J Neurophysiol 120: 1186–1197, 2018.
First published June 20, 2018; doi:10.1152/jn.00153.2018.

RESEARCH ARTICLE | *Neural Circuits*

Interhemispheric dominance switching in a neural network model for birdsong

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Submitted 2 March 2018; accepted in final form 18 June 2018

Galvis D, Wu W, Hyson RL, Johnson F, Bertram R. Interhemispheric dominance switching in a neural network model for birdsong. *J Neurophysiol* 120: 1186–1197, 2018. First published June 20, 2018; doi:10.1152/jn.00153.2018.—Male zebra finches produce a sequence-invariant set of syllables, separated by short inspiratory gaps. These songs are learned from an adult tutor and maintained throughout life, making them a tractable model system for learned, sequentially ordered behaviors, particularly speech production. Moreover, much is known about the cortical, thalamic, and brain stem areas involved in producing this behavior, with the premotor cortical nucleus HVC (proper name) being of primary importance. In a previous study, our group developed a behavioral neural network model for birdsong constrained by the structural connectivity of the song system, the signaling properties of individual neurons and circuits, and circuit-breaking behavioral studies. Here we describe a more computationally tractable model and use it to explain the behavioral effects of unilateral cooling and electrical stimulations of HVC on song production. The model demonstrates that interhemispheric switching of song control is sufficient to explain these results, consistent with the hypotheses proposed when the experiments were initially conducted. Finally, we use the model to make testable predictions that can be used to validate the model framework and explain the effects of other perturbations of the song system, such as unilateral ablations of the primary input and output nuclei of HVC.

NEW & NOTEWORTHY In this report, we propose a two-hemisphere neural network model for the bilaterally symmetrical song system underlying birdsong in the male zebra finch. This model captures the behavioral effects of unilateral cooling and electrical stimulations of the premotor cortical nucleus HVC during song production, supporting the hypothesis of interhemispheric switching of song control. We use the model to make testable predictions regarding the behavioral effects of other unilateral perturbations to the song system.

birdsong; computational model; neural network; zebra finch

INTRODUCTION

Complex sequential behaviors such as speech and the production of music rely on carefully timed movements produced by hierarchically organized neural networks. An example of such a behavior is birdsong, which consists of an ordered sequence of vocalizations called syllables separated by silent gaps. Male zebra finch song is often studied because, unlike many other songbirds, the bird learns a single set of syllables in a specific order, called a motif, and maintains that song throughout its life. The premotor cortical nucleus HVC (proper name) is a key element of the neural song system and is necessary for adult song production (Nottebohm et al. 1976; Simpson and Vicario 1990). HVC coordinates song production via an efferent pathway to the cortical motor nucleus robust nucleus of the arcopallium (RA), which directly controls the respiratory and vocal musculature (see Fig. 1). The role of HVC in syllable timing was established by experiments in which HVC was bilaterally cooled, resulting in a nearly uniform stretch of the song (Long and Fee 2008). More recent experiments showed that syllables stretch more than gaps when HVC is cooled (Fig. 2 in Andalman et al. 2011), which may reflect the participation of brain nuclei other than HVC.

In a recent publication (Galvis et al. 2017), we proposed a model for the neural coding of birdsong in the male zebra finch. Key elements of this model are a distributed network with roles for nuclei in addition to HVC in song production, as in Gibb et al. (2009b), chainlike timing structures within HVC that code for both syllables and the gaps between syllables, and differential roles for the medial and lateral portions of HVC. As expected from any model of the song system, this model accounts for motif production utilizing sparse coding within HVC (Hahnloser et al. 2002). It also accounts for the data on bilateral HVC cooling and data from our laboratory showing that bilateral ablation of lateral HVC results in dropped syllables whereas bilateral ablation of medial HVC results in atypical syllable transitions (Basista et al. 2014). That model does not, however, account for the apparent distribution of song coding between left and right HVC. That is, motor dominance appears to switch back and forth between the two hemispheres during song production. One piece of evidence for this is the finding that unilateral cooling led to stretching of some portions of the song but not others and the portions stretched by cooling the right hemisphere were anticorrelated with portions stretched by cooling the left hemisphere (Long and Fee 2008). Another study showed that unilateral stimulation of HVC led to song perturbations such as syllable distortions, syllable truncations, song stops, and song restarts (Wang et al. 2008). These stimulations were only effective during some portions of the song, and portions where left stimulation

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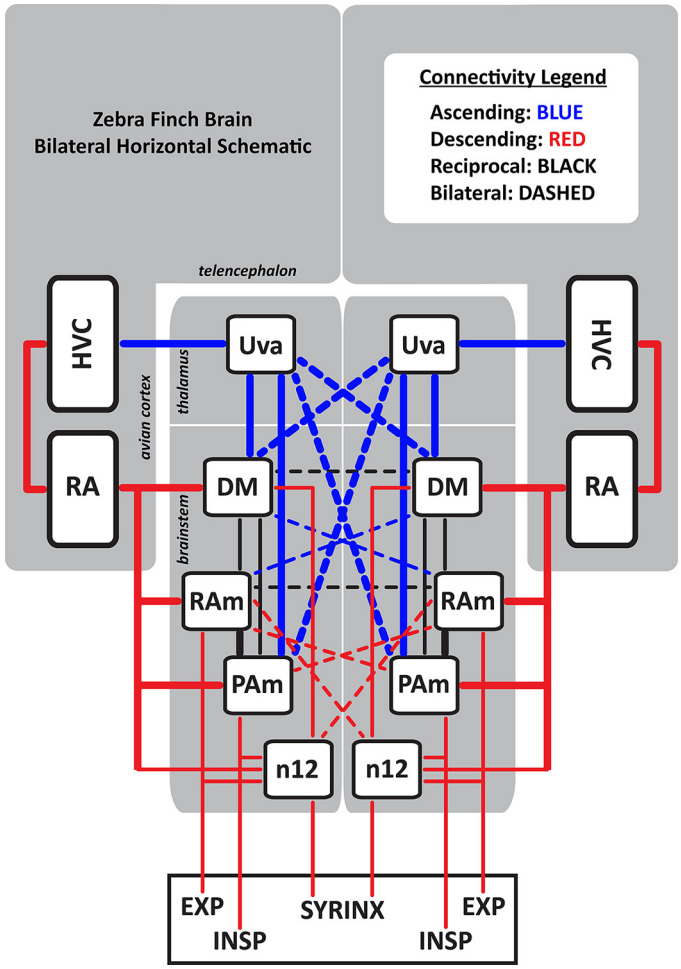

Fig. 1. Zebra finch song system showing descending, ascending, and reciprocal connectivity. Connections that are explicitly used by our model are shown in bold. Ipsilateral connections are shown as solid lines, while contralateral connections are shown with dashed lines. The respiratory nuclei in the brain stem, dorsomedial nucleus of the intercollicular complex (DM), nucleus retroambigualis (RAm), and nucleus paraambigualis (PAm), provide contralateral coupling that, in the model, is the synchronizing element of the distributed network. Signals from respiratory brain stem control the respiratory nerves (insp/exp) and converge with signals from robust nucleus of the arcopallium (RA) on the vocal nerve nucleus (n12). Uva, uvaeform nucleus of thalamus.

was effective were anticorrelated with portions where right stimulation was effective (Wang et al. 2008).

In this report, we illustrate how dominance switching can occur in the neural song system, extending our previous recurrent network model by including model neurons from each hemisphere. A challenge faced by any model of distributed coding is that of synchronization. This challenge is particularly acute here, since birds lack a corpus callosum, so the left and right HVC (as well as the left and right RA) have no direct connections. If parts of the song are coded in left HVC and parts are coded in right HVC, how do they coordinate their activity? It has been suggested that this coordination occurs through the nuclei in the brain stem, in particular the expiratory nucleus retroambigualis (RAm), the inspiratory nucleus paraambigualus (PAm), and the dorsomedial nucleus of the intercollicular complex (DM), which are bilaterally connected to the uvaeform nucleus of the thalamus (Uva), an input to HVC (Ashmore et al. 2008a). Since these nuclei are elements in a recurrent loop from HVC onto itself, they provide a natural synchronizing center that we utilize in our model to coordinate activity in the two hemispheres.

Although feedforward, or "synfire," chains of coupled HVC projection neurons are elements common to models of the zebra finch song system (Andalman et al. 2011; Galvis et al. 2017; Gibb et al. 2009a, 2009b; Long et al. 2010; Long and Fee 2008; Weber and Hahnloser 2007) and multiple experiments have been conducted to support such a model (Benezra et al. 2018; Long et al. 2010; Lynch et al. 2016; Picardo et al. 2016), it has never been clear whether these synfire chains should code for the entire motif, the individual syllables and gaps that make up the motif, or just segments of the syllables and gaps. The data on unilateral electrical stimulation of HVC suggest that syllables are composed of segments, each of which is coded separately (Wang et al. 2008). Also, representing HVC timing as a chain with many small segments results in a good fit to spike correlation data (Weber and Hahnloser 2007). In our model, we hypothesize that synfire chains code for syllable segments and separate chains code for intersyllable gaps. This provides the flexibility needed to account for the electrical stimulation data as well as the data on unilateral HVC cooling. Finally, we describe several stimulation and cooling experimental manipulations that could be performed to test and refine the model.

METHODS AND NETWORK MODEL

The computer code described below can be downloaded from https://www.math.fsu.edu/~bertram/software/birdsong.

Neural ensemble models. We use firing rate models of neural subpopulations or ensembles, where an ensemble is described by the variable X, which can take on a value in the interval 0 to 1. If $X = 0$ the ensemble is silent, whereas if $X = 1$ it is maximally active. Time is discretized into steps of length 0.1 ms, and at each time step the activity of each ensemble is updated to reflect inputs that are themselves the activity levels of other ensembles at previous time steps. To highlight the time dependence, we will often use the notation $X(t)$ to denote the activity level of an ensemble at time t. This firing rate representation is used as a simplification of the biophysical Hodgkin-Huxley representation that we employed in an earlier study (Galvis et al. 2017). Computer simulations with the firing rate models are orders of magnitude faster than with the biophysical models, but they capture the network behavior. A similar simplification was employed in a Markov model of HVC and RA activity (Weber and Hahnloser 2007).

The state of a postsynaptic ensemble depends on the firing rates of the presynaptic neurons, as well as the coupling strength α_j, which also takes values between 0 and 1. If two nodes are not connected $\alpha_j = 0$, and unless otherwise noted connected nodes take $\alpha_j = 1$. The action potential latency from pre- to postsynaptic neurons, which we refer to as the synaptic delay, is set at $\delta = 3$ ms, as suggested in Ashmore et al. (2008b). Thus

$$X_{\text{post}}(t) = \sum_j \alpha_j X_j(t - \delta) \tag{1}$$

where the summation is over all presynaptic neuron ensembles.

In some cases (the RA transition neurons), we assume that ensemble activity occurs only if the input is above a threshold, and in this case the activation is maximal. These are described by a Boolean function:

$$X_{\text{post}}(t) = \begin{cases} 1 \text{ if } \sum_j \alpha_j X_j(t - \delta) \geq 0.5 \\ 0 \text{ otherwise} \end{cases} \tag{2}$$

In an earlier version of the model, HVC neurons that project to RA (HVC_{RA}) and PAm neurons were represented by intrinsic bursting

neurons that activate in the presence of a sufficiently large input, stay active for a short time, and turn off even if the input continues (Galvis et al. 2017). To capture this behavior, these ensembles are set according to *Eq. 1*, but if activated, X_{post} stays on for 6 ms before inactivating. Once inactivated, it cannot be reactivated until after a 20-ms refractory period. The length of the active period is based on in vivo data showing that HVC_{RA} neurons burst for ~6 ms when the bird is singing (Hahnloser et al. 2002).

There are separate ensembles of inhibitory neurons. An ensemble postsynaptic to an inhibitory ensemble is set to $X_{post}(t) = 0$ if $X_{pre}(t - \delta) > 0$.

Single-hemisphere recurrent network model. In this study, we extend our previous recurrent network model (Galvis et al. 2017) in which transitions from syllables to gaps and vice versa occur through the bilateral pathway: HVC→dorsal RA (RA_d)→respiratory nuclei (RAm/PAm/DM)→Uva→HVC (bold links in Fig. 1). (See Galvis et al. 2017 for the rationale for the exclusion of other nuclei from the model.) In this model, each syllable and gap is encoded by a synaptic chain of HVC_{RA} neurons. Model songs are chosen randomly with 4–8 syllables and syllable and gap lengths averaging 106.0 ± 16.7 ms and 52.3 ± 9.0 ms, respectively. These lengths depend on the length of syllable and gap chains, with longer chains resulting in longer song units. The chain lengths were chosen so that syllable and gap lengths match the experimentally determined averages of 107 ± 13.7 ms and 50.4 ± 3.2 ms, respectively (Andalman et al. 2011). More details on how the parameters were chosen can be found in the APPENDIX.

The first ensemble in each HVC chain is activated by an ensemble in the thalamic nucleus Uva (Fig. 2, Fig. 3). This is consistent with the findings that Uva is necessary for adult song production (Coleman and Vu 2005), as well as the recent finding that Uva is maximally active before syllable onset (Danish et al. 2017). Indeed, our model suggests that Uva should be active immediately after a gap begins, since $Uva_{(S\text{-}G)}$ is activated after PAm, as well as before a syllable begins, since $Uva_{(G\text{-}S)}$ is activated before PAm is inactivated (Fig. 2; Galvis et al. 2017). As in the previous model, we assume that Uva ensembles mutually inhibit one another, preventing more than one chain from being activated at a time (Galvis et al. 2017). Furthermore, if any Uva ensemble becomes active, none of the other Uva ensembles can fire until after a refractory period. This refractory period can be thought of as a slow inhibition that prevents multiple song units from becoming

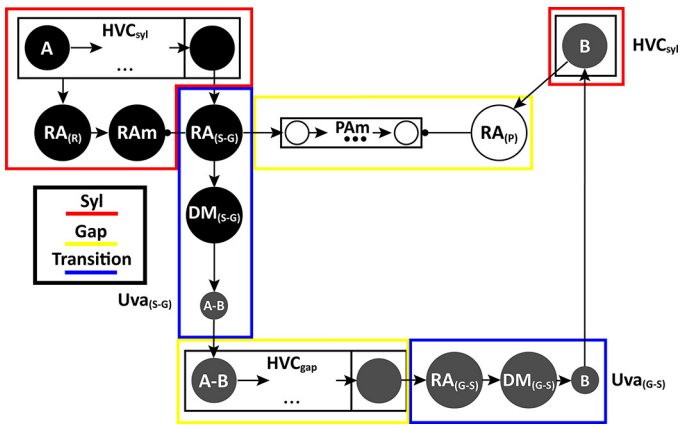

Fig. 2. Single-hemisphere recurrent network model. Red box depicts the moment-by-moment control of expiration by HVC. Each ensemble in the HVC_{syl} chains (*A* shown) signals an ensemble of robust nucleus of the arcopallium (RA) neurons that project to RAm [$RA_{(R)}$ neurons] that innervate an ensemble of expiratory nucleus retroambigualis (RAm) neurons. At the end of a syllable, an HVC_{syl} ensemble sends a signal to an RA transition ensemble (S-G) that turns off RAm and activates inspiration in nucleus paraambigualis (PAm) (*top* yellow box). Blue box on *left* depicts the synchronizing transition through the brain stem pathway that activates HVC_{gap} chains. *Bottom* yellow box depicts the activity of the network during gaps. PAm neurons are arranged in a chain, which captures the external control of the inspiratory wave. The length of each HVC_{gap} chain modulates the length of each gap. Finally, each HVC_{gap} chain activates an RA transition ensemble (G-S), which leads to a synchronizing transition through the brain stem (blue box on *right*) and activation of the next syllable. When that syllable begins, a signal is sent through an $RA_{(P)}$ ensemble that inactivates inspiration in PAm.

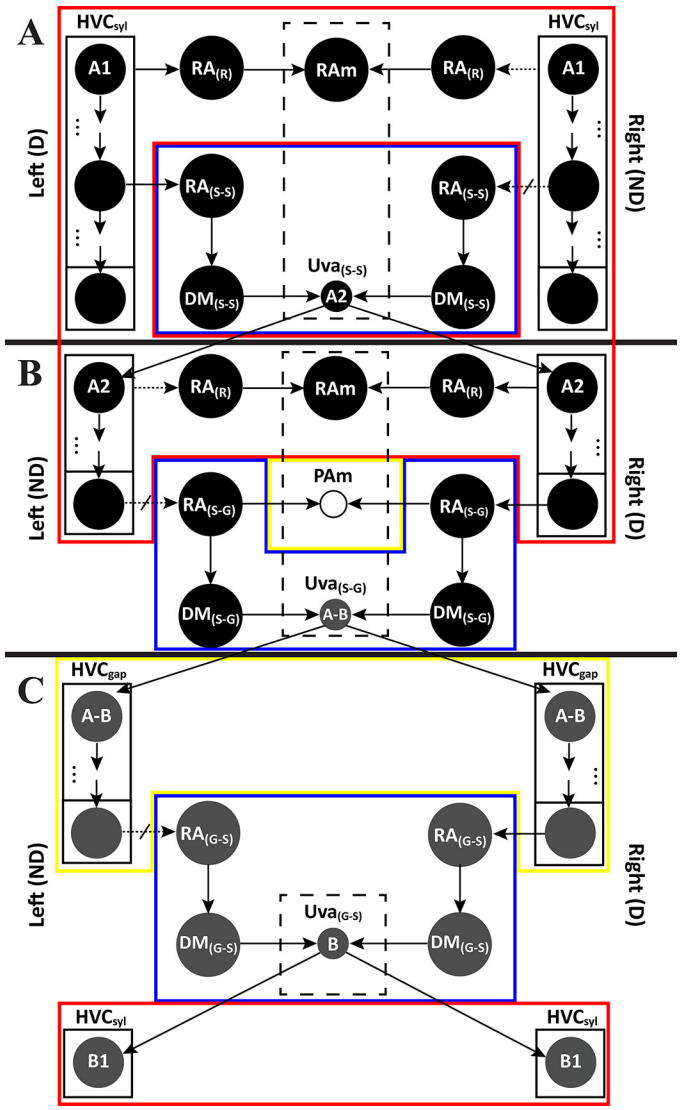

Fig. 3. Two-hemisphere network incorporating interhemispheric dominance switching. Colors as in Fig. 2. *A*: both left and right HVC contain syllable chains for the complete song (red box). Each syllable is represented by 1 or more segments (A_1, A_2....) depending on the number of dominance switches. In this example, the left HVC is dominant for the first syllable segment (A_1), sending a stronger signal to downstream nuclei than right HVC. A neuron in the dominant HVC chain sends a signal through the brain stem pathway (blue box) that initiates the next segment (A_2). The HVC neuron ensemble that sends this signal is set so that the first neurons in the A_2 ensemble fire immediately after the last ensemble in A_1 stops firing. *B*: dominance switches from one segment to the next, so right HVC is dominant for A_2. The last HVC ensemble in the final segment on the dominant hemisphere sends a signal through the brain stem pathway (blue box), inhibiting nucleus retroambigualis (RAm) (not shown), activating nucleus paraambigualis (PAm) (yellow box), and activating the *A-B* gap chains in HVC. *C*: both left and right HVC contain gap chains for the complete song (yellow box). In this example, the *A-B* gap is dominated by right HVC. The last ensemble in the dominant HVC chain sends a signal through the brain stem network (blue box) activating the next syllable. D, dominant; ND, nondominant; DM, dorsomedial nucleus of intercollicular complex; RA, robust nucleus of arcopallium; Uva, uvaeform nucleus of thalamus.

active. When Uva activates an HVC chain, it also inhibits each of the other HVC chains. These model assumptions are included to ensure that the two hemispheres are synchronized whenever a signal is sent through the recurrent network. Moreover, a previous study by Hahnloser et al. (2008) suggests that Uva is capable of both exciting and inhibiting neurons in HVC (and RA indirectly).

Transitions from gaps to syllables (G-S) or syllables to gaps (S-G) are mediated through the recurrent network: first passing through $RA_{(S\text{-}G)}$ or $RA_{(G\text{-}S)}$, then through DM, and finally through the Uva ensemble representing the next song unit (Fig. 2, Fig. 3). The chain for syllable *A* activates the gap for *A–B*, which in turn activates syllable *B*, and so on. Threshold functions are used for the activity in $RA_{(S\text{-}G)}$ and $RA_{(G\text{-}S)}$, activating the chain in the sequence only if the input is large enough (*Eq. 2*).

During syllable production, HVC chains send a moment-by-moment signal through RA neurons that project to RAm, denoted as $RA_{(R)}$ neurons, that control the waveform of expiration (Fig. 2, Fig. 3; Andalman et al. 2011; Galvis et al. 2017). In the present model, each HVC ensemble connects to a distinct $RA_{(R)}$ ensemble that represents the group of muscle-controlling neurons that would be activated at that time. In turn, each $RA_{(R)}$ ensemble connects to a distinct RAm ensemble representing the set of neurons that would be recruited to produce the necessary expiratory output. This mode allows us to visualize the behavioral effects of perturbations in sequence or activity of HVC neurons by looking at changes in the order of activity of RAm neurons. That is, the RAm neuron ensembles act as the readout for the song system.

As mentioned, each $RA_{(R)}$ and RAm ensemble described above represents the subset of neurons that would fire at a particular time during each rendition of the song. However, no single $RA_{(R)}$ neuron need fire throughout the entirety of a syllable segment, and it is possible for one $RA_{(R)}$ neuron to fire during multiple syllable segments. Indeed, voltage traces from RA neurons exhibited multiple time-locked bursts of activity during each motif (Hahnloser et al. 2002).

During gap production, HVC chains control the timing of activity in PAm, while PAm itself controls the inspiratory waveform (Fig. 2, Fig. 3; Andalman et al. 2011). When the terminal end of an HVC syllable chain signals $RA_{(S\text{-}G)}$, expiration in RAm is turned off, inspiration in PAm is activated, and an HVC gap chain is activated through $Uva_{(S\text{-}G)}$ (Fig. 2, Fig. 3). After the termination of that gap chain and activation of the next syllable chain, a signal is sent through another population of RA neurons, denoted $RA_{(P)}$ neurons, to turn off PAm (Galvis et al. 2017). The presence of a chain of neural ensembles in PAm is a simple implementation of a timing circuit, and it is very likely that the actual circuitry is more complex. That some timing circuit does exist in PAm is supported by extracellular recordings of PAm neurons in anesthetized zebra finches; different populations of neurons fire with maximum rate or entirely during specific phases of inspiration and expiration (McLean et al. 2013).

Two-hemisphere extension of recurrent network model. In the two-hemisphere extension of the model, we suppose that HVC chains are simultaneously active in both hemispheres, which is consistent with experimental results showing continuous, roughly synchronous neural activity in both hemispheres during song production (Schmidt 2003). Each element of an HVC syllable chain sends an ipsilateral connection to an $RA_{(R)}$ ensemble (Fig. 3, *A* and *B*). While the HVC syllable chains are active, each hemisphere will have active $RA_{(R)}$ neurons that converge at a set of downstream RAm neurons. Because the left and right RAm are known to be reciprocally connected (Fig. 1), we treat RAm as a single nucleus.

Our model achieves interhemispheric dominance switching through the brain stem feedback pathway (Fig. 3, *A* to *B*). A dominance switch can occur between syllables or within a syllable, in which case we partition the syllable into subsyllables, which we call "segments" (e.g., A_1 and A_2 if a single switch occurs in syllable *A*). As in a transition between syllable and gap a midsyllable transition (S-S) utilizes the RA→DM→Uva pathway, in this case an $RA_{(S\text{-}S)}$ ensemble is activated, which activates an $DM_{(S\text{-}S)}$ ensemble, which indirectly activates an $Uva_{(S\text{-}S)}$ ensemble. The $Uva_{(S\text{-}S)}$ ensemble then excites an HVC chain in each hemisphere that codes for the next portion of the syllable, but dominance is transferred from one hemisphere to the other. We assume that there are dominant and nondominant HVC_{gap} chains, but because the gaps are much shorter than the syllables, we assume that no midgap switching occurs. For model songs, the number of dominance switches is chosen randomly for each syllable (see APPENDIX). For simplicity, we assume that the hemispheres are equally likely to be dominant for a given syllable segment. However, the finding that lesions of right HVC exert a larger effect on song than lesions of left HVC (Williams et al. 1992) implies that the right HVC could be dominant for a greater proportion of the song.

In the two-hemisphere extension, HVC neurons send an ipsilateral signal to $RA_{(S\text{-}S)}$ to initiate a dominance switch, to $RA_{(S\text{-}G)}$ to initiate a gap, or to $RA_{(G\text{-}S)}$ to initiate a new syllable (Fig. 3). These RA neurons then send an ipsilateral connection to $DM_{(S\text{-}S)}$, $DM_{(S\text{-}G)}$, or $DM_{(G\text{-}S)}$, respectively. DM sends a bilateral signal to $Uva_{(S\text{-}S)}$, $Uva_{(S\text{-}G)}$, or $Uva_{(G\text{-}S)}$, respectively. We treat Uva as a single nucleus because it receives bilateral input from DM and PAm. Left Uva sends a signal to start the next segment, gap, or syllable on the left side and right Uva does the same, but it is shown as a single Uva nucleus in Fig. 3.

In this model, we incorporate the prediction that the dominant hemisphere has stronger connections between HVC and RA. We use $\alpha = 0.9$ for dominant connections and $\alpha = 0.1$ for nondominant connections (chosen so that they sum to 1). At each time during a syllable there is an active dominant HVC ensemble on one hemisphere and a nondominant HVC ensemble on the other hemisphere, both of which take the value $X = 1$ (Fig. 4). These ensembles both send output to an ipsilateral $RA_{(R)}$ ensemble, which takes the value $X = 0.9$ on the dominant side (since $\alpha = 0.9$ for the presynaptic ensemble) and $X = 0.1$ on the nondominant side (Fig. 4). Connections from $RA_{(R)}$ to RAm are strong ($\alpha = 1$), and RAm receives input from both hemispheres, so during normal song activated RAm neurons take the value $X = 1{\cdot}0.1 + 1{\cdot}0.9 = 1$ (Fig. 4). Connections from HVC to $RA_{(S\text{-}S)}$, $RA_{(S\text{-}G)}$, or $RA_{(G\text{-}S)}$ are modeled in a similar fashion, except that the transition RA neurons are threshold functions. Only the RA ensemble on the dominant side gets activated by the HVC chain and sends a signal through the recurrent network to start a new segment, syllable, or gap.

When the transition is from a syllable to a gap, $RA_{(S\text{-}G)}$ activates inspiratory activity in PAm ($X = 1$), which we treat as a single unit because left and right PAm are indirectly connected through bilateral connections with RAm (Fig. 2, Fig. 3*B*). $RA_{(S\text{-}G)}$ also inhibits expiratory activity in RAm (Fig. 2).

The model assumes that expiratory RAm and inspiratory PAm ensembles are mutually inhibitory, but the circuitry for this is not specified. This assumption prevents the model from displaying the mutually exclusive behaviors of inspiration and expiration simultaneously. This becomes relevant during the stimulation portion of RESULTS, where syllable and gap chains in HVC become active at the same time.

In our previous model, atypical transitions occurred when a PAm chain reached its end. In that case, a signal was sent from PAm to Uva initiating a new, sometimes atypical syllable (Galvis et al. 2017). This connection is maintained in this version of the model, through bilateral connectivity from PAm to Uva in both hemispheres.

Stimulation of HVC. Two types of model HVC stimulations are simulated. The first type is inactivation of active chains, where during some point in the song and while a syllable or gap chain is active we reset the value of the active HVC chain ensemble in one hemisphere. This results in all of the subsequent elements of that chain taking the same new value (e.g., $X = 0.2$ rather than the unstimulated value $X = 1$). This lowers the value of the input to downstream RA neurons from that chain. The other type of HVC stimulation is activation of a previously inactive chain, where an ensemble is increased from $X = 0$

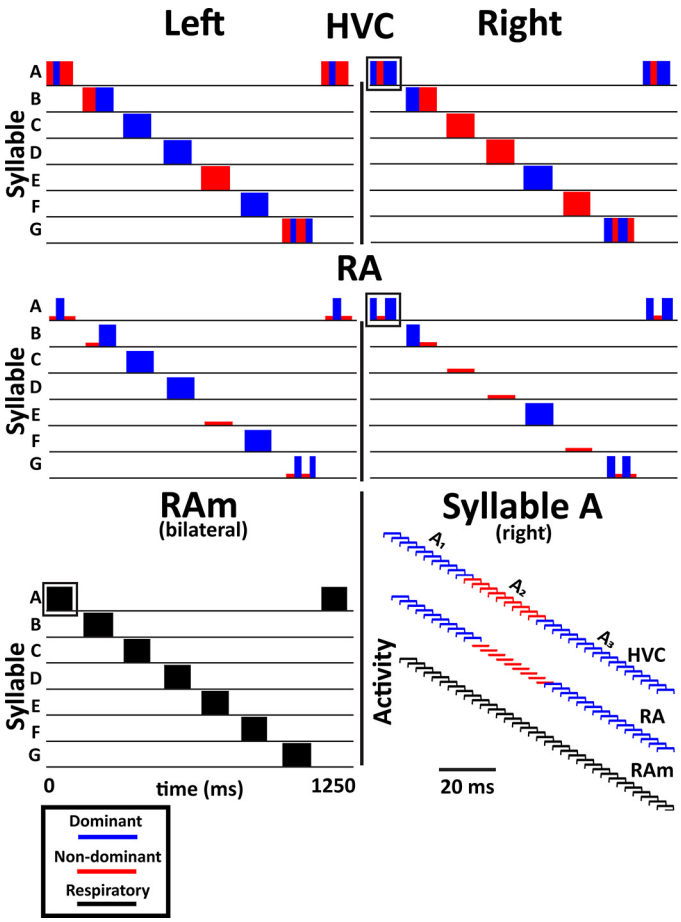

Fig. 4. Behavior of the two-hemisphere model under normal conditions. The ensembles within dominant HVC chains send strong signals to robust nucleus of the arcopallium (RA) neurons that project to nucleus retroambigualis (RAm) [$RA_{(R)}$ neurons], while the ensembles within nondominant HVC chains send weak signals to $RA_{(R)}$ neurons. In this example, right HVC is dominant for A_1 and A_3, while left HVC is dominant for A_2. During any syllable, the weakly active RA on the nondominant side and the strongly active RA on the dominant side converge onto RAm, which is fully active as a result (see METHODS AND NETWORK MODEL). *Bottom right*: individual ensembles for the right hemisphere of syllable *A*.

to a nonzero value. This results in activation of that chain and its downstream RA neurons.

RESULTS

Behavior of two-hemisphere network model during song production. The two-hemisphere network model produces sequences of activity during a motif, with dominance over sound production frequently switching between hemispheres. As an example, Fig. 4 shows a raster plot of neural ensemble activity during a 7-syllable model song. Each syllable is shown as a block, with an expanded view in Fig. 4, *bottom right*. During syllable *A* there is an active HVC_{syl} chain in both the left and right hemispheres; however, only the right hemisphere is dominant during the first syllable segment, A_1. Because the right hemisphere is dominant in A_1, $RA_{(R)}$ neurons in the right hemisphere are strongly activated. In contrast, the left hemisphere is nondominant in A_1; therefore $RA_{(R)}$ neurons on the left hemisphere are weakly activated. Left and right $RA_{(R)}$ project to neural ensembles in RAm and together fully activate them. There is a dominance switch when the dominant HVC chain representing A_1 sends a signal through the brain stem pathway that activates the A_2 syllable segment in the left hemisphere. During this portion of the song, $RA_{(R)}$ in the right hemisphere is weakly active while $RA_{(R)}$ in the left hemisphere is strongly activated. Syllable *A* switches dominance again for the production of segment A_3 about midway through the syllable.

When the final HVC syllable *A* segment reaches the end of the chain, the dominant (right) hemisphere initiates gap *A-B* through the recurrent network (Fig. 2, Fig. 3). PAm neuron ensembles also become active during this transition (see METHODS AND NETWORK MODEL and Galvis et al. 2017). In this example, the left hemisphere is the dominant hemisphere for gap *A-B* (not shown), which means that the left side initiates syllable *B* through the recurrent pathway. Syllable *B* has two segments with dominance beginning in the right hemisphere in this example. This bird's song contains 7 syllables, ending on syllable *G*. After syllable *G* is produced, gap *G-A* is initiated, which leads to the beginning of a new motif.

Simulating effects of unilateral and bilateral HVC temperature manipulations. The first manipulation to HVC that we simulate with the two-hemisphere model is unilateral or bilateral cooling. Cooling was implemented in our previous model by increasing the time constants and decreasing the maximum values of the channel conductances (Galvis et al. 2017). We found that this model could capture the behavioral effects of bilateral cooling of HVC on song (Long and Fee 2008), including the finding that gaps stretch significantly less than syllables (Andalman et al. 2011). (Note that the earlier study by Long and Fee 2008 found the opposite.) In the network model, the recurrent network includes nuclei other than HVC that are unaffected by cooling. Gaps stretch less than syllables because the electrical signals for the transition into the gap phase (the syllable-gap or S-G transition) and out of the gap phase (the gap-syllable or G-S transition) pass through the recurrent network, which is not cooled. The transition times are a larger fraction of the gap duration than are analogous transitions into and out of syllables.

The two-hemisphere version of the model uses the same framework, but now cooling is implemented by increasing the action potential latency or delay parameter δ for chain neurons in HVC (see APPENDIX). This is analogous to the original model, where the song stretch is due to an increase in the time between the onsets of bursts in the chain. Scaling of delay in the chain neurons was set so that a 10°C decrease in temperature ($\Delta T = -10°C$) results in a 25% song stretch to match the experimentally determined stretch found in Hamaguchi et al. (2016) (see APPENDIX). A major benefit of the simplified mathematical model is that analytic expressions can be derived relating change in temperature to stretch (or contraction) in gaps and syllables (see APPENDIX). For example, there are eight connections between the activation of PAm and the subsequent activation of RAm outside of HVC, so a portion of the gap with duration 8δ does not stretch or contract. Furthermore, syllables stretch proportionally to the temperature change unless there is a dominance switch. Each switch requires a pass through the recurrent network, adding a length of 4δ that does not stretch or contract (see APPENDIX). We simulated bilateral cooling/heating of HVC in 10 randomly selected model birds over temperature changes between $\Delta T = -10°C$ and $\Delta T = 3°C$ (Fig. 5). As in the original model, cooling HVC stretches the

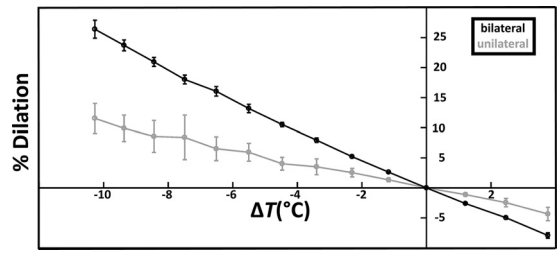

Fig. 5. Effects of unilateral and bilateral cooling and heating of HVC on song duration. Bilateral cooling stretches or contracts the song nearly uniformly. Parameters were chosen so that $\Delta T = -10°C$ results in an ~25% increase in song length. Unilateral cooling results in approximately half the dilation that occurs during bilateral cooling because, on average, half of the song is dominated by each hemisphere.

song, whereas heating HVC contracts the song (Fig. 5 and Galvis et al. 2017). The variation shown by the standard deviation bars in Fig. 5 reflects the difference in the number of gaps and lengths of syllable and gap chains between randomly selected motifs.

Experimental studies have shown that although bilateral cooling of HVC leads to stretching of the whole song, unilateral cooling of HVC only stretches some portions of the song (Long and Fee 2008). In fact, the stretch due to cooling of left HVC was significantly anticorrelated with stretch during right cooling (Long and Fee 2008). Furthermore, unilateral cooling did not result in song degradation, despite the possibility of desynchronization between left and right HVC (Long and Fee 2008). These observations led to the hypothesis that left HVC is responsible for the timing of some portions of the song and right HVC is responsible for others (Long and Fee 2008).

Our model is consistent with this hypothesis, since synchronization occurs at the transitions between syllable segments and between syllables and gaps. Furthermore, each hemisphere is responsible for the timing of the portions of the song that it dominates, and unilateral cooling of HVC only slows the portions of the song for which the cooled hemisphere is dominant. As an example, consider a cooling of $\Delta T = -10°C$ of the right hemisphere. During the production of segment A_1 HVC in the right hemisphere is dominant, and since it is cooled the chain of activity propagates more slowly than that in the left hemisphere. Only the dominant right HVC can activate the recurrent pathway, so the nondominant left HVC chain propagates to the end of its chain first but cannot activate the next segment. The next segment starts once the dominant HVC chain reaches its end.

For quantification, we used 10 model birds and in each randomly cooled/heated either the right or left HVC. This was done for a range of values of ΔT. Over the full range, unilateral cooling stretched the song approximately half as much as bilateral cooling (Fig. 5). This is because, on average, half of the segments and gaps will be stretched and the other half propagate at normal speed. The standard deviation is much larger for unilateral cooling, however, because the songs are chosen randomly and often one hemisphere dominates more of the song than the other. If this is the case, unilateral cooling of the more dominant hemisphere results in stretch closer to the bilateral cooling, whereas cooling the less dominant hemisphere results in a stretch closer to the uncooled song.

Simulating effects of unilateral electrical stimulation of HVC. Wang et al. (2008) found that unilateral electrical stimulation of HVC can result in syllable distortion and truncation as well as motif stops and restarts. They also found that the effect of stimulation depends on stimulation timing. Stimulations that were made at similar points in time during a motif often resulted in similar perturbations to the song. In some cases, stimulation at two similar but not identical time points led to identical syllable truncations (Wang et al. 2008). Furthermore, they found that the phases of the motif where stimulation of left HVC was effective in causing behavioral perturbations were anticorrelated with those where stimulation of right HVC was effective.

It is not at all clear what effects electrical microstimulation will have on the activity of neurons. One study using optical techniques found that microstimulation activated neurons hundreds of micrometers or even millimeters from the stimulating electrode, but the affected neurons were sparsely distributed. Increasing the magnitude of the stimulation did not increase the diameter of influence but instead increased the density of neurons that were activated within the same diameter. The activation was largely independent of synaptic transmission and instead appeared to be due to direct depolarization of neural processes that were close to the stimulating electrode (Histed et al. 2009). Such stimulation could activate either excitatory or inhibitory neurons, since the mechanism for activation (a change in the extracellular potential) is the same for both.

We consider perturbations of model HVC activity that could potentially result from unilateral electrical stimulation of HVC during song production. There are two ways that stimulation could interfere with propagation across a network. One way is to activate chains at the inappropriate time in the sequence. We call this a "strengthening stimulation." A second way is for stimulation to arrest propagation in an ongoing chain. This could be achieved by activating inhibitory neurons, by putting component neurons in a refractory period so that they fail to propagate activity at the appropriate time, or by other mechanisms such as inducing short-term synaptic depression or depolarization block. Regardless of mechanism, this sort of stimulation would prevent an active chain from continuing to completion. We call this a "weakening stimulation." To simulate a weakening stimulation, we reduce the activity value of a previously active HVC ensemble. To simulate a strengthening stimulation, we increase the activity level of a previously inactive ensemble. We tacitly assume that all effects on song are the result of changes to the RA-projecting HVC ensembles, although at the level of detail of the model effects of the area X-projecting neurons are not precluded.

Unilateral weakening stimulations: inactivation of syllable chains. The stimulation of an active syllable chain (Fig. 6) is achieved by changing the "normal" value of an active ensemble, $X_n(t) = 1$, to a "perturbed," smaller value, X_p. The remainder of the chain also takes this new value as the signal moves through it. While the perturbed chain is active, it sends a smaller input to RA, which also manifests as decreased activity in RAm. This reduction in RAm activity can result in spectral distortion, which lasts only as long as the syllable segment during which the stimulation was applied. Transient syllable distortions have been reported after unilateral HVC stimulation (Wang et al. 2008). Inactivation of a nondominant HVC_{syl}

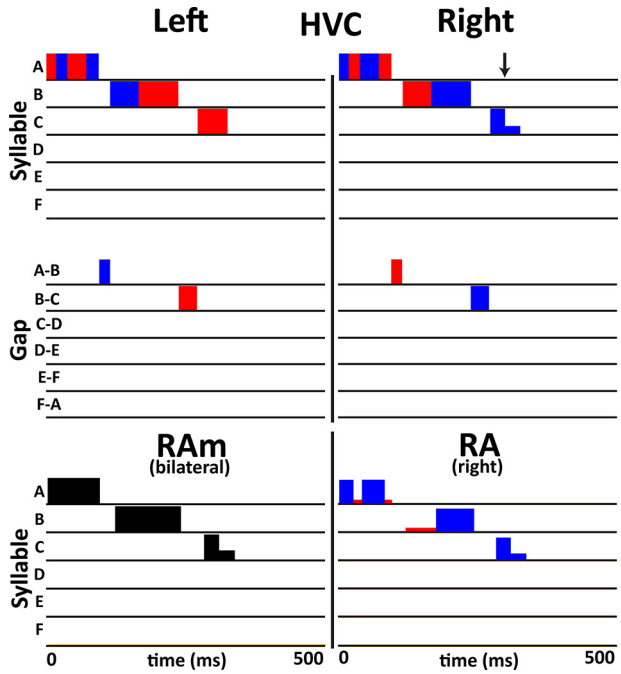

Fig. 6. Inactivation of an active syllable chain in the dominant hemisphere. During production of syllable *C*, electrical stimulation of the right hemisphere results in a weakened dominant syllable chain, which sends a weaker signal through the robust nucleus of the arcopallium (RA) to nucleus retroambigualis (RAm), potentially resulting in spectral distortion. When the chains reach the end, the dominant chain is not capable of sending a signal to initiate the next segment. Therefore, the song terminates after a truncation of syllable *C*. Arrow indicates the time of stimulation of the right hemisphere HVC.

chain has a smaller effect, since the contribution to overall output of downstream RA on the nondominant side is always smaller than the contribution from the dominant side.

A more serious possible effect of a weakening stimulation is that the chain may become too weak ($\alpha X_p < 0.5$) to overcome the threshold function required to activate the next segment or gap and can only occur if the weakening is applied to the dominant hemisphere for the syllable segment (the nondominant chain cannot activate the threshold function even under normal conditions). When this occurs, the song terminates prematurely, and the syllable during which the stimulation was applied may be truncated. Figure 6 shows an example where the first segment in syllable *C* is perturbed on the dominant right side. The signal sent by the dominant right HVC chain through RA to RAm is too weak to overcome the threshold function for activation of the next segment. This results in a song ABC_1 followed by termination of the song. If syllable *C* had only one segment the full syllable *C* would have been produced, but afterwards the song would have prematurely terminated.

Such syllable truncations and premature song terminations were reported by Wang et al. (2008). They also found that stimulations at nearby points in the motif led to similar behavioral perturbations, which is consistent with our hypothesis that song syllables consist of segments each of which is coded by a chain of neural ensembles. Electrical stimulations that occur on the dominant hemisphere at any time during that segment will have the same effects on the song. Furthermore, at any point in the song, stimulation is only effective on the dominant side (Fig. 7), consistent with the finding that the effectiveness of left and right stimulation is anticorrelated throughout the song (Wang et al. 2008).

Unilateral weakening stimulations: inactivation of gap chains. Because gaps are much shorter than syllables, the probability that randomly applied unilateral stimulation would occur during a gap is low; however, it can happen. What prediction does the model make in response to stimulation during a gap? If the stimulation is applied to the nondominant hemisphere for that gap, then there will be no effect. However, if applied to the dominant hemisphere, the stimulation could result in an atypical syllable transition, where the next syllable sung is different from that in the unperturbed motif. Therefore, as in the case of inactivation of syllable chains, the effectiveness of left and right stimulation is anticorrelated throughout the song (Fig. 7).

To understand the model prediction of an atypical transition, we recall a key element of our previous biophysical model for the song network (Galvis et al. 2017), which was developed partly to explain the finding that atypical syllable transitions occur when there are bilateral medial ablations of HVC (Basista et al. 2014). In that model, as in the present model, it was

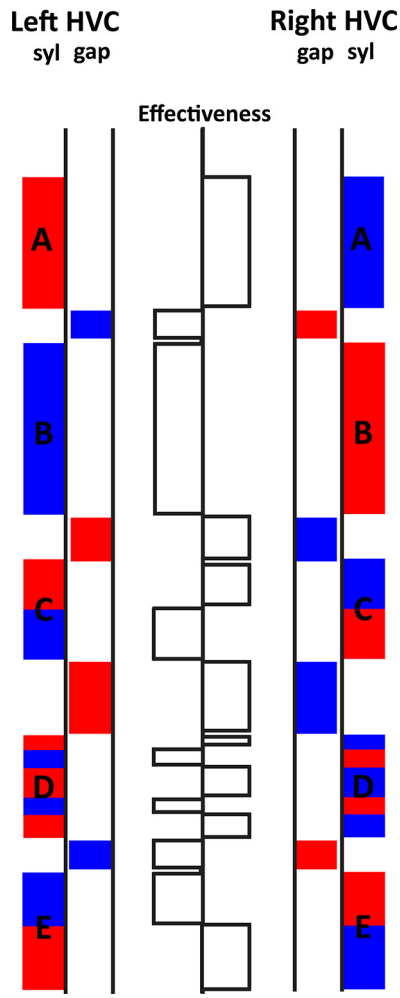

Fig. 7. Effectiveness of left and right stimulation of HVC is anticorrelated in weakening stimulations. Unilateral stimulations are only ever effective in the hemisphere that is dominant for a section of the song. The effectiveness curve shows portions of the song where left or right stimulation can lead to perturbations of the song assuming that the HVC chain is sufficiently weakened ($\alpha X_p < 0.5$).

assumed that the final ensemble of a PAm chain innervates Uva and, if activated, can induce a new syllable chain in HVC. Normally, activity in a PAm chain is terminated before the final ensemble by a signal emanating from an HVC_{gap} chain through $RA_{(P)}$. However, if this does not happen, the terminal point in the PAm chain is reached and a new syllable, usually not the expected syllable, is produced. Returning to the case at hand, if an HVC_{gap} chain is weakened to a subthreshold level ($\alpha X_p < 0.5$), then it will not trigger the normal next syllable and will not terminate the PAm chain. Instead, the PAm chain will reach its terminal point and trigger a new syllable, likely an atypical transition.

This phenomenon is illustrated in Fig. 8, where the *A*-*B* gap chain is inactivated on the dominant left side. This results in a long gap, as shown in Fig. 8, *bottom right*, and an atypical transition from *A* to *F*. Furthermore, the long gap occurs because PAm has to reach the end of its chain before signaling a new syllable (Galvis et al. 2017).

Wang et al. (2008) did not mention the occurrence of atypical transitions after unilateral excitation of HVC. However, Basista et al. (2014) suggest that only medial stimulation of HVC would result in atypical transitions, and the stimulation locations were not reported by Wang et al. (2008). Moreover, since stimulation occurring during a gap in the song would be a rare event, it is likely that a medial stimulation during a gap did not occur. Also, if the atypical transition occurs onto the syllable *A*, then the atypical transition would be indistinguishable from a restart, which they did report. Furthermore, Hamaguchi and Mooney (2012) found that augmentation of the lateral magnocellular nucleus of the anterior nidopallium

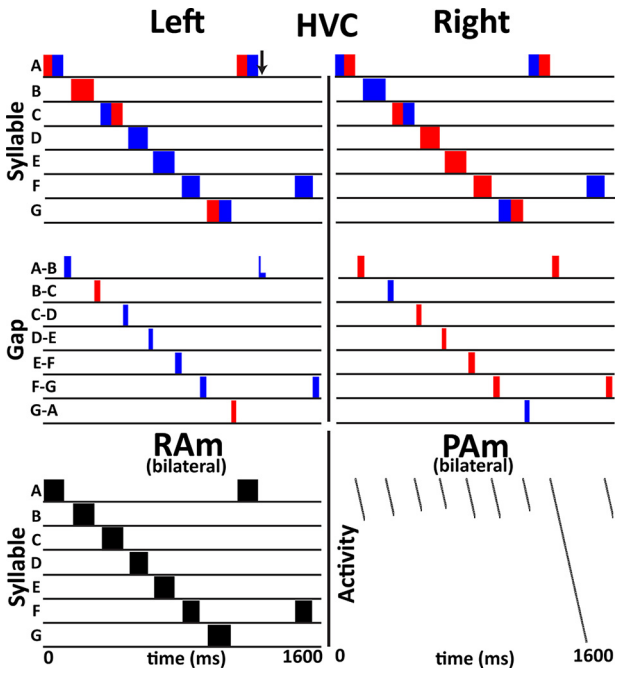

Fig. 8. Inactivation of an active HVC_{gap} chain in the dominant hemisphere. During production of the *A*-*B* gap, electrical stimulation of the left hemisphere results in a weakened dominant gap chain, which cannot signal the production of syllable *B*. The downstream nucleus paraambigualis (PAm) chain is therefore not deactivated by the activation of syllable *B* and reaches its terminal point. PAm signals an atypical transition from *A* to *F* following a long gap. Arrow indicates time of stimulation of the left hemisphere HVC. RAm, nucleus retroambigualis.

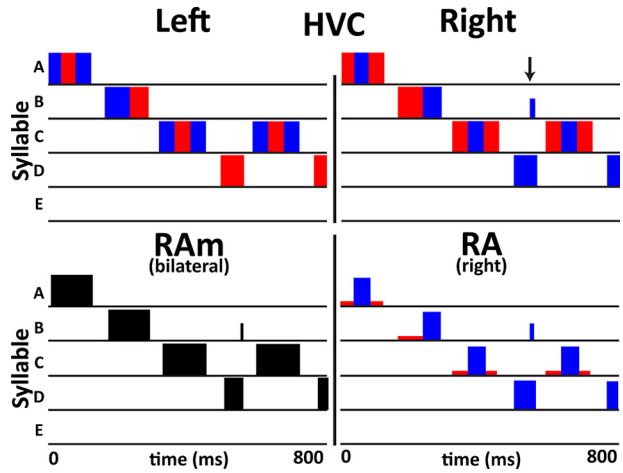

Fig. 9. Activation of a dominant syllable chain during syllable production. During the production of syllable *D*, electrical stimulation of the right hemisphere strengthens the dominant syllable segment B_2. The B_2 chain reaches its end before syllable *D* does, resulting in activation of the *B*-*C* gap chain followed by syllable *C*. Syllable *D* gets truncated when the *B*-*C* gap is activated, which results in the syllable sequence $ABCD_{partial}CD$. A spectral distortion is observed in nucleus retroambigualis (RAm) while both syllable B_2 and *D* chains are active. RA, robust nucleus of arcopallium.

(LMAN) with bicuculline methiodide resulted in atypical transitions during song through an indirect and potentially complex circuit leading from LMAN onto HVC. Therefore, some perturbation of HVC is most likely capable of causing atypical transitions and may occur via a weakening stimulation of active gap chains.

Unilateral strengthening stimulations: activation of a chain during a syllable. The stimulation of an inactive chain is achieved by changing the "normal" value of a randomly selected inactive ensemble, $X_n(t) = 0$, to a "perturbed" active value, X_p. As in the weakening stimulations, the remainder of the chain will take on the perturbed value as the signal propagates through it. This can occur in tandem with a previously active syllable or gap chain, depending on the stimulation time. When a strengthening stimulation activates a syllable chain during syllable production, two chains send signals to RAm through RA in the stimulated hemisphere (Fig. 9). This leads to a spectral distortion of the syllable, as observed experimentally (Wang et al. 2008). In contrast to the distortion observed in the weakening stimulations, this distortion is observed as the activation of unexpected RAm ensembles rather than the weakening of expected RAm ensembles. Strengthening a chain in the dominant hemisphere results in a larger distortion than strengthening a chain in the nondominant hemisphere, because it sends a stronger signal to RAm.

If a dominant syllable segment is activated, it is sometimes activated with enough strength to send a signal through the recurrent pathway. In this case, the HVC chain that reaches the end first will determine which portion of the motif occurs next. If the normally active HVC chain reaches its end first, then the song will proceed normally and the distortion will end as soon as the next segment or gap is activated. However, if the perturbed HVC chain reaches its end first, it will truncate the syllable and activate an atypical segment or gap. Figure 9 shows the case in which a dominant segment B_2 is activated in the right hemisphere during production of syllable *D*. The HVC chain representing B_2 reaches the end of the chain before

D and sends a signal through the recurrent pathway turning off syllable *D* and activating the *B-C* gap. The result is a syllable sequence $ABCD_{partial}CD$.

If the perturbed HVC chain is not the last segment in a particular syllable, the perturbed chain signals the next segment rather than a gap. For example, had A_2 (also right dominant) been activated instead of B_2, the resulting syllable sequence would have been $ABCD_{partial}A_3B$ with $D_{partial}A_3$ occurring without a gap between.

Another possibility is that a dominant gap chain gets activated during production of a syllable. If the perturbed gap chain reaches its end first, it sends a signal through the recurrent network, truncating the normal syllable and activating a new syllable. In this case, the atypical transition is always from a truncated syllable to the first segment of the atypical syllable. Furthermore, there is no gap during the transition because the normal syllable is truncated at the same time the new syllable is activated. Unlike in the weakening cases and Wang et. al. (2008), stimulation of either hemisphere can result in these atypical transitions regardless of the point in the song.

Unilateral strengthening stimulations: activation of a chain during a gap. Strengthening a syllable chain while an HVC_{gap} chain is active does not result in a spectral distortion of the song because expiration in RAm cannot become active while inspiration in PAm is active. Therefore, the behavioral output does not change unless the strengthened HVC_{syl} chain is dominant and reaches the end of its chain before the gap chain reaches its end. This is expected to be a low probability for two reasons. First, gaps take up a small portion of the song relative to syllables; therefore randomly applied stimulations will usually occur during syllables. Second, gap chains are short relative to syllable chains. The probability that a syllable segment is activated at an ensemble close enough to the end of the chain so that it reaches its end before the gap chain does is low.

Figure 10 shows the case in which the last segment in syllable *D* becomes active during the *B-C* gap. In this case, the syllable segment sends a signal through the brain stem turning off the normally active gap chain and activating the *D-A* gap chain. This results in a gap that is approximately twice as long as a normal gap followed by an atypical transition from *B* to *A*. In this case, the syllable sequence that is produced is *ABAB*.

The final possibility is that a dominant gap chain becomes active during a gap. If the perturbed gap chain reaches its end first, this results in an atypical transition between two complete syllables.

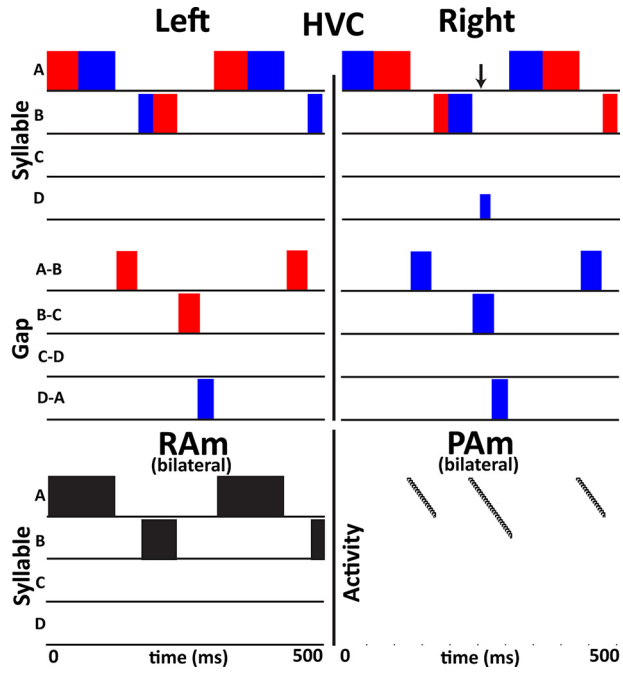

Fig. 10. Activation of a dominant syllable chain during gap production. During production of the *B-C* gap, stimulation of the right hemisphere results in strengthening of the dominant syllable *D* chain. The syllable *D* chain reaches its end before the *B-C* gap chain, resulting in termination of the *B-C* gap chain and activation of the *D-A* gap chain. This produces an atypical transition from *B* to *A* and the syllable sequence *ABAB*. Inspiration in nucleus paraambigualis (PAm) is longer than normal because it starts before the activation of the *B-C* gap chain but does not inactivate until after a portion of the *B-C* gap chain and the full *D-A* gap chains have reached the end. Furthermore, no spectral distortion is observed in nucleus retroambigualis (RAm) because PAm suppresses the RAm activity even when the syllable *D* chain is active.

DISCUSSION

In this study, we have developed a two-hemisphere, recurrent network model that can account for the dominance switching hypothesis proposed by Long and Fee (2008) and Wang et al. (2008) while maintaining the network structure described in our previous model (Galvis et al. 2017). In this model, dominance switching occurs multiple times throughout the song through a synchronizing bilateral signal between respiratory nuclei in the brain stem (PAm and DM) and thalamus (Uva). We propose that the dominant hemisphere is characterized by stronger innervation of RA by HVC, which results in a one-sided control over the motor program and transitions between song units. This could mean more neurons in dominant HVC populations than in nondominant populations or greater synaptic coupling between dominant HVC neurons and RA neurons than for nondominant HVC neurons. At the level of this model the two are equivalent, and to our knowledge such differences have not been investigated experimentally. It is also possible that dominance comes at the level of connections from the RA to the brain stem.

We simulated temperature manipulation of HVC in this model by altering the delay time between chain neurons. These simulations showed that bilateral cooling of HVC results in nearly uniform slowing of the signal propagation of the neural network for the song, except that the portion of the network coding for transitions between gaps and syllables is not cooled and thus not slowed. These results are similar to results obtained previously with a network model utilizing conductance-based neurons (Galvis et al. 2017), which was designed to capture bilateral cooling data showing uniform stretching of syllables and somewhat less stretching of gaps (Andalman et al. 2011). [Hamaguchi et al. (2016) found that bilateral cooling of Uva also results in song stretch, which we cannot explain with our model.] The two-hemisphere version of the model allowed us to consider the effect of unilateral cooling of HVC. In the model, cooling a single hemisphere results in stretching of all portions of the song for which the cooled hemisphere is dominant. The rest of the song is not stretched. This is consistent with experimental results showing that unilateral cooling results in stretching of some portions of the song but not others and that the portions stretched by cooling of the right

HVC are anticorrelated with those stretched by cooling of the left HVC (Long and Fee 2008). This contrasts with cooling of the HVC in the Bengalese finch, which affects the transition probabilities between syllables (Zhang et al. 2017).

We next investigated the possible effects of unilateral HVC stimulation during singing on the network behavior. Since it is not known whether the primary effect of electrical stimulation performed in the study of Wang et al. (2008) was to activate inactive RA-projecting neurons or to inactivate active ones, we explored both possibilities. We found that weakening stimulations of the dominant hemisphere during syllables caused syllable distortions, syllable truncations, and song stops. If a song stop was followed by a reactivation, this stimulation could also result in a restart. Furthermore, because these stimulations are only effective on the dominant hemisphere, we found that stimulation could only be effective in one hemisphere at any point in the song. These results are consistent with the effects of stimulation described by Wang et al. (2008).

Weakening stimulations of the dominant hemisphere during gaps resulted in atypical transitions of the type described by our previous model after medial ablations (Basista et al. 2014; Galvis et al. 2017). While the weakening stimulation does fit the result that left and right stimulation effectiveness is anticorrelated, Wang et al. (2008) did not document atypical transitions despite attempting stimulations throughout the entire song. In our model, signaling through the recurrent networks occurs during approximately half of the duration of each gap, so even though gaps take up approximately one-third of the song, gap chains are active for only approximately one-sixth of the song. If this is the case in the actual birds, then few randomly timed weakening stimulations would occur when gap chains were active. Furthermore, medial and lateral ablation of HVC (Basista et al. 2014) suggests that only a stimulation of a medial, dominant gap chain will have the effect of an atypical transition. Wang et al. (2008) did not discuss the placement of their electrodes within HVC. Another possibility is that these weakening stimulations occur simultaneously with strengthening stimulations of some of the HVC neurons that inactivate PAm. In this case, one would expect a song stop rather than an atypical transition.

Strengthening stimulations resulted in syllable distortion and atypical transitions. The atypical transitions occurred even when stimulations were applied during syllables, which was never seen in the Wang et al. (2008) data. Furthermore, the effects of left and right stimulation were not anticorrelated. Therefore, our model predicts that the primary effect of the unilateral HVC stimulations performed in Wang et al. (2008) was to inactivate active HVC chains. This could be due to the activation of HVC interneurons, which would otherwise be inactive. Indeed, Kosche et al. (2015) showed that interneuron spiking patterns exhibit pauses in activity during song production, which could potentially be overcome by electrical stimulation.

One hypothesis underlying our two-hemisphere network model is that each hemisphere has copies of chains for each syllable segment and gap (or gap segment). The only difference between the two is that the dominant hemisphere for a segment/gap has a stronger connection to nucleus RA. Thus there are two complete copies of the recurrent network for song, with rapid switching in dominance between the two. Is there any evidence for this? Perhaps, in unilateral ablation studies. In one study, Uva was unilaterally ablated in adults, which resulted in recovery of the song over time (Coleman and Vu 2005). In a second study, unilateral ablation of RA in juveniles did not prevent normal song acquisition, although it did prevent song production if the ablation was done in an adult bird (Ashmore et al. 2008a). How can the bird recover its song when the primary input (Uva) and output (RA) of the timing nucleus HVC are removed in one hemisphere? This would not be hard to explain if there are two copies of the necessary circuitry, as in our model. Compensation for unilateral ablation of either Uva or RA could be achieved by strengthening any weak connections from HVC to RA on the side contralateral to the ablation. This hemisphere would then become dominant for the entire song, and there would be no dominance switching. In this case, one would predict that cooling the HVC contralateral to the ablation should result in slowing of the song, comparable to bilateral cooling in the intact bird. Furthermore, unilateral electrical stimulation of contralateral HVC should be effective over a larger proportion of the song than would be the case before ablation.

Another testable prediction made by this model is that stimulation effectiveness curves should be positively correlated with portions of the song that exhibit cooling related stretch when both perturbations are performed to HVC in the same hemisphere. Furthermore, one would expect the onsets of these effective regions to be correlated with increased synchrony between the hemispheres due to the transition through the recurrent network. Experimental results have suggested that synchronization is correlated with the onsets of syllables, onsets of intrasyllabic segments called "notes," and in many instances gaps (Schmidt 2003). In our model, the onsets of syllables require a synchronizing signal through the recurrent network, but an interesting possibility is that the transitions between notes could also occur through a segment-to-segment transition. If so, it is possible that transitions between notes would be correlated with dominance switching as well.

We have focused exclusively on unilateral control of song segments or gaps with interhemispheric switching, since this is the most difficult scenario to explain. However, while the results of Long and Fee (2008) and Wang et al. (2008) do show anticorrelated behavior between left and right cooling and stimulations, respectively, there was also evidence for bilateral control of at least some song segments in some birds. For example, there were a significant number of instances where stimulation of either left or right HVC perturbed the song (see Supplemental Table 2 in Wang et al. 2008). In our model, bilateral control could be instantiated by setting the parameter $\alpha = 0.5$ for HVC→RA connections in both hemispheres. If this is done to the RAm-projecting $RA_{(R)}$ neurons, then both hemispheres would be contributing equally to the vocal output during syllable production. Moreover, if this is done to the threshold transition RA neurons [$RA_{(S\text{-}G)}$, $RA_{(S\text{-}S)}$, or $RA_{(G\text{-}S)}$], then either hemisphere could initiate a transition if the requirement for activation of threshold neurons is that presynaptic input (αX) be ≥ 0.5.

What would be the result of unilateral cooling on a bilaterally controlled syllable segment like that described above? The expected result would be rapid degradation of that segment because both hemispheres would be driving their respective motor behaviors at different rates. This would persist only until the end of the segment, when interhemispheric synchronization

would take place. The segment duration would not change, since the uncooled side would initiate the transition (since its chain would reach its terminal point sooner). With unilateral heating, the segment would be shorter, since now the chain in the heated hemisphere would finish first and trigger the transition.

APPENDIX

Expressions for durations of syllables, gaps, and motifs. As in the previous model, the length of each syllable is determined by the time between RAm activation and the subsequent activation of inspiration in PAm. Likewise, the length of each gap is determined by the time between activation of PAm and activation of expiration in RAm (Galvis et al. 2017). A benefit of the present model with the simpler neural dynamics is that expressions can be derived for syllable, gap, and motif lengths. For example, the length of any syllable is given by

$$L_{\text{syl}} = \delta\left(n_{\text{syl}} - 1\right) \tag{A1}$$

where n_{syl} is the number of neuron ensembles in the HVC chains for that syllable. This is because for a chain of length n_{syl} there are exactly $n_{\text{syl}} + 1$ connections between the first HVC ensemble and the first ensemble in the PAm chain. Therefore, it takes $\delta(n_{\text{syl}} + 1)$ ms for PAm to begin firing after an HVC syllable chain has been activated. It takes exactly 2δ ms for RAm to begin firing after activation of the first HVC ensemble in the chain because of the two connections between HVC and RAm. Therefore, RAm is active for $\delta(n_{\text{syl}} + 1) - 2\delta = \delta(n_{\text{syl}} - 1)$ ms. The inclusion of multiple segments in a syllable does not change the syllable length. The four connections required to switch between segments is offset because the fourth from last ensemble in the chain activates the switch. Therefore, the first neuron ensemble in the new segment activates precisely when it would have if the segments were combined.

Using an analogous argument, each gap length is given by the equation

$$L_{\text{gap}} = \delta\left(n_{\text{gap}} - 1\right) + 8\delta \tag{A2}$$

The extra 8δ ms is due to connections through the recurrent network that occur while PAm is active (Fig. 2) and lead to the activation of RAm.

The length of each motif is the sum of all of the gap and syllable lengths:

$$L_{\text{motif}} = \delta(n_{\text{tot}} - 2S) + 8\delta S \tag{A3}$$

where S is the number of syllables in the motif and n_{tot} is the sum of all neuron ensembles in the syllables and gaps that make up the motif.

For this study, we use randomly selected songs with $S = 6 \pm 2$, $n_{\text{syl}} \approx 37$, and $n_{\text{gap}} \approx 10$. The number of ensembles in each syllable and gap is chosen randomly from a Poisson distribution with these averages. The number of syllables was chosen from a discrete uniform distribution. The variable n_{tot} is given by $n_{\text{tot}} = S(n_{\text{syl}} + n_{\text{gap}})$. The average number of segments for each syllable is $c \approx 2$ but is also chosen randomly from a Poisson distribution for each run of the model.

Temperature manipulation in HVC. In our previously published model, temperature changes were accomplished by increasing channel activation time constants and maximum conductance values in the HVC neurons (Galvis et al. 2017). In the present model, temperature manipulation is achieved by changing the synaptic delay value δ for chain connections. We either cool bilaterally, which increases the delay in both hemispheres, or unilaterally, which increases the delay in a single hemisphere. The modified delay is then

$$\delta_{\Delta T} = Q_{10}(\Delta T)\delta \tag{A4}$$

where

$$Q_{10}(\Delta T) = (Q_{10})^{-\Delta T/10} \tag{A5}$$

This equation has been used frequently to model the effects of temperature dependence (Galvis et al. 2017; Hamaguchi et al. 2016; Hille 2001), where Q_{10} refers to the ratio of change in time constant over 10°C change in temperature. In the previous model, gaps stretched less than syllables because they contain passes through the recurrent network (Galvis et al. 2017). Here we can use the expressions above to see why this is the case. From *Eq. A1*, the syllable lengths are stretched by HVC cooling to a length of

$$L_{\text{syl}}(\Delta T) = Q_{10}(\Delta T)\delta(n_{\text{syl}} - 1) \tag{A6}$$

if a syllable has only one segment (does not switch dominance). If the syllable does switch dominance, then there is an unstretched portion of the syllable due to four connections through the recurrent network. This gives the expression

$$L_{\text{syl}}(\Delta T) = Q_{10}(\Delta T)\delta\left[n_{\text{syl}} - 1 - 4(c-1)\right] + 4\delta(c-1) \tag{A7}$$

where c is the total number of syllable segments. Note that if $c = 1$ we get the original expression.

Similarly, the gap length is described by

$$L_{\text{gap}}(\Delta T) = Q_{10}(\Delta T)\delta(n_{\text{gap}} - 1) + 8\delta \tag{A8}$$

so the syllable length has a component $4\delta(c-1)$ that is unaffected by HVC cooling, whereas the gap length has a component 8δ that is unaffected. Unless there are $c = 3$ or more segments in a syllable, more of the unstretched portion of the song occurs during gaps because $4\delta(c-1) < 8\delta$. Furthermore, gaps are less than half as long as syllables, which implies that the percent stretch of gaps will be less than syllables even if $c = 3$. By dividing the unstretched portion of gaps and syllables by the full lengths of gaps and syllables, respectively, we arrive at an inequality relating the necessary condition for gaps to stretch less than syllables $\dfrac{4\delta(c-1)}{\delta(n_{\text{syl}}-1)} < \dfrac{8\delta}{\delta(n_{\text{gap}}-1)+8\delta}$. Using the average values for n_{syl} and n_{gap} (δ cancels), we find that as long as $c < 6$, gaps will stretch less than syllables. The motif length is described by

$$L_{\text{motif}}(\Delta T) = Q_{10}(\Delta T)\delta\left[n_{\text{tot}} - 2S - 4(c_{\text{tot}} - S)\right] + 4(c_{\text{tot}} - S) + 8\delta S \tag{A9}$$

where c_{tot} is the sum of segments in all syllables. The expressions above are valid when HVC is bilaterally cooled to the same degree in both hemispheres. With unilateral cooling, only the gaps and segments that are dominant on the cooled hemisphere are stretched.

Hamaguchi et al. (2016) found that the Q_{10} for song motifs was $Q_{10\text{-song}} \approx 1.25$, which implies that $\Delta T = -10$°C should result in an ~25% motif stretch. We used this as a constraint to find the remaining free parameter, $Q_{10\text{-}\delta}$:

$$L_{\text{motif}}(-10\,^{\circ}\text{C}) = 1.25 L_{\text{motif}}(0\,^{\circ}\text{C})$$

where the average values for c_{tot}, n_{tot}, and S were used in *Eq. A9*. We found that $Q_{10\text{-}\delta} = 1.37$. The difference between the Q_{10} value for delay of HVC chains and the smaller Q_{10} value for song reflects the portions of song that are not stretched.

GRANTS

This research was supported by National Science Foundation Grant IOS-1456965 awarded to F. Johnson, R. Bertram, R. L. Hyson, and W. Wu.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

D.G., F.J., and R.B. conceived and designed research; D.G. performed experiments; D.G. analyzed data; D.G., W.W., R.L.H., F.J., and R.B. interpreted results of experiments; D.G. prepared figures; D.G. drafted manuscript; W.W., R.L.H., F.J., and R.B. edited and revised manuscript; D.G., W.W., R.L.H., F.J., and R.B. approved final version of manuscript.

ENDNOTE

At the request of the authors, readers are herein alerted to the fact that additional materials related to this manuscript may be found at the institutional Web site of the authors, which at the time of publication they indicate is: https://www.math.fsu.edu/~bertram/software/birdsong. These materials are not a part of this manuscript and have not undergone peer review by the American Physiological Society (APS). APS and the journal editors take no responsibility for these materials, for the Web site address, or for any links to or from it.

REFERENCES

Andalman AS, Foerster JN, Fee MS. Control of vocal and respiratory patterns in birdsong: dissection of forebrain and brainstem mechanisms using temperature. *PLoS One* 6: e25461, 2011. doi:10.1371/journal.pone.0025461.

Ashmore RC, Bourjaily M, Schmidt MF. Hemispheric coordination is necessary for song production in adult birds: implications for a dual role for forebrain nuclei in vocal motor control. *J Neurophysiol* 99: 373–385, 2008a. doi:10.1152/jn.00830.2007.

Ashmore RC, Renk JA, Schmidt MF. Bottom-up activation of the vocal motor forebrain by the respiratory brainstem. *J Neurosci* 28: 2613–2623, 2008b. doi:10.1523/JNEUROSCI.4547-07.2008.

Basista MJ, Elliott KC, Wu W, Hyson RL, Bertram R, Johnson F. Independent premotor encoding of the sequence and structure of birdsong in avian cortex. *J Neurosci* 34: 16821–16834, 2014. doi:10.1523/JNEUROSCI.1940-14.2014.

Benezra SE, Narayanan RT, Egger R, Oberlaender M, Long MA. Morphological characterization of HVC projection neurons in the zebra finch (*Taeniopygia guttata*). *J Comp Neurol* 526: 1673–1689, 2018. doi:10.1002/cne.24437.

Coleman MJ, Vu ET. Recovery of impaired songs following unilateral but not bilateral lesions of nucleus uvaeformis of adult zebra finches. *J Neurobiol* 63: 70–89, 2005. doi:10.1002/neu.20122.

Danish HH, Aronov D, Fee MS. Rhythmic syllable-related activity in a songbird motor thalamic nucleus necessary for learned vocalizations. *PLoS One* 12: e0169568, 2017. doi:10.1371/journal.pone.0169568.

Galvis D, Wu W, Hyson RL, Johnson F, Bertram R. A distributed neural network model for the distinct roles of medial and lateral HVC in zebra finch song production. *J Neurophysiol* 118: 677–692, 2017. doi:10.1152/jn.00917.2016.

Gibb L, Gentner TQ, Abarbanel HD. Brain stem feedback in a computational model of birdsong sequencing. *J Neurophysiol* 102: 1763–1778, 2009a. doi:10.1152/jn.91154.2008.

Gibb L, Gentner TQ, Abarbanel HD. Inhibition and recurrent excitation in a computational model of sparse bursting in song nucleus HVC. *J Neurophysiol* 102: 1748–1762, 2009b. doi:10.1152/jn.00670.2007.

Hahnloser RH, Kozhevnikov AA, Fee MS. An ultra-sparse code underlies the generation of neural sequences in a songbird. *Nature* 419: 65–70, 2002. [Erratum in *Nature* 421: 294, 2003.] doi:10.1038/nature00974.

Hahnloser RH, Wang CZ, Nager A, Naie K. Spikes and bursts in two types of thalamic projection neurons differentially shape sleep patterns and auditory responses in a songbird. *J Neurosci* 28: 5040–5052, 2008. doi:10.1523/JNEUROSCI.5059-07.2008.

Hamaguchi K, Mooney R. Recurrent interactions between the input and output of a songbird cortico-basal ganglia pathway are implicated in vocal sequence variability. *J Neurosci* 32: 11671–11687, 2012. doi:10.1523/JNEUROSCI.1666-12.2012.

Hamaguchi K, Tanaka M, Mooney R. A distributed recurrent network contributes to temporally precise vocalizations. *Neuron* 91: 680–693, 2016. doi:10.1016/j.neuron.2016.06.019.

Hille B. *Ion Channels of Excitable Membranes*. Sunderland, MA: Sinauer, 2001.

Histed MH, Bonin V, Reid RC. Direct activation of sparse, distributed populations of cortical neurons by electrical microstimulation. *Neuron* 63: 508–522, 2009. doi:10.1016/j.neuron.2009.07.016.

Kosche G, Vallentin D, Long MA. Interplay of inhibition and excitation shapes a premotor neural sequence. *J Neurosci* 35: 1217–1227, 2015. doi:10.1523/JNEUROSCI.4346-14.2015.

Long MA, Fee MS. Using temperature to analyse temporal dynamics in the songbird motor pathway. *Nature* 456: 189–194, 2008. doi:10.1038/nature07448.

Long MA, Jin DZ, Fee MS. Support for a synaptic chain model of neuronal sequence generation. *Nature* 468: 394–399, 2010. doi:10.1038/nature09514.

Lynch GF, Okubo TS, Hanuschkin A, Hahnloser RH, Fee MS. Rhythmic continuous-time coding in the songbird analog of vocal motor cortex. *Neuron* 90: 877–892, 2016. doi:10.1016/j.neuron.2016.04.021.

McLean J, Bricault S, Schmidt MF. Characterization of respiratory neurons in the rostral ventrolateral medulla, an area critical for vocal production in songbirds. *J Neurophysiol* 109: 948–957, 2013. doi:10.1152/jn.00595.2012.

Nottebohm F, Stokes TM, Leonard CM. Central control of song in the canary, Serinus canarius. *J Comp Neur* 165: 457–486, 1976. doi:10.1002/cne.901650405.

Picardo MA, Merel J, Katlowitz KA, Vallentin D, Okobi DE, Benezra SE, Clary RC, Pnevmatikakis EA, Paninski L, Long MA. Population-level representation of a temporal sequence underlying song production in the zebra finch. *Neuron* 90: 866–876, 2016. doi:10.1016/j.neuron.2016.02.016.

Schmidt MF. Pattern of interhemispheric synchronization in HVc during singing correlates with key transitions in the song pattern. *J Neurophysiol* 90: 3931–3949, 2003. doi:10.1152/jn.00003.2003.

Simpson HB, Vicario DS. Brain pathways for learned and unlearned vocalizations differ in zebra finches. *J Neurosci* 10: 1541–1556, 1990. doi:10.1523/JNEUROSCI.10-05-01541.1990.

Wang CZ, Herbst JA, Keller GB, Hahnloser RH. Rapid interhemispheric switching during vocal production in a songbird. *PLoS Biol* 6: e250, 2008. doi:10.1371/journal.pbio.0060250.

Weber AP, Hahnloser RH. Spike correlations in a songbird agree with a simple markov population model. *PLoS Comput Biol* 3: e249, 2007. doi:10.1371/journal.pcbi.0030249.

Williams H, Crane LA, Hale TK, Esposito MA, Nottebohm F. Right side dominance for song control in the zebra finch. *J Neurobiol* 23: 1006–1020, 1992. doi:10.1002/neu.480230807.

Zhang YS, Wittenbach JD, Jin DZ, Kozhevnikov AA. Temperature manipulation in songbird brain implicates the premotor nucleus HVC in birdsong syntax. *J Neurosci* 37: 2600–2611, 2017. doi:10.1523/JNEUROSCI.1827-16.2017.