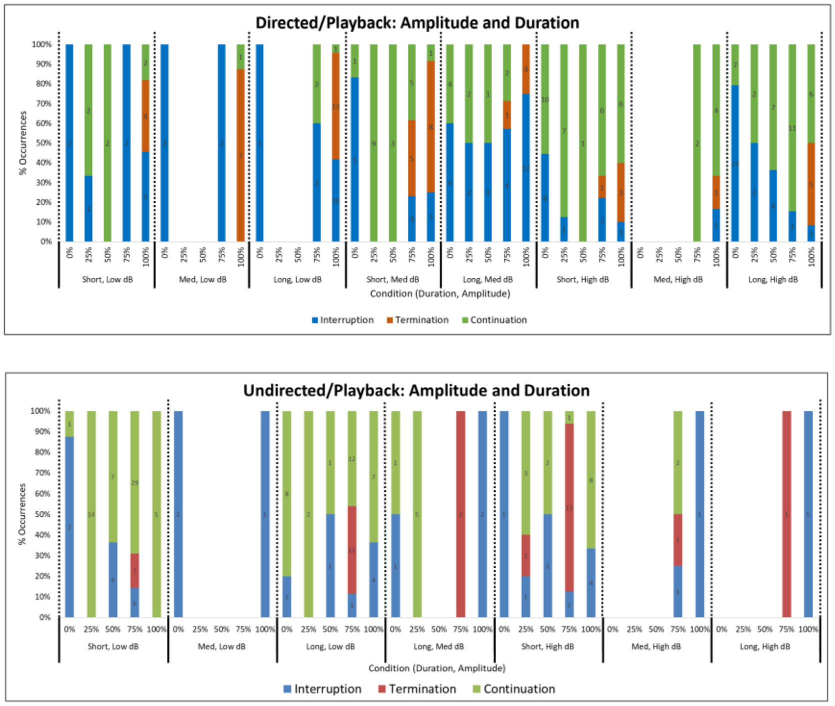


Our second analysis focuses on the effect of amplitude and duration on interruptibility. We expected more salient stimuli to have greater effects on song motif disruption; therefore, white noise with greater amplitudes and durations should lead to increased interruption and termination. We analyzed frequencies of interruption, termination, and continuation as a function of song progression with varying amplitudes and durations of playback. Figure 5 shows the percentage of syllables that interrupted, continued, or terminated with varying amplitude/duration combinations of white noise. Syllables within the song motif were approximated as 0%, 25%, 50%, 75%, or 100% of song progression.

We defined short duration as 50-100 ms, medium duration as 250 ms, and long duration as 500 ms. We defined low amplitude as 70-80 dB, medium amplitude as 80-90 dB, and high amplitude as 90-100 dB. Within each amplitude level, we explored differences in the duration of playback to determine whether amplitude and duration of the sound were systematically related to interruption and termination of song. In Figure 5, **A)** and **B)** demonstrate that interruption occurred across most conditions; the frequencies of interruption were highest for introductory notes, which is consistent with the data in Figure 4. Song termination in earlier syllables occurred with increasing amplitudes of white noise.

Figure 5

Amplitude and Duration Effects on Interruption, Termination, and Continuation



Figures 6-11 depict the amplitude and duration effects on interruptibility for each bird in the study. The data clarify that interruption occurs across the song motif and that, in some cases, higher amplitude noise relates to an increase in termination before 100% of song progression.

Figure 6

Amplitude and Duration Effects on Interruption, Termination, and Continuation: ZFW3

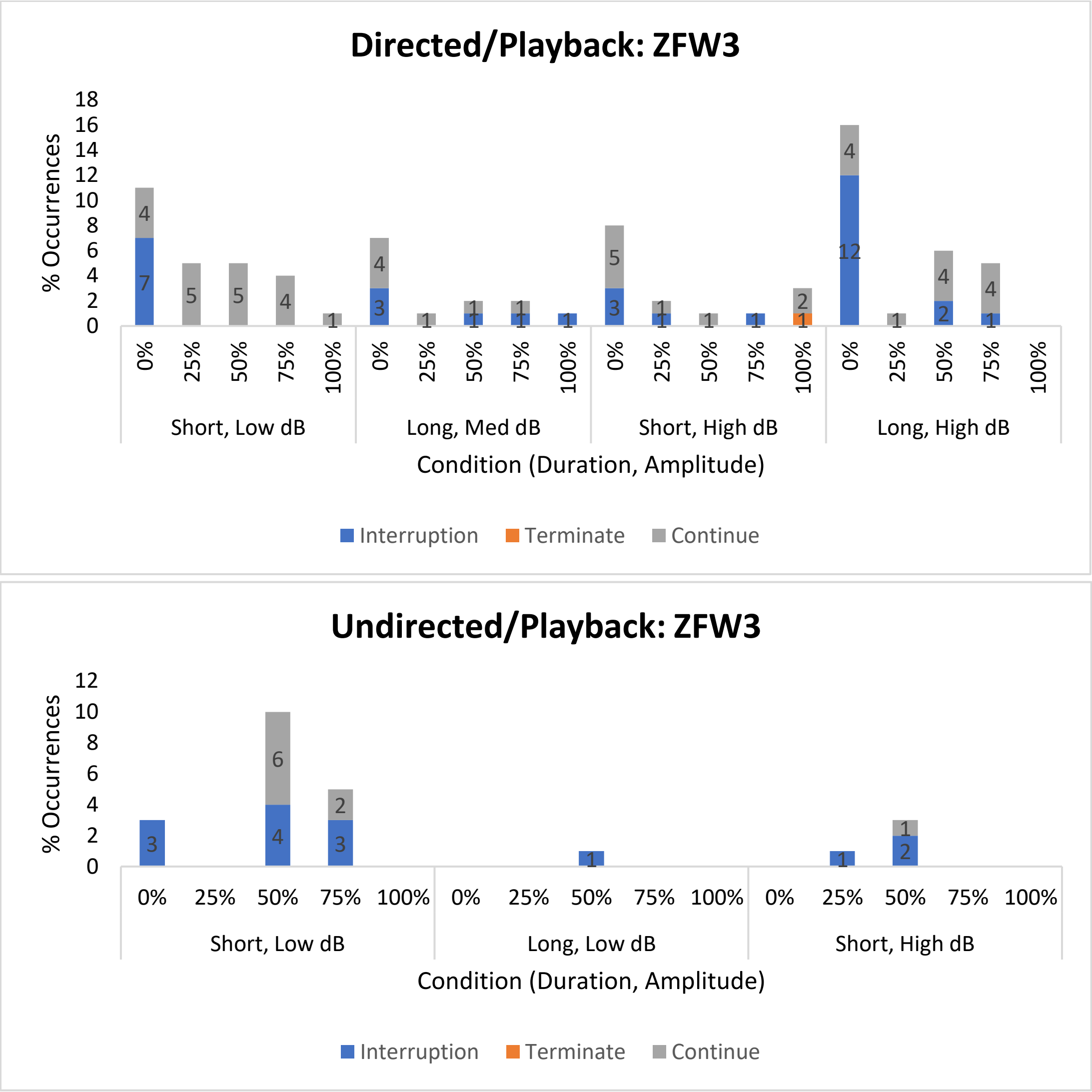


Figure 7

Amplitude and Duration Effects on Interruption, Termination, and Continuation: ZFG435

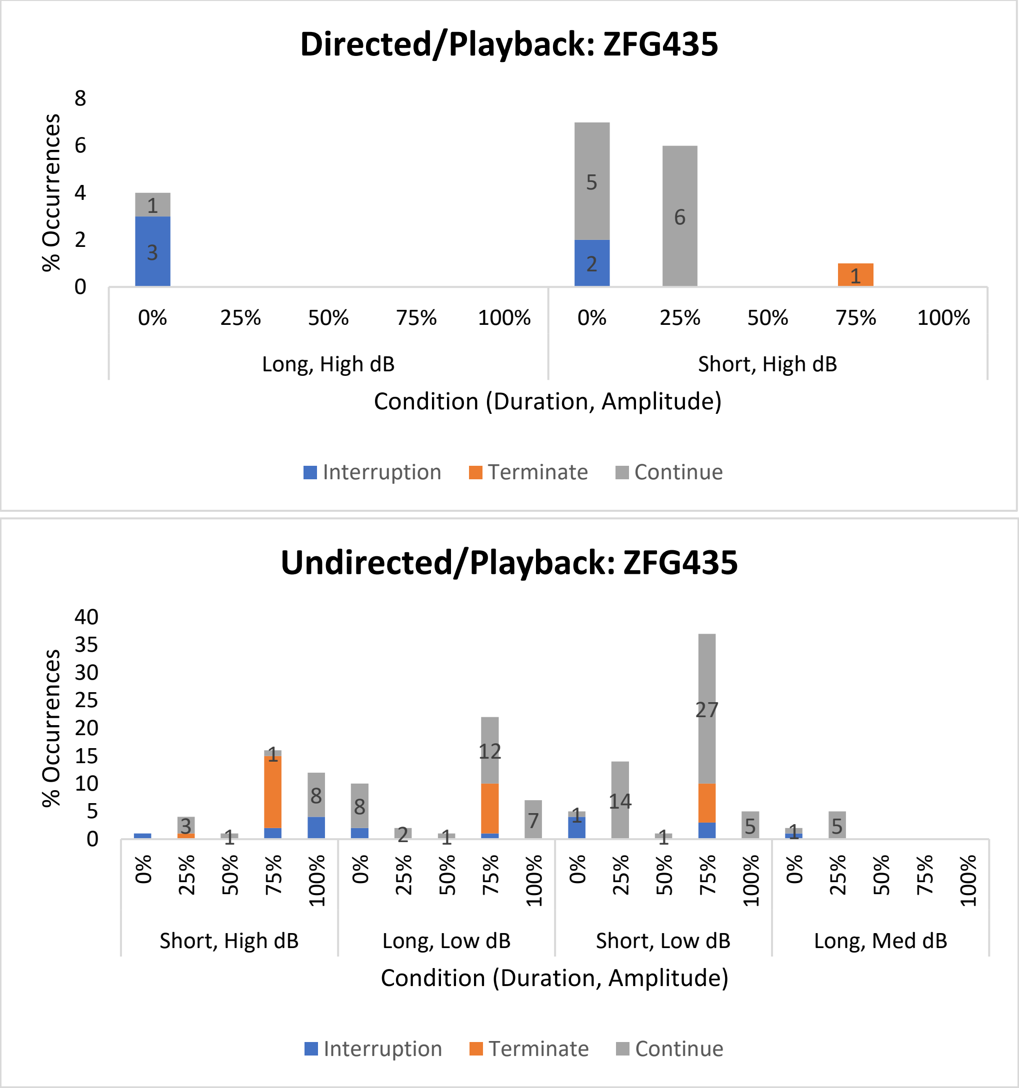


Figure 8

Amplitude and Duration Effects on Interruption, Termination, and Continuation: ZFW6

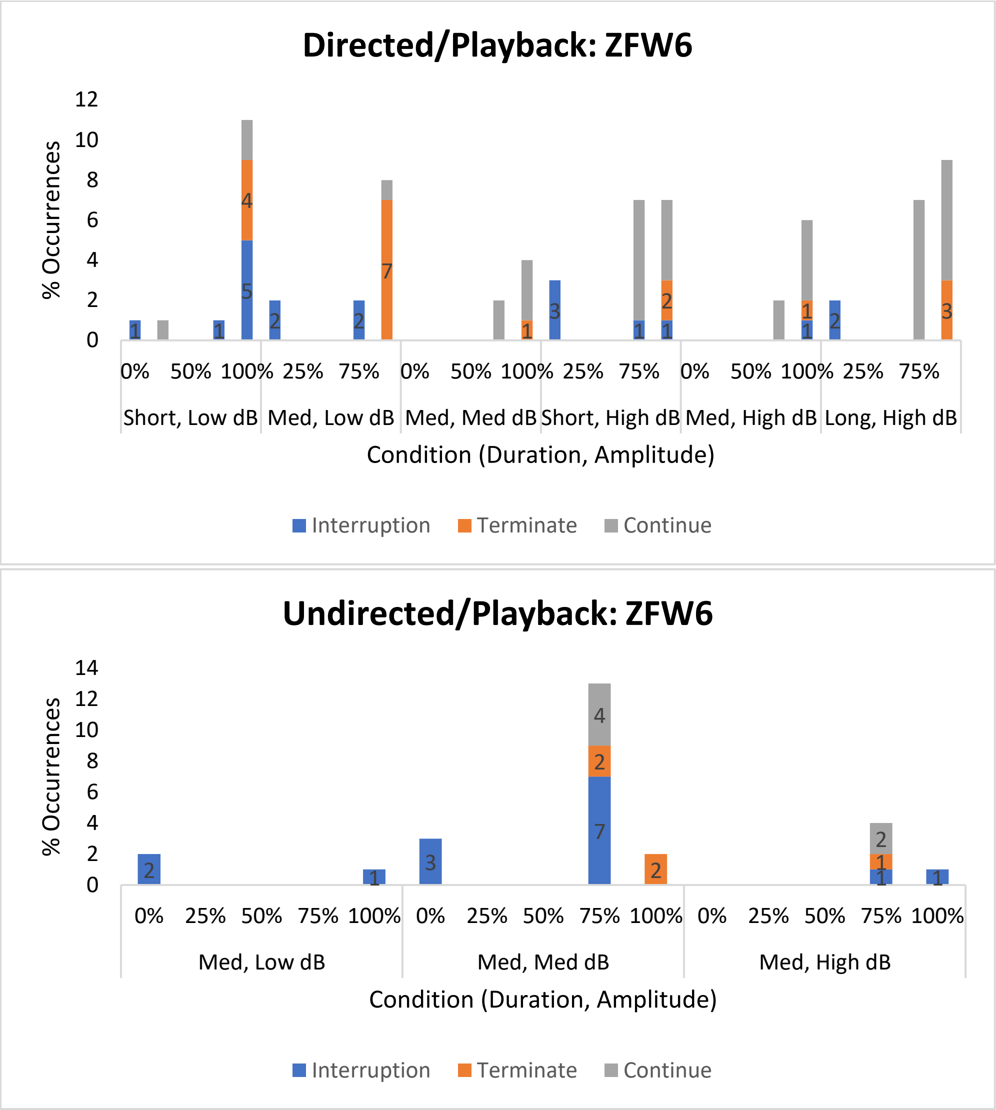


Figure 9

Amplitude and Duration Effects on Interruption, Termination, and Continuation: ZFP78

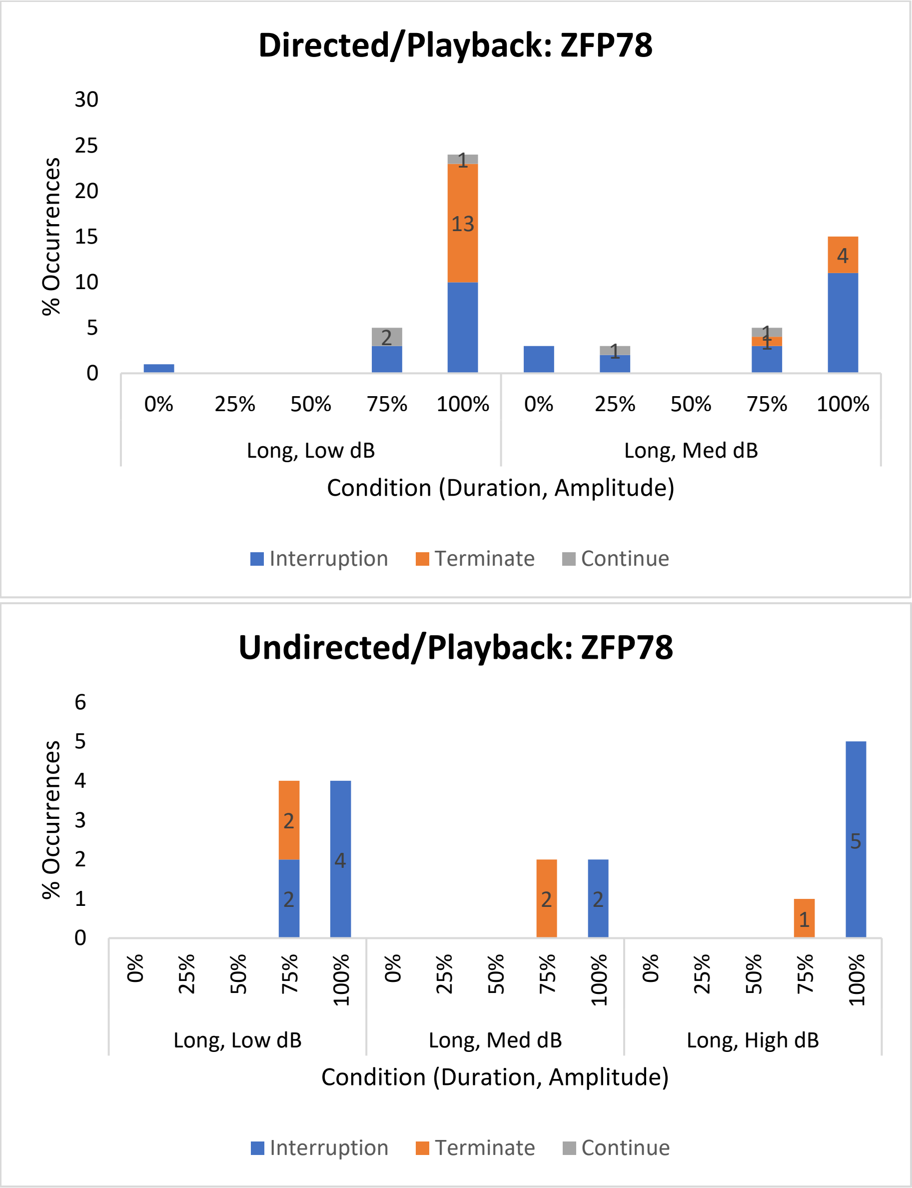


Figure 10

Amplitude and Duration Effects on Interruption, Termination, and Continuation: ZFR108

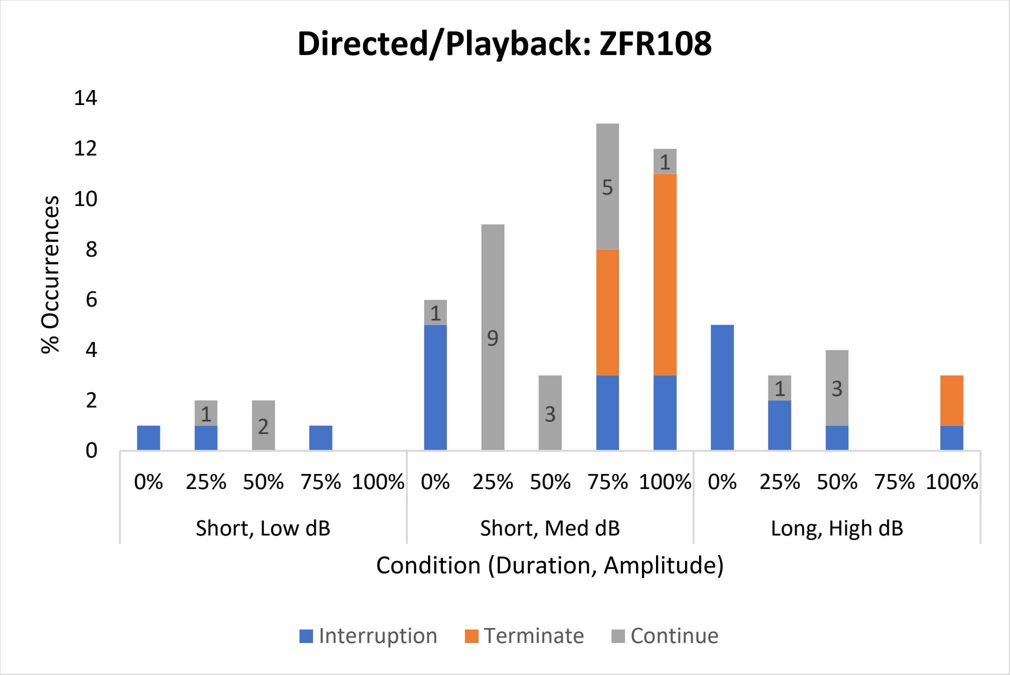
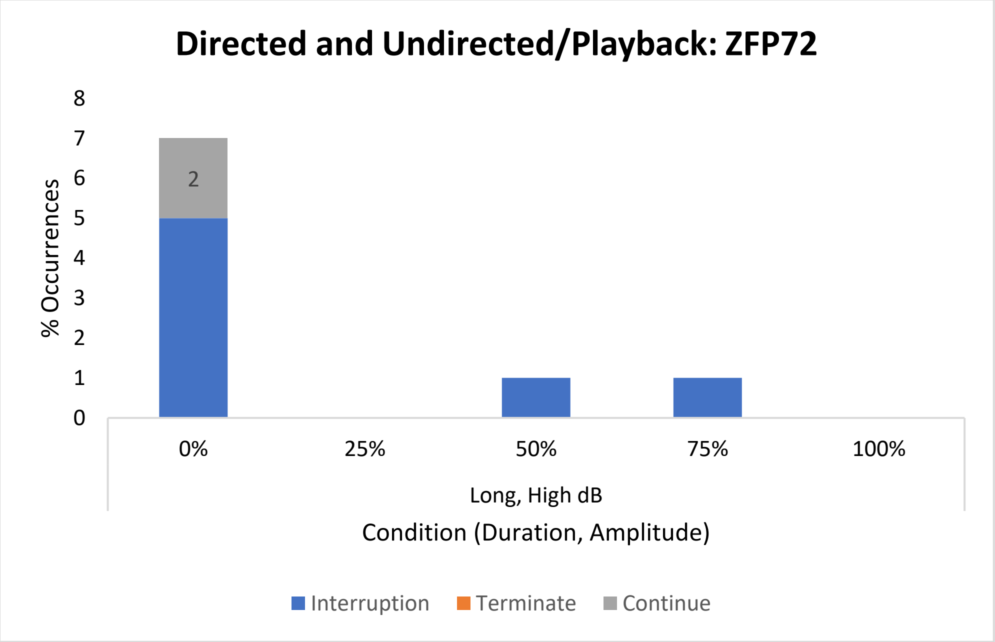


Figure 11

Amplitude and Duration Effects on Interruption, Termination, and Continuation: ZFP72



DISCUSSION

The interruptibility of zebra finch song with white noise illustrates that environmental sounds can be used to disrupt song progression. Male zebra finches sing for various reasons, the most prominent of which is mate attraction (Byers & Kroodsma, 2009). Environmental noise has been shown to disrupt zebra finch song learning, impair mate preferences, and alter foraging behavior (Evans & Kight, 2018; Potwin et al., 2016; Swaddle & Page, 2007). The interruption of song with white noise is likely an ethologically relevant behavior to avoid predation by rapidly responding to environmental stimuli.

Our data largely supports our hypothesis of introductory notes as motor preparation for song. We predicted that the most interruptible parts of the song would correspond to the song preparatory phase. Our results indicate that introductory notes are more susceptible to interruption than the song syllables; the percent change from baseline was highest for interruption of introductory notes in both the directed and undirected conditions and largely uniform across the remainder of the song. Thus, we can determine that introductory notes correspond to motor preparation that precedes the learned execution of song.

We found little effect of amplitude and duration on interruption frequencies. However, higher-amplitude noise showed increased mid-song termination compared to lower-amplitude playback sound, indicating that a more salient stimulus might be more successful in terminating the song motor pattern. Determining the complete effects of amplitude and duration on the interruptibility of song requires further study due to the small sample size for the playback events. The challenge with this experiment is that birds sing a variable number of times, and we sought to minimize the effects of habituation to the stimulus presentation by reducing the number of playbacks. Further, air pressure can only be measured for a few days due to fluid accumulation in the cannula. This prevents long-term investigation of interruption events in the birds. Therefore, it is difficult to have a comparable number of playback trials for each bird in every condition.

We also hypothesized to observe increased variability during the song progression with undirected song compared to directed song. The subtle variations between directed and undirected song in zebra finches are analogous to social cues in human speech syntax appropriate to various social contexts (Woolley & Doupe, 2008). For reasons not limited to increased motivation in the presence of a female bird, we anticipated the birds in our study to exhibit decreased interruptibility overall in the directed condition compared to the undirected condition. However, due to limited data in the undirected condition, further study is needed to determine if there are differences between directed and undirected conditions in song interruption events.

Overall, our study helps us better understand how birds prepare to sing. Since introductory notes are more variable gestures within song, they serve as motor preparation for learned sequences of syllables. Several human disorders, including speech apraxia, aphasia, stuttering, autism spectrum disorder, and developmental coordination disorder, involve a motor planning component as part of the symptomatic deficits (Chow, 2023; Trupe, 2013; van Swieten, 2010). We hope understanding zebra finch song syntax and preparation for song will lead to a more comprehensive understanding of vocal fluency in both songbirds and humans.

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