

## Introduction

The philosophical concept of free will is often highly debated. Benjamin Libet, an academic in experimental philosophy, discovered through recordings of cerebral activity that there is neural activity that correlates with a decision prior to our conscious declaration of that decision (Libet et al., 1983). In songbirds, previous studies have begun to show an increase in neuronal and respiratory activity in the seconds prior to song production, indicating preparatory action before performing this learned behavior (Daliparthi et al., 2019).

Singing in songbirds is a learned behavior that is passed down from one generation to the next via imitative learning. Birds initiate song in response to the presentation of a female bird (directed) or spontaneously when in isolation from other birds (undirected). The production of song requires the control of respiratory, vocal organ, and upper vocal tract motor systems; these motor systems are controlled by the activation of neural networks within specific areas of the brain leading to subsequent activation of the expiratory muscles roughly 20 ms after.

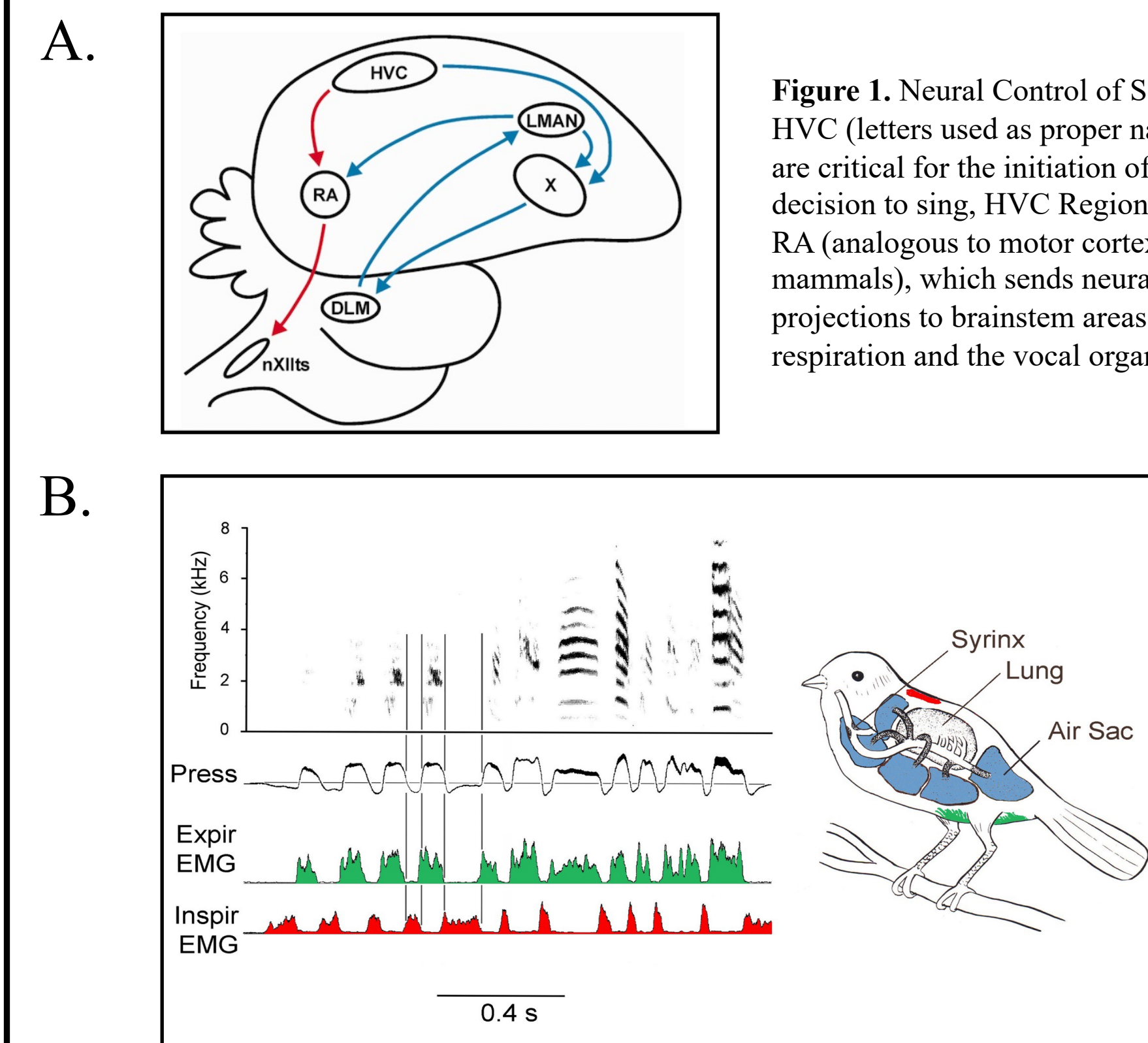
In this study, the preparation for the learned motor behavior (song production) is compared against an unlearned control (defecation) in Zebra Finches.

Electromyography (EMG) of respiratory muscles is employed as an additional technique to provide more detailed exploration of preparatory motor activity compared to previous studies. Our analysis of EMG recordings focused on the six expirations that occur prior to song production, because previous research has shown that there is an acceleration of the respiratory rhythm occurring in the last three respiratory cycles before song (Méndez et al., 2022). By measuring the electrical activity in the muscle, we hope to provide a more detailed understanding of how birds prepare for their upcoming song. Overall, this study aims to explore motor responses to determine whether expiratory muscular activation is preparatory for and predictive of an upcoming behavioral event. The larger goal of this study is to be able to “read a bird’s mind” by establishing physiological models for predicting behavior before the decision has occurred.

## Method

Subsyringeal air pressure was recorded from two zebra finches. Each bird was accustomed to holding a pressure transducer on its back held in place by an elastic band. The weight of the transducer was off-set by a counterbalance arm to facilitate free movement of the bird around the cage. A cannula was then inserted surgically into the bird, allowing for measurement of subsyringeal air pressure changes inside the anterior thoracic air sac before, during, and after spontaneously generated song events and defecation.

Additionally, two fine wire electrodes were inserted into the abdominal expiratory muscles in order to collect data regarding the activation of expiratory muscles. Collectively, this allowed for EMG, air pressure, and acoustic record data collection.

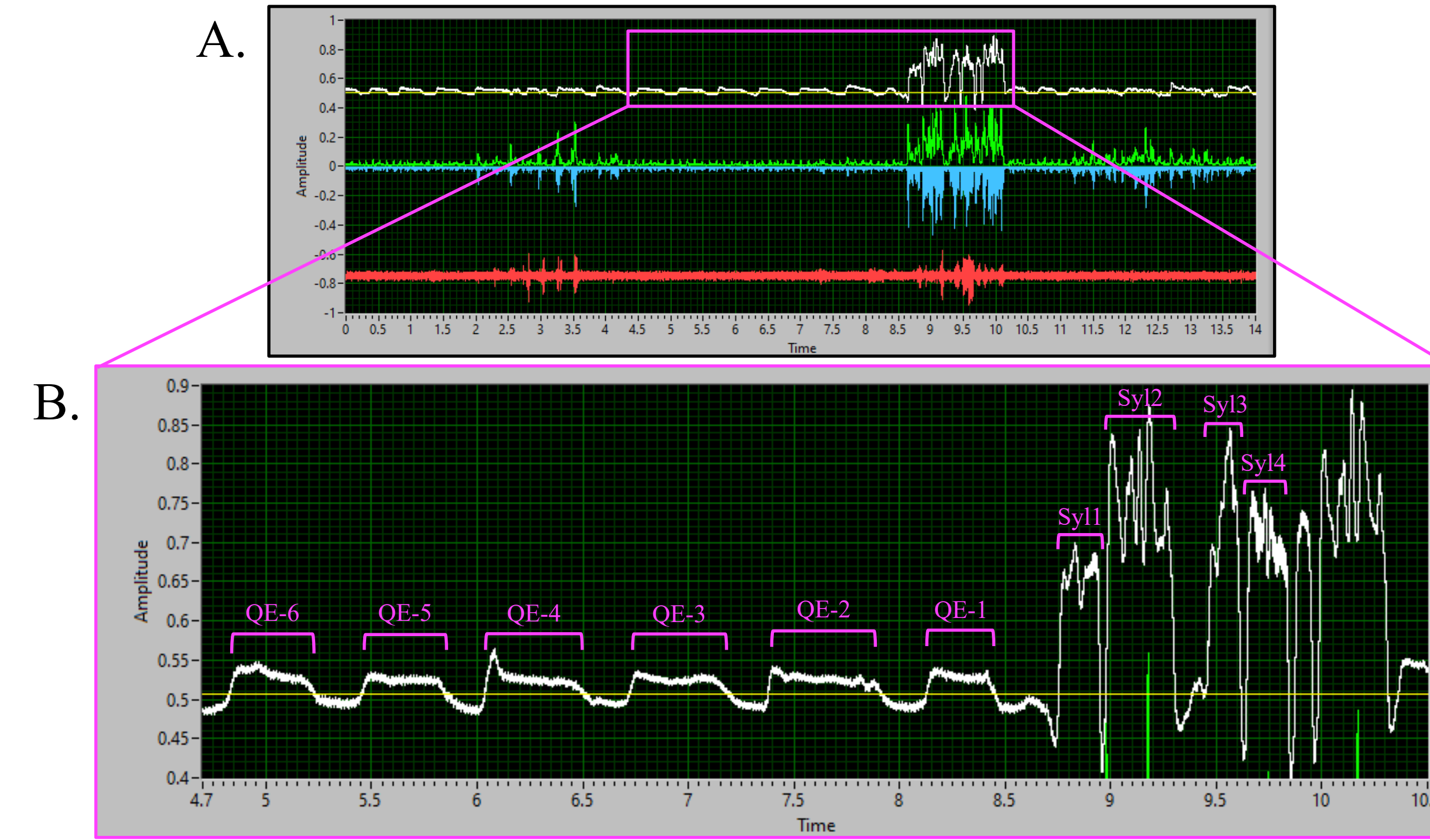


**Figure 1.** Neural Control of Song. The HVC (letters used as proper name) nuclei are critical for the initiation of song. In decision to sing, HVC Region projects to RA (analogous to motor cortex in mammals), which sends neural projections to brainstem areas controlling respiration and the vocal organ.

**Figure 2.** Respiratory Control of Song. In Aves, both expiration and inspiration are active muscular processes. The syrinx (avian vocal organ) contains two sets of vibratory tissues that regulate airflow for expiration, inspiration, and vocalization occurs almost exclusively during expiration. Song respiratory are faster tempo and higher amplitude compared to quiet respiration.

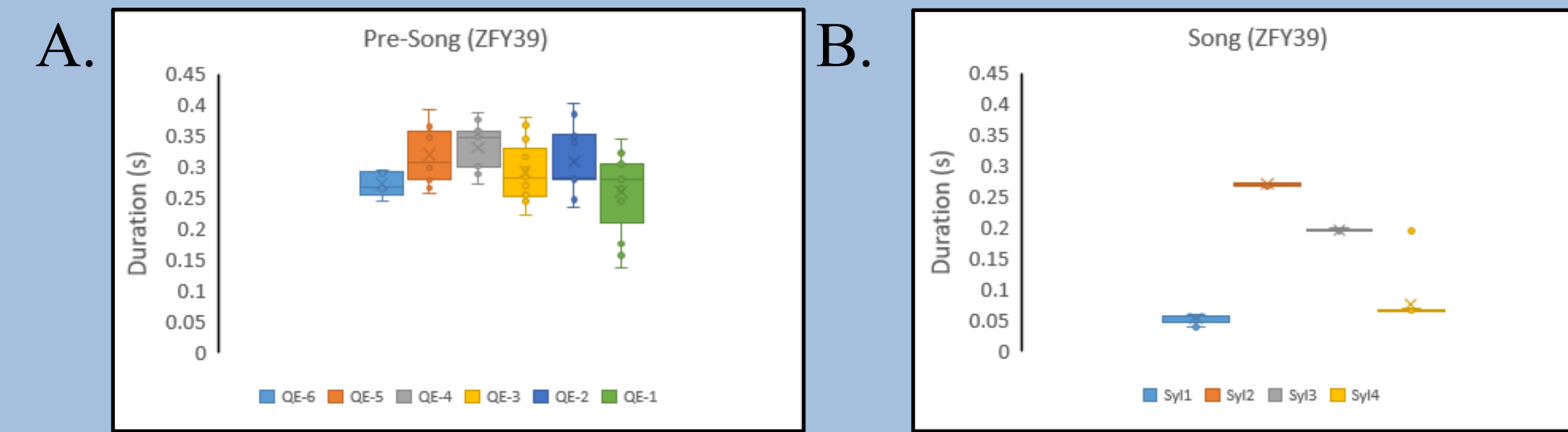
## Can we use the past to predict the future?

### 1. EMG analyses of the zebra finch expirations prior to and during song.



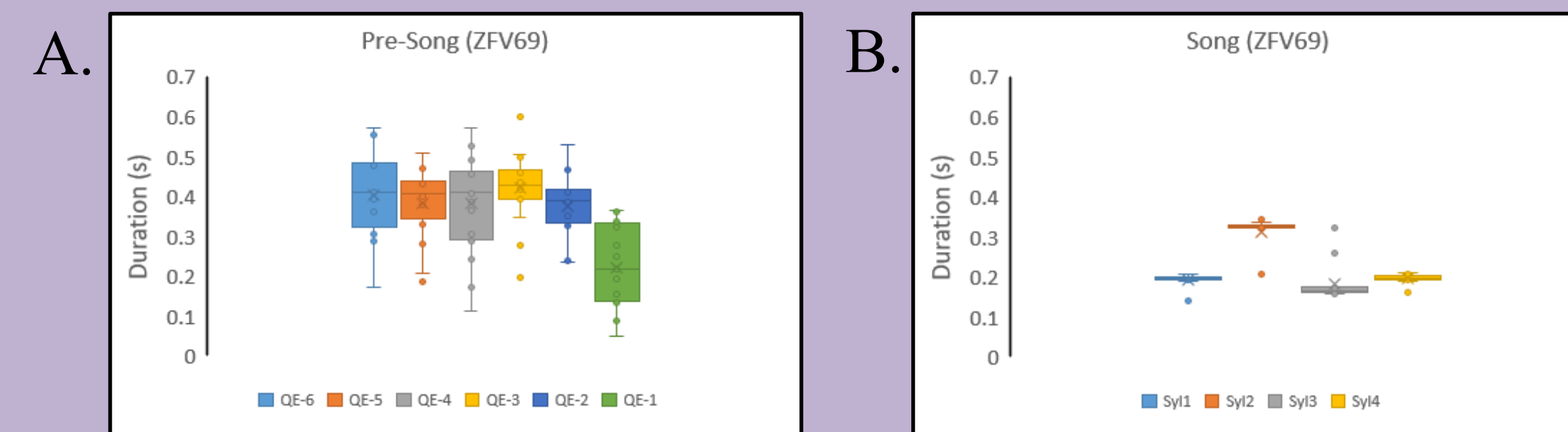
**Figure 3.** A) View of song motif with air pressure (white), ambient pressure (yellow), raw EMG (blue), averaged EMG (green), and acoustic record (red). B) Focus on quiet expirations (QE-s) and song syllables (Syls).

#### Expiratory Duration:



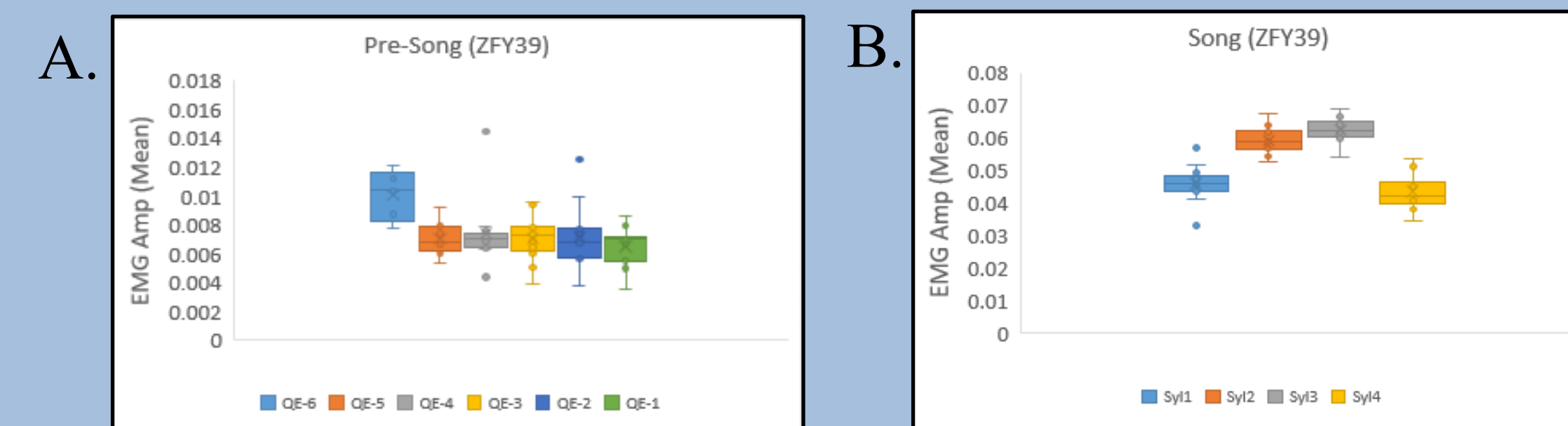
**Figure 5.** Average duration of A) quiet expirations, and B) song production in bird ZFY39.

Expiratory duration is less stereotyped compared to song. Although, there is a marked drop in duration of the expiration immediately preceding song that is consistent among the birds.



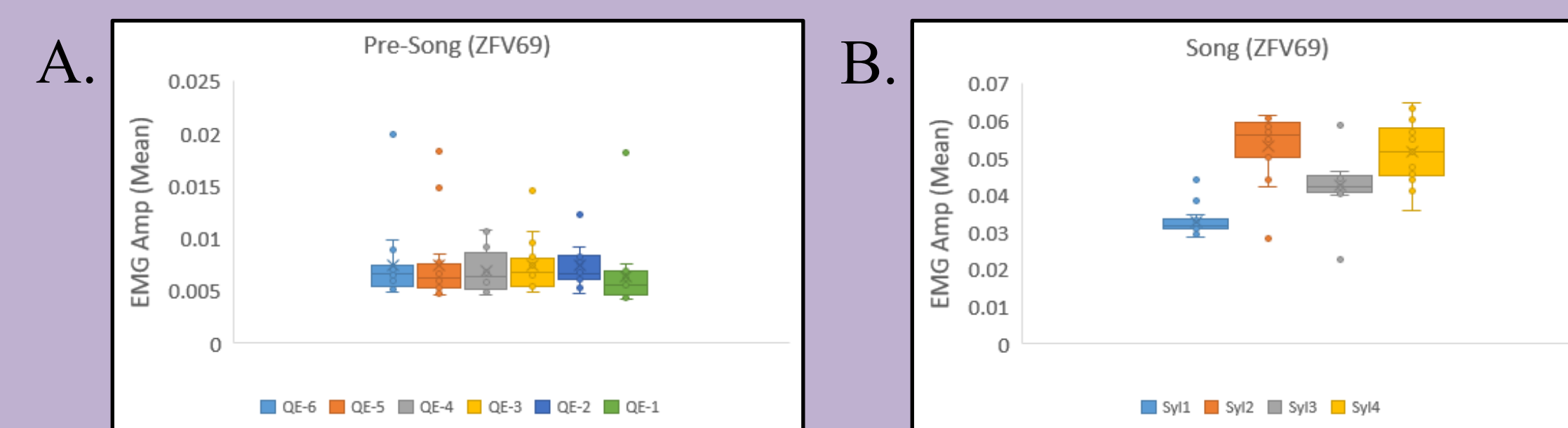
**Figure 6.** Average duration of A) quiet expirations, and B) song production in bird ZFV69.

#### Expiratory EMG:



**Figure 7.** Expiratory muscle activation in bird ZFY39. A) Average EMG amplitude during quiet expirations. B) Average EMG amplitude during song production.

Average EMG amplitude is lower in preparatory expirations compared to song. The greatest effect is seen in the expiration immediately preceding song, in which there is a clear decrease in expiratory muscle activation.

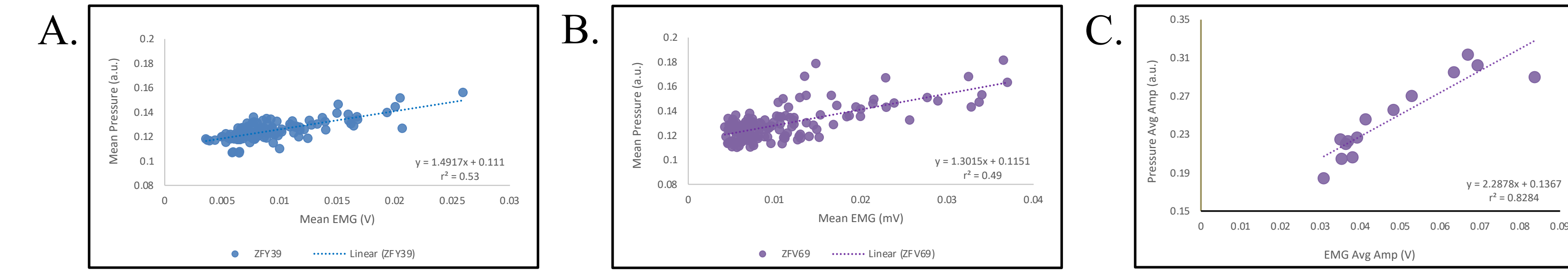


**Figure 8.** Expiratory muscle activation in bird ZFV69. A) Average EMG amplitude during quiet expirations. B) Average EMG amplitude during song production.

## Data Validation Analysis

● = ZFY39 ● = ZFV69

A concern with low amplitude signals, like quiet breathing, is that the resolution may not be sufficient for detecting changes in the signal that would correspond to changes in behavior. We explored whether EMG Mean Amplitude predicted the outcome variable mean air pressure amplitude in multiple birds and across conditions.



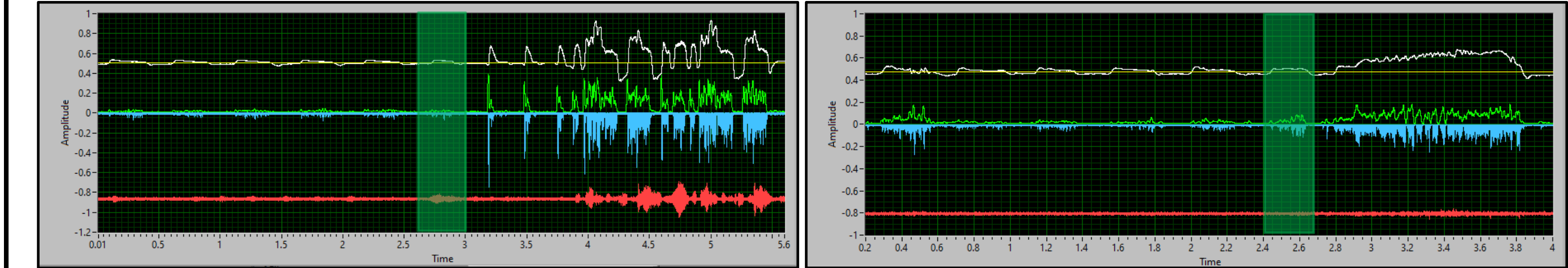
**Figure 4.** Regressions between average EMG and air pressure amplitudes. Average respiratory EMG signal was sufficient to predict the average air pressure amplitude for A) song preparatory expirations in bird ZFY39, B) song preparatory expirations in ZFV69, and C) during defecation.

## Can we use the past to predict the future?

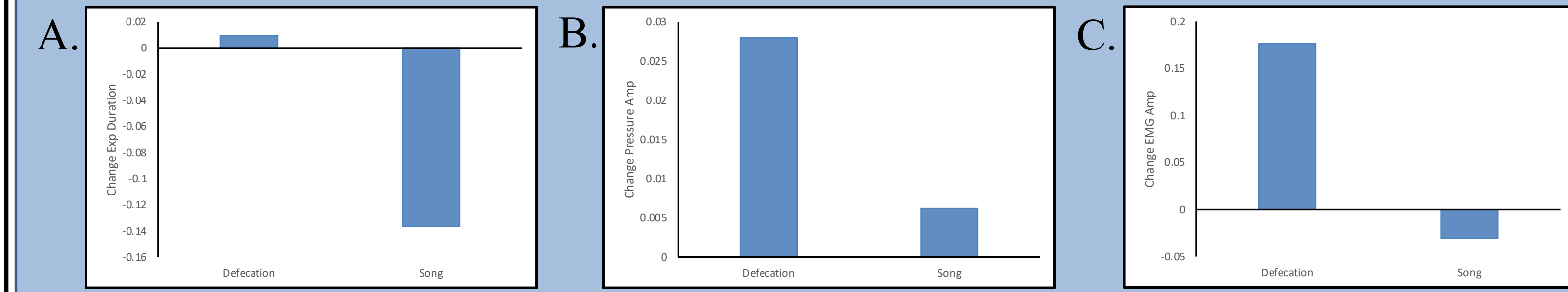
### 2. EMG analyses of the zebra finch song preparatory expirations compared to an unlearned control, defecation.

Last Quiet Expiration Before Song

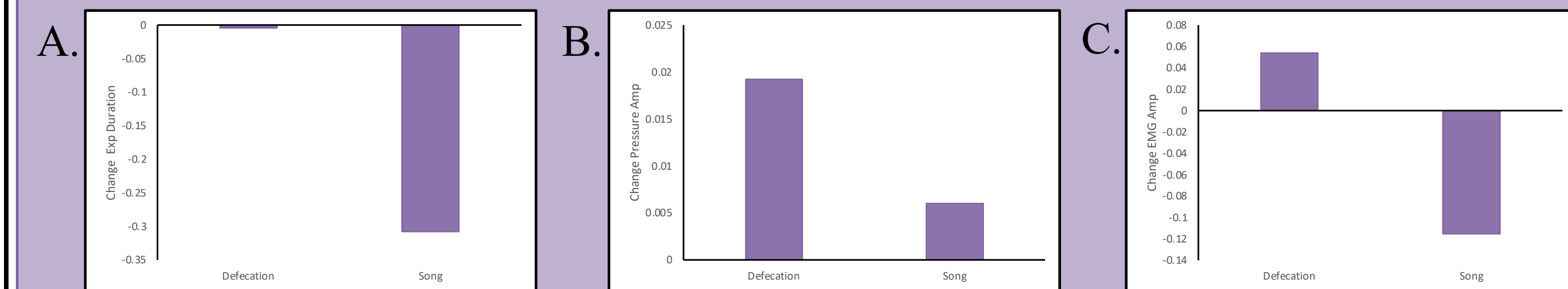
Last Quiet Expiration Before Defecation



Song preparatory expirations were compared to defecation by quantifying the changes between QE-2 and QE-1 in each condition. The change scores reflect an increased pressure during defecation, most likely due to the syrinx being closed. Further, expirations prior to song show an observable reduction in duration and EMG amplitude, each reflecting decreased expiratory muscle activation.



**Figure 9.** Change scores from QE-2 to QE-1 in defecation and song conditions for bird ZFY39.



**Figure 10.** Change scores from QE-2 to QE-1 in defecation and song conditions for bird ZFV69.

## Conclusions

1. Validation analysis shows EMG signal as a valid measure of activation of expiratory muscles during quiet breathing before song, illustrating that depending on level syringeal gating of air flow, muscle activation can predict 50% to 100% of the resultant air pressure.
2. Consistent change in respiratory rhythm that matches previously published results, and a reduction in the activation of the expiratory muscles. This suggests that before birds begin to sing, the neural system begins by inhibiting brainstem respiratory circuits.
3. Reduction is exaggerated compared to an experience-independent behavior (defecation), suggesting that learned motor behaviors require more extensive planning for the upcoming motor actions.
4. Future neural models should be developed to incorporate respiratory control that precedes vocalization, as there are significant changes in respiration in the seconds prior to vocal production. New models may help identify and predict song events, and better understand how fluent vocalizations are shaped by preparatory motor processes.

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