Knowing When to Shut Up: Specific Neurons Control the Suppression of Vocal Respiration

Nguyen, M. N., Arnold, R. L., & Cooper, B. G.
Psychology Department, Texas Christian University, Fort Worth, TX 76129

INTRODUCTION

Human language is an action wherein one plans for, produces, and terminates sound production. Errors in motor planning for initiation and cessation of speech lead to vocal dysfluency. Motor control of respiration is critical for a myoelastic-aerodynamic sound generation mechanism that is used by humans and many other vocalizing animals. Developing our understanding of how the forebrain assumes control of brainstem respiratory circuitry is essential for understanding language initiation, execution, and termination. Songbirds are an animal model for speech production in humans because of the numerous similarities between song learning and production and language acquisition and speech production. Zebra finches sing a learned song that is composed of a motif of 4-7 syllables. The motif is repeated multiple times to form a song bout. Here we explore how a cell-type specific class of neurons control sound termination in zebra finches (*Taeniopygia guttata*). In songbirds, motor production of song requires activity in premotor and motor cortical analogues HVC (letters used as proper name) and RA (robust nucleus of the arcopallium), respectively. Using an AAV (adeno-associated virus) as a viral vector for expression of a red-shifted opsin (ChRmine), neurons in premotor cortex inserted soma-targeted, membrane-bound ion channels that allowed for light-dependent manipulation of ongoing cellular activity. Using real-time recording of singing, optical stimulation was timed with the production of individual song syllables. Light stimulation was delivered while birds produced self-initiated and female-directed songs, as well as during quiet respiration. We measured respiratory pressure in birds while they were singing by inserting a small piece of silastic tubing into the anterior thoracic air sac. Our goal was to determine how aberrant activation of HVC_{RA} neurons controls song-related respiratory patterns.

METHODS

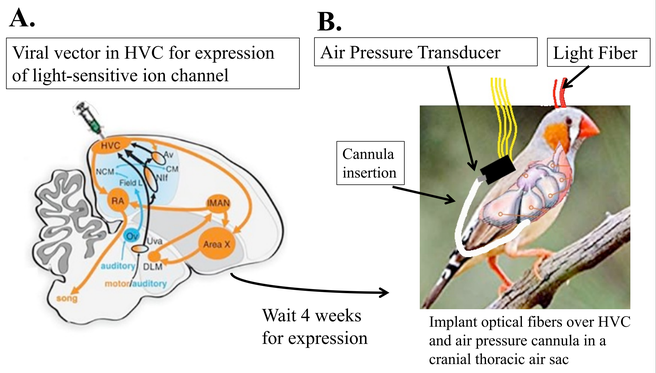

Figure 1: Methods and procedure.

- A. The premotor region HVC (proper name) was targeted for expression of light sensitive ion channels (ChRmine) using an AAV vector
- B. After allowing time for expression, light fibers were placed above the surface of the brain and a cannula was used to measure song-related respiratory patterns.
- Software identified the acoustic features of specific song syllables that allowed for the precisely timed administration of light pulses to optogenetically activate HVC neurons during song.
- Resultant changes in song respiration induced by optogenetic activation of HVC neurons were compared to the normal variation that occurred during unstimulated song renditions.

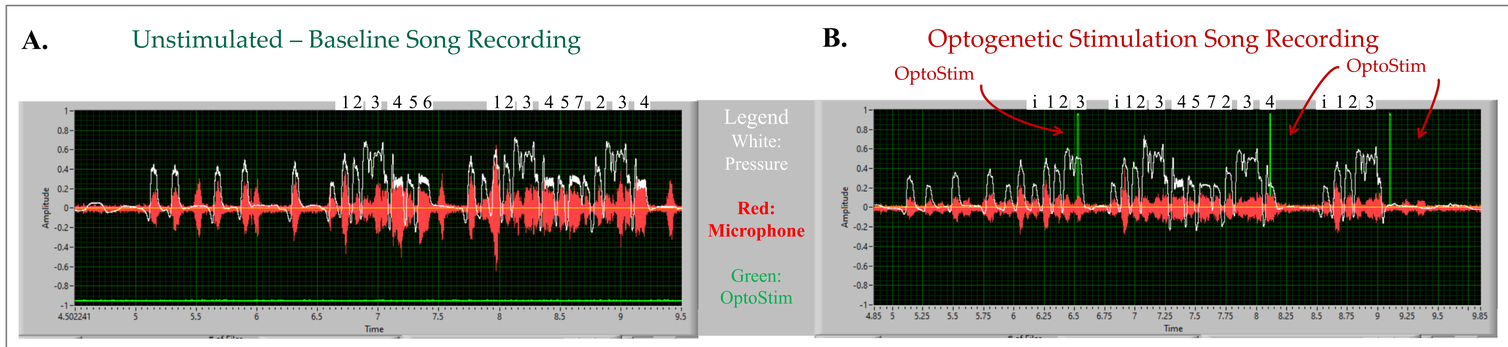

Figure 2: Example recording traces. **A.** The bird's song respiration (white, air pressure, red microphone) are displayed for an unstimulated song recording. The yellow line indicates ambient pressure, dividing air pressure into expiratory and inspiratory phases of respiration. **B.** A song during which optical stimulation of HVC neurons (OptoStim) was performed. During normal song, the bird sang a sequence of 6-7 syllables (motif), but optogenetic activation of HVC neurons leads to a truncation of the ongoing syllable, a variable pause, and then the bird resumed singing back at the start of the motif.

RESULTS – HVC Opto-Stimulation Disrupts Song Respiration

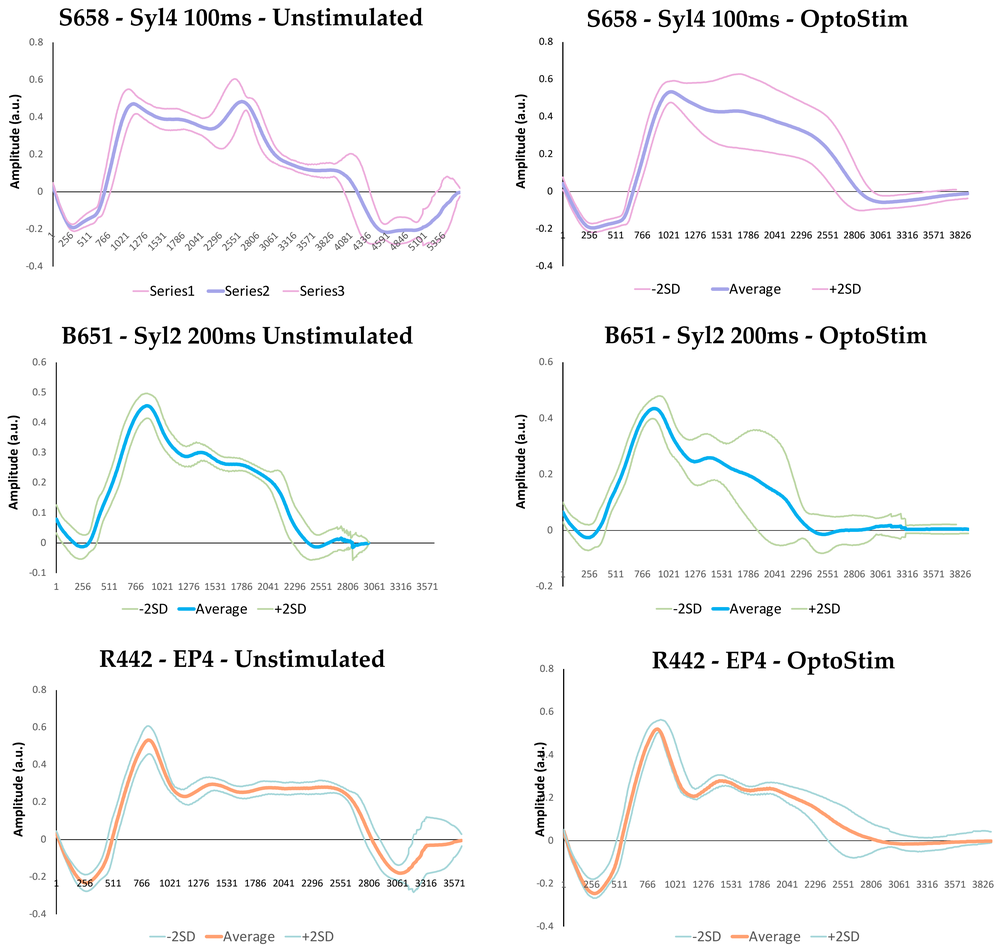

Figure 3: HVC Optogenetic stimulation results in a suppression of ongoing respiration. Expiratory pulses of air generate song syllables. The average ±2SD is displayed for individual syllables when they are unstimulated (left) and during optogenetic stimulation (right). A clear suppression of ongoing song-related respiration is observed following optogenetic activation of HVC.

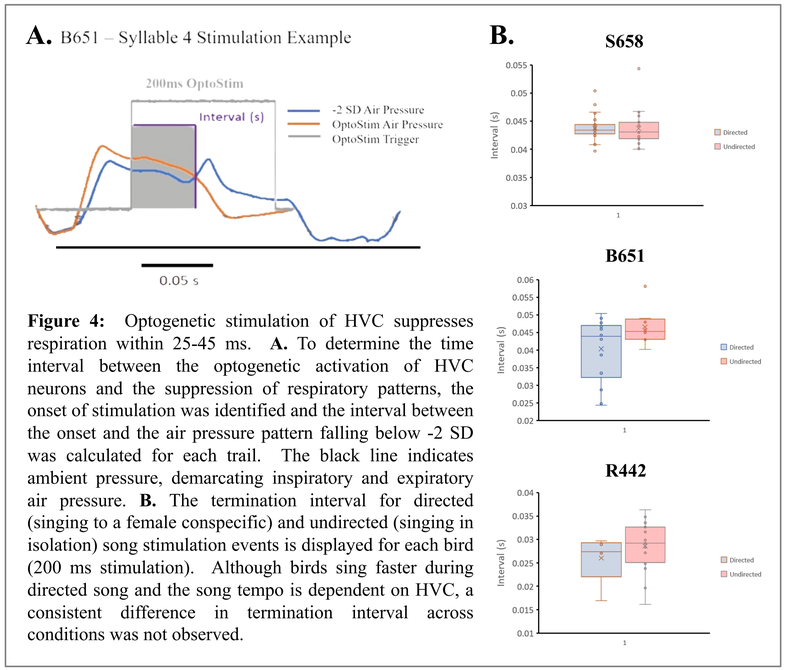

Figure 4: Optogenetic stimulation of HVC suppresses respiration within 25-45 ms. **A.** To determine the time interval between the optogenetic activation of HVC neurons and the suppression of respiratory patterns, the onset of stimulation was identified and the interval between the onset and the air pressure pattern falling below -2 SD was calculated for each trail. The black line indicates ambient pressure, demarcating inspiratory and expiratory air pressure. **B.** The termination interval for directed (singing to a female conspecific) and undirected (singing in isolation) song stimulation events is displayed for each bird (200 ms stimulation). Although birds sing faster during directed song and the song tempo is dependent on HVC, a consistent difference in termination interval across conditions was not observed.

CONCLUSIONS

- The premotor nucleus HVC is critical for maintaining the normal song tempo and sequence of song syllables.
- Optogenetic activation of HVC during song caused a truncation of the ongoing song respiratory patterns, a short latency pause, and then a resetting of song back to the beginning of the bird's motif.
- The typical song syllables are produced with little variation, but optogenetic stimulation of HVC causes a more variable song pressure pattern and a suppression of the normal duration of the ongoing vocal breath.
- The range of stimulation disruption intervals was 25-45 ms. The differences in intervals across birds may be related to viral expression patterns. Singing context did not systematically shift the interval, although there is a trend towards doing so in 2 of the 3 birds.
- These data illustrate that volitional respiration in songbirds is controlled by premotor cortical region HVC, and that song termination arises from a suppression of brainstem respiratory patterns.

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