Efficacy Modeling of Passive Muscimol Diffusion into HVC

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Introduction

Birdsong, like human speech, is a vocal behavior that requires birds to precisely coordinate the activation of respiratory and vocal organ motor systems in order to generate sufficient subsyringeal air pressure for phonation. Inability to exert sufficient muscle contraction or to time the activity of these motor systems results in a impaired, often lower amplitude, vocalization.

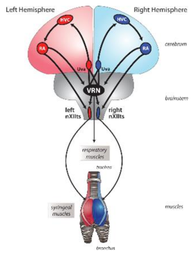


Figure 1. Avian brain areas involved in vocal production. Premotor nucleus HVC and downstream nuclei (e.g. motor nucleus RA and brainstem nuclei) are key for generating audible vocalizations. Via RA, HVC can control vocal muscles through nXIIts and respiratory muscles through the ventral respiratory network (VRN).

In songbirds, generating sufficient internal driving air pressure involves activation of the telencephalic nuclei in the song system; we have found that HVC (proper name) regulates air pressure amplitude via the motor nucleus RA and respiratory motoneurons in the midbrain and brainstem (Figure 1). In both juvenile and adult songbirds, ablation or inactivation of HVC (proper name) neural activity disrupts temporal features of song. Consistent with the role of respiration in controlling song tempo, we have previously demonstrated that HVC contributes to song respiratory features in adult male Bengalese finches (*Lonchura striata domestica*, Urbano & Cooper, 2015).

Here we further explored these findings by recording subsyringeal air sac pressure in singing birds prior to, during, and following recovery from HVC inactivation. Dialysis probes were implanted into either left or right HVC in adult male Bengalese finches (N=5), allowing for reversible suppression of neural activity with the GABA (gamma-aminobutyric acid) agonist muscimol.

Here we used an established measure of efficacy (Beggs & Dobrovolny 2015) to model the change in peak song EP amplitude during HVC inactivation and we were able to produce consistent estimates for the variables in our model. We then ran a Monte Carlo simulation and parameter stability analysis to determine that the chosen model is robust and truth-conducive in predicting future values for peak EP amplitude under continued diffusion of muscimol into the tissue. We tested this model against simpler models to rule out alternative explanations.

Experimental Methods

•Surgical, drug administration, and recording procedures

- A reverse microdialysis probe was implanted into either left or right HVC (Figure 2) and filled with saline daily.
- Acoustic and air sac pressure were simultaneously recorded in a multi-channel .wav file . Recording occurred 24/7, triggered by a custom threshold.
- Once birds resumed singing, probes were filled with muscimol (1.5 mg/mL, 1.125mg/mL). After 4-6 hours, the drug was flushed and replaced with saline.
- Respiratory air pressure amplitude was measured before and during drug infusion.

Reverse microdialysis in HVC

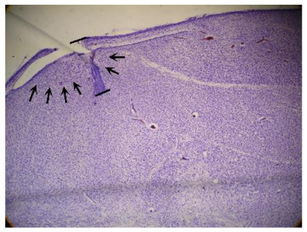


Figure 2. Example verification of dialysis probe implant. Sagittal sections 50 µm thick were stained with cresyl violet for identifying location of the dialysis implant. The dialysis membrane was 1mm long with an exposed area of 500 µm. 200 µm from probe tip was nonpermeable. Probes were inserted at a depth of 900 µm ventral to tissue surface (black lines).

Computational Methods

•Optimization of parameters with competing models

- Two models were selected to predict the change in expiratory pressure amplitude.
- One based on a constant dosage over the course of the experiment, and one on an exponential decay.
- The *least_squares* method from the *scipy.optimization* library within the *python* scripting language was used to optimize the parameters in both models.
- This method is efficient, yet greedy; it will choose the first set of parameters that fits the criteria of being a local minimum with respect to the sum of squared residuals.
- We randomized the initial guess of parameters within predetermined bounds to ensure that the set of optimized parameters was stable.

•Creating and verifying a computational model

- We borrowed an efficacy model from Beggs & Dobrovolny:

$$e(t) = \frac{e_{max} D(t)}{e_{max} + IC_{50}}$$

- The parameter e_{max} represents the maximum efficacy, D(t) represents the dosage as a function of time, and IC_{50} is the concentration where 50% inhibition is observed.
- Then we substituted an exponential decay equation for the dosage function, and a constant function for the competing model, which reduces to just a constant (c).

$$e(t) = \frac{e_{max} A_0 e^{r(t-t_0)}}{e_{max} + IC_{50}}$$

$$e(t) = c$$

- A_0 here is the initial relative max EP, r is the rate of clearance, and t_0 represents a small time shift correcting for the wide variation in when the first data point is collected.

Prolonged passive diffusion exhibits nonlinear recovery

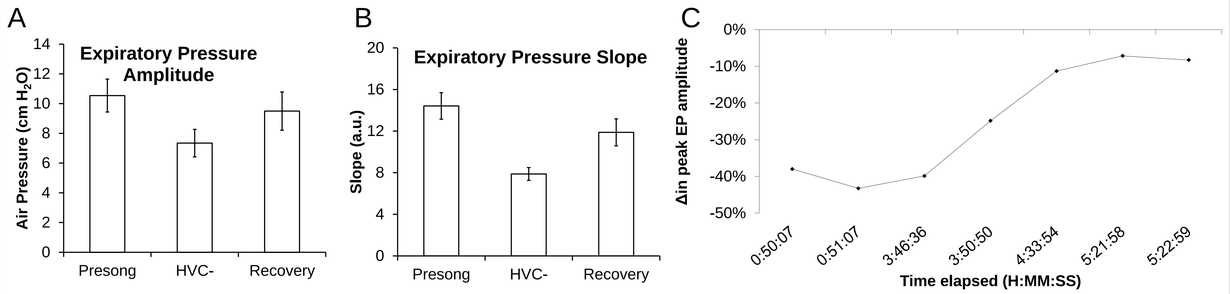
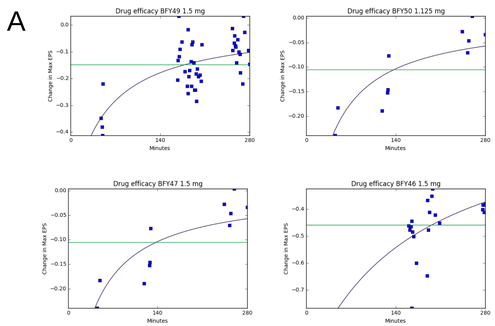


Figure 3. Musicmol diffusion into HVC causes a transient reduction in song air expiratory pressure amplitude and slope. A) Decline and recovery in EP amplitude (One-way ANOVA: $F(2, 16) = 22.55$, $p < 0.001$). B) Decline and recovery in EP slope ($F(2, 16) = 23.88$, $p < 0.001$) Presong = 1-4 hours of presong recording; HVC- = 4-6 hours of GABA-ergic suppression of HVC neural activity; Recovery 4-6 hours of recording after saline flush. C) Peak EP amplitude of sequential song bouts during HVC-. EP amplitude of the first few songs dropped by 40-45%, but showed nonlinear recovery over time.

Results



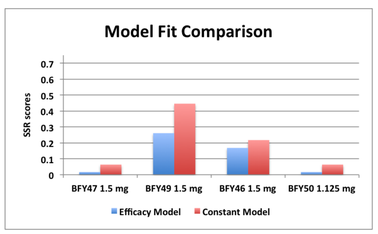


Figure 4. Qualitative assessment and preliminary results
A) It's clear that the exponential model of efficacy wins out over the constant diffusion model. However, a quantitative measure was also used as a second check against which model should be chosen (B). Each data set produced a better fit by measure of a low SSR score for the exponential decay diffusion.

Further Research

- We expected a constant efficacy (change in max EP), however clearly a different model predicts the data better. That points to the fact that another mechanism may be at hand.
- It may be the case that the muscimol from the muscimol-saline solution contained in the reservoir is diffusing out of the saline and into the brain causing the dosage at later times to be pure saline and ineffective.
- A secondary explanation is that an unaccounted for clearance mechanism such as astrocytes and glial cells have a rate of action faster than the dosage was administered. Compounded with this may be up or down regulation of neuronal activity. If that were true, it would explain the fact the unexpected behavior as down regulated neurons would not be affected by muscimol injection. We will investigate further on this matter.

SciCom Summary

Birdsong is a behavior driven by a particular area of the avian brain called the HVC. We infused an inhibitory drug into this area of the brain and observed that while the drug was still being infused, birdsong gradually returned to normal, which is very peculiar behavior. We fit an already established model to our data and found evidence that an undiscovered mechanism may be responsible.