

INTRODUCTION

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 was 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.



• Respiratory events were recorded using the surgical insertion of a pressure cannula into the anterior thoracic air sac.

- Prior to insertion, each bird was acclimated to the weight of the pressure transducer and counter-balance arm.
- A cannula was inserted into an anterior thoracic air sac under isoflurane anesthesia, then connected to a pressure transducer, to measure changes in respiratory pressure.
- A recording microphone was placed above the bird's cage. After baseline song and air pressure were recorded (2-3 days), white noise playback was triggered by a repeated sequence of 75-150 ms of sound-silence-sound events measured from the high-pass filtered air pressure recording.
- One hour playback trials were conducted in the morning and afternoon each day when song was produced in isolation (undirected) or female-directed song. Playback during song segments were isolated for analysis and compared to baseline song without playback.
- Motif is a stereotyped sequence of song syllables.
- Bout is one or more motifs, followed by one or more breaths and/or introductory notes before the onset of the subsequent song bout.

Stereotypy and Variability Predict Interruptibility of Zebra Finch Song

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What is the effect of amplitude and duration on interruptibility? Which points in song are the most interruptible? 2. Comparison of interruption, termination, and continuation as a function of **1.** Comparison of interruption, termination, and continuation as a song progression with varying amplitudes and durations of playback function of song progression with and without white noise playback **A**. Directed/Playback: Amplitude and Duration Fig. 2 – Demonstration of song interruption (A), termination (B), and continuation (C). The microphone (red), air pressure (white), and the playback trigger (green, with the timing during playback song 50% highlighted). Syllables are numbered 40% consecutively with i representing introductory **A**). notes interruption on an introductory note occurs during playback, changing the 25% 50% 00% 50% 75% 75% 0% 5% 0% syllable air pressure amplitude and temporal modulation. In **B**), the bird terminates after playback on syllable MI INA MI MINM Interruption Termination Continuation 4; in C), the bird continues singing after playback on syllable 2. **B**. Undirected/Playback: Amplitude and Duration 90% 80% 70% D. Undirected/Playback: Interruption **Directed/Playback: Interruption** 60% 100% 50% 40% 30% 60% 50% 50% 20% 40% 40% 30% 20% 10% 25% 50% 75% 1 25% Short, Low dB Med, Low dB Long, Low dB Long, Med dB % Song Progression % Song Progression E. **Undirected/Playback: Termination Directed/Playback: Termination** Interruption Termination Continuation





Fig. 3 – Average percent change from baseline frequencies of interruption (**A**, **D**), termination (**B**, **E**), and continuation (C, F) at different points during song. 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 during introductory notes because of song interruption events.

Fig. 4 – 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.

Short duration = 50-100 ms, medium duration = 250 ms, long duration = 500 ms. Low amplitude = 70-80 dB, medium amplitude = 80-90 dB, high amplitude = 90-100 dB. 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 Fig. 3. Song termination in earlier syllables occurred with increasing amplitudes of white noise.

CONCLUSIONS

- 1. These data illustrate that environmental sounds can be used to interrupt the stereotyped zebra finch song. This is likely an ethologically relevant behavior to avoid predation by rapidly responding to environmental stimuli.
- 2. We hypothesized that the preparatory phase of song would be the most easily interrupted part of song. 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. Amplitude and duration had little effect on interruption frequencies. However, higher amplitude noise showed increased mid-song termination than lower amplitudes, indicating that a more salient stimulus might be more successful in disrupting the song motor pattern.
- 3. We expect to observe increased variability during the song progression with undirected song compared to directed song. 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.

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