Inhibition, recurrent excitation, and neural feedback in computational models of sparse bursting and birdsong sequencing

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Inhibition, Recurrent Excitation, and Neural Feedback in Computational Models of Sparse Bursting and Birdsong Sequencing

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in Neurosciences with a Specialization in Computational Neurobiology

by

Leif Gibb

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Terrence J. Sejnowski

2009
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The Dissertation of Leif Gibb is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2009
DEDICATION

To my mother, Roberta Gibb, who has always been my greatest source of love, support, and inspiration; my grandmother, Jean Gibb, who helped her raise me and wanted to live to see me earn my PhD; and my grandfather, Prof. Thomas R. P. Gibb, who helped inspire my love of science.
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I also thank my co-advisor, Tim Gentner, for giving me the opportunity to work part time in his lab from 2006 to 2007. I learned a tremendous amount from setting up the first rig in his lab for chronic microstimulation and neural recording; writing data acquisition and control software; and performing chronic electrode implantation, microstimulation, and recordings. I am grateful to Eric Vu of the Barrow Neurological Institute in Phoenix for allowing me to spend a week in his lab in December 2006 and for visiting UCSD for four days in January 2007 to teach me stereotaxic electrode placement and histological identification of nucleus Uva.

I thank my thesis committee for their guidance and support. I am especially grateful to Terry Sejnowski and Bill Kristan for their advice over the years. I thank Terry for his informative e-mail replies to my many questions.

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2003 to 2004 helped our lab to begin its research on birdsong and coauthored three papers with us.

I thank Michael Brainard for giving me the opportunity to do a 12-week experimental rotation in his lab in the winter of 2005 and Richard Mooney of Duke University for giving me the opportunity to do a 6-week experimental rotation in his lab in the spring of 2005. Both of these rotations were extraordinary learning experiences, which I will never forget. I am grateful to Massimo Scanziani and Harvey Karten for highly informative experimental rotations at UCSD, and Dan Margoliash of the University of Chicago for giving me the opportunity to spend a week in his lab in September 2007.

I also thank Richard Mooney for discussions and ideas for experimental tests of the sparse bursting model (Chapter 1); Thomas Nowotny for providing the basic C++ neural simulation framework and programming advice; Micah Richert for help with statistics; and two anonymous reviewers of the material in Chapters 1 and 2 for the Journal of Neurophysiology, whose comments have significantly improved this dissertation. I am also grateful for helpful discussions and communications with Robin Ashmore, Maxim Bazhenov, Melissa Coleman, Michale Fee, Richard Hahnloser, John Kirn, Michael Long, David Perkel, Mikhail Rabinovich, Constance Scharff, Steven Shea, Michele Solis, Marc Schmidt, David Vicario, and Martin Wild.
I thank my friends and family members for their love, support, and encouragement. I am also grateful to the National Science Foundation’s IGERT program for the fellowship that supported me for my first years in the Computational Neurobiology program; to the Training Program in Cognitive Neuroscience of the Institute for Neural Computation at UCSD, supported by the National Institutes of Mental Health, for the fellowship that supported me from 2007 to 2008; and to the NSF-sponsored Center for Theoretical Biological Physics at UCSD, which supported me in the fall of 2008.

Chapter 1, in part, has been submitted for publication in the Journal of Neurophysiology: Gibb L, Gentner TQ, Abarbanel HD. Inhibition and recurrent excitation in a computational model of sparse bursting in song nucleus HVC, 2008. The dissertation author was the primary investigator and author of this material. The Supplementary Material section of Chapter 1 has been submitted as supplementary material for this article.

Chapter 2, in part, has also been submitted for publication in the Journal of Neurophysiology: Gibb L, Gentner TQ, Abarbanel HD. Inhibition and neural feedback in a computational model of birdsong sequencing, 2008. The dissertation author was the primary investigator and author of this material. The Supplementary Material section of Chapter 2 has been submitted as supplementary material for this article.
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Thesis project:  
Inhibition, Recurrent Excitation, and Neural Feedback in  
Computational Models of Sparse Bursting and Birdsong Sequencing

Research Summary:  
My research is motivated by two fundamental questions: How does the brain produce sequenced premotor activity? And how does the brain learn to do this? In my thesis research, I have chosen to focus on the first of these questions. To address the question of how the brain produces sequenced premotor activity, I am developing large-scale, biologically plausible models of sparse burst generation and syllable sequencing in the song system of oscine songbirds, zebra finches in particular. A central goal is to forge close ties between computational modeling and experimental investigation of the brain—and the song system in particular—at multiple levels of analysis.

Education:  
2009  
PhD in Neurosciences with a Specialization in Computational Neurobiology  
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2003-2008  
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2001  Outstanding Achievement Award in Biology: Animal Physiology and Neuroscience
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Research Experience in the Computational Neurobiology Graduate Program:

6/05-11/08  Thesis research, computational
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Created computational models of the songbird brain (C++ and MATLAB) and intensively researched the literature. Wrote and revised two papers on this work (Gibb et al. 2008a,b).

4/06-8/07  Experimental research
Timothy Gentner (co-advisor)
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12/06 & 1/07  Specialized training, Eric Vu. Barrow Neurological Institute, Phoenix, AZ and UCSD. Learned stereotaxic electrode placement and histological identification of nucleus Uva of the zebra finch (BNI: 1 week; UCSD: 4 days).

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1/05-4/05  Laboratory rotation
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Keck Center for Integrative Neuroscience, Departments of Physiology and Psychiatry, UCSF
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4/04-12/04 Computational research
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Created computational models of neurons and sparsely bursting networks in the premotor nucleus HVC of songbirds (C and MATLAB). Created network models of the slow repetitive bursting evoked in X-projecting HVC neurons in slices by antidromic stimulation of HVC-RA axons in the presence of picrotoxin. Modeling also suggested a novel explanation of the plateau potential evoked in X-projecting neurons in low Ca\(^{2+}\) in the presence of Co\(^{2+}\).

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Harvey Karten
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10/03-1/04 Laboratory rotation
Massimo Scanziani
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1/07-3/07 BIGN 260, Neurodynamics  
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C/C++, MATLAB, and LabVIEW programming  
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Publications:
Gibb L, Gentner TQ, Abarbanel HD. Inhibition and recurrent excitation in a computational model of sparse bursting in song nucleus HVC. Submitted, 2008a.  
Gibb L, Gentner TQ, Abarbanel HD. Inhibition and neural feedback in a computational model of birdsong sequencing. Submitted, 2008b.  

**Other Activities:**
12/04-2007  Played a leadership role in bringing about changes to the content and organization of the Computational Neurobiology program’s core courses and the addition of a course to the curriculum. Served on the curriculum subcommittee.
ABSTRACT OF THE DISSERTATION

Inhibition, Recurrent Excitation, and Neural Feedback in
Computational Models of Sparse Bursting and Birdsong Sequencing

by

Leif Gibb

Doctor of Philosophy in
Neurosciences with a Specialization in Computational Neurobiology

University of California, San Diego, 2009
Professor Henry D. I. Abarbanel, Chair

Mapping the functional diversity of interneurons and uncovering the roles of
neural feedback in the brain are two active areas of experimental research. The
telencephalic nucleus HVC is situated at a critical point in the pattern-generating
premotor circuitry of oscine songbirds and receives neural feedback from both
forebrain and brainstem areas. A striking feature of HVC’s premotor activity is that its projection neurons burst extremely sparsely.

In Chapter 1 of this dissertation, I present a computational model of HVC embodying several central hypotheses: (1) sparse bursting is generated in bistable groups of recurrently connected RA-projecting (HVC\textsubscript{RA}) neurons; (2) inhibitory interneurons terminate bursts in the HVC\textsubscript{RA} groups; and (3) sparse sequences of bursts are generated by the propagation of waves of bursting activity along networks of HVC\textsubscript{RA} neurons. This model of sparse bursting places HVC in the context of central pattern generators and cortical networks utilizing inhibition, recurrent excitation, and bistability. Importantly, the unintuitive result that inhibitory interneurons can precisely terminate the bursts of HVC\textsubscript{RA} groups while showing relatively sustained activity throughout the song is made possible by a specific constraint on their connectivity. I use the model to make novel predictions that can be tested experimentally.

In Chapter 2, I present a computational model of HVC and associated nuclei that builds on the model of sparse bursting presented in Chapter 1. This model embodies the hypotheses that (1) different networks in HVC control different syllables or notes of birdsong, (2) interneurons in HVC not only participate in sparse bursting but also provide mutual inhibition between networks controlling syllables or notes, and (3) these syllable networks are stimulated by neural feedback via the brainstem and the afferent thalamic nucleus Uva, or a similar feedback pathway. I discuss the
model’s ability to unify physiological, behavioral, and lesion results, and I use it to make novel predictions that can be tested experimentally.
Chapter 1: Inhibition and Recurrent Excitation in a Computational Model of Sparse Bursting in Song Nucleus HVC

Abstract

The telencephalic premotor nucleus HVC is situated at a critical point in the pattern-generating premotor circuitry of oscine songbirds. A striking feature of HVC’s premotor activity is that its projection neurons burst extremely sparsely. Here we present a computational model of HVC embodying several central hypotheses: (1) sparse bursting is generated in bistable groups of recurrently connected RA-projecting (HVC_{RA}) neurons; (2) inhibitory interneurons terminate bursts in the HVC_{RA} groups; and (3) sparse sequences of bursts are generated by the propagation of waves of bursting activity along networks of HVC_{RA} neurons. Our model of sparse bursting places HVC in the context of central pattern generators and cortical networks utilizing inhibition, recurrent excitation, and bistability. Importantly, the unintuitive result that inhibitory interneurons can precisely terminate the bursts of HVC_{RA} groups while showing relatively sustained activity throughout the song is made possible by a
specific constraint on their connectivity. We use the model to make novel predictions that can be tested experimentally.

**Introduction**

In oscine songbirds, the telencephalic nucleus HVC (used as the proper name; Reiner et al. 2004) is a key structure of the premotor pathway, projecting both to the premotor nucleus RA (the robust nucleus of the arcopallium) and to a basal ganglia nucleus, Area X, which forms the first step of a basal ganglia-thalamocortical pathway essential for song learning (the anterior forebrain pathway; Bottjer et al. 1984; Sohrabji et al. 1990; Scharff and Nottebohm 1991; Brainard and Doupe 2000; Abarbanel et al. 2004a,c; Perkel 2004).

HVC contains three broad classes of neuron: RA-projecting (HVC\textsubscript{RA}), X-projecting (HVC\textsubscript{X}), and interneurons (HVC\textsubscript{I}) (Nixdorf et al. 1989; Fortune and Margoliash 1995; Dutar et al. 1998; Kubota and Taniguchi 1998; Mooney 2000; Rauske et al. 2003; Shea 2004). Using paired intracellular recordings and antidromic stimulation in slices, Mooney and Prather (2005) have found connections between members of all three of these classes and among HVC\textsubscript{RA} neurons.
HVC<sub>RA</sub> neurons, which are the HVC projection neurons that participate directly in the adult song control pathway, have been shown to exhibit temporally sparse bursting during singing in zebra finches (Hahnloser et al. 2002; Kozhevnikov and Fee 2007): in this study, each neuron bursts at most once per song motif. Each burst consists of 4.3 ± 1.3 spikes and has a duration of 5.1 ± 1.8 ms (Kozhevnikov and Fee 2007). Similar sparse bursting also occurs spontaneously during sleep (Hahnloser et al. 2002; Hahnloser et al. 2006, Hahnloser and Fee 2007). By contrast, HVC<sub>1</sub> neurons spike and burst densely throughout the song (Hahnloser et al. 2002; Kozhevnikov and Fee 2007). We and others have previously utilized this sparse bursting in models of birdsong (Abarbanel et al. 2004b; Fiete et al. 2004; Fiete et al. 2007). The basis of sparse bursting in the neuronal circuitry of HVC remains unknown and is the focus of the model that we describe in the present paper. In the following companion paper (Gibb et al. 2008), we present a model of the potential role of neural feedback to HVC in syllable sequencing.

The present study has the goal of developing a numerical model of HVC sparse bursting in which inhibitory interneurons play a central role. In our HVC model, the sparse bursting associated with any syllable is generated by the propagation of a wave of activity along a network of locally excitatory HVC<sub>RA</sub> neurons interacting with globally inhibitory HVC<sub>1</sub> neurons. We represent this network organization of HVC<sub>RA</sub> neurons as a chain of bistable clusters. We see our model as a set of hypotheses, based
on experimental data and expressed in quantitative language, from which we can derive predictions to be tested experimentally.

A portion of this work has appeared previously in abstract form (Gibb and Abarbanel 2006).

**Methods**

We implemented all models in C++ using a neural simulation framework developed by T. Nowotny and extended by L. Gibb, using a Runge-Kutta 6(5) algorithm with a relative error of $10^{-6}$, and we performed analyses of model output in MATLAB. We also tested some of the models in Fortran.

*Basic spiking model*

All neurons in our model are based on a single-compartment Hodgkin-Huxley type neuron with just Na$^+$, K$^+$, and leak currents (Traub and Miles 1991; Destexhe et al. 1998a; Destexhe and Sejnowski 2001). The membrane potential of this basic spiking model follows the equation:
\[
C_m \frac{dV(t)}{dt} = -g_N m(t)^3 h(t)(V(t) - E_{Na}) - g_K n(t)^4 (V(t) - E_K) \\
- g_L (V(t) - E_L) - I_{syn} + I_{DC},
\]

where \(V(t)\) is the membrane potential; \(g_{Na}, g_K,\) and \(g_L\) are the maximal conductances of the \(Na^+\), \(K^+\), and leak currents; \(E_{Na}, E_K,\) and \(E_L\) are the reversal potentials of the \(Na^+\), \(K^+\), and leak currents; \(I_{syn}\) is the sum of synaptic currents; and \(I_{DC}\) is the value of an injected current (\(I_{DC} = 0\) unless otherwise noted). The gating variables \(X(t) = \{m(t), h(t), n(t)\}\) are taken to satisfy the first-order kinetics

\[
\frac{dX(t)}{dt} = \alpha_X(V(t))(1 - X(t)) - \beta_X(V(t))X(t),
\]

where \(\alpha_X(V)\) and \(\beta_X(V)\) are given in Table 1.1.

Although the ionic currents of song system neurons have not yet been well characterized, in many cases the responses of neurons to depolarizing and hyperpolarizing current pulses have been recorded in vitro. In order to match such data from \(HVC_{RA}\) and \(HVC_I\) neurons, we included and modified appropriate currents characterized in mammalian neurons. This level of modeling is appropriate to our long-term goal of spanning cellular, circuit, network and systems levels of analysis in the song system. As the model develops and new data is obtained, we will replace these neurons with more complex ones where appropriate. We will also explore simplified models to determine which details are essential to the network behavior.
TABLE 1.1. Rate functions of model neurons (V in mV). \( V_T = -53 \) mV for HVC\textsubscript{RA} neurons; \( V_T = -63.4 \) mV for HVC\textsubscript{I} neurons.

Basic spiking model (Traub and Miles 1991)

\[
\begin{align*}
\alpha_m(V) &= \frac{-0.32(V - V_T - 13)}{e^{-(V - V_T - 13)/4} - 1} \\
\beta_m(V) &= \frac{0.28(V - V_T - 40)}{e^{(V - V_T - 40)/5} - 1} \\
\alpha_h(V) &= 0.128e^{-(V - V_T - 17)/18} \\
\beta_h(V) &= \frac{4}{e^{-(V - V_T - 40)/5} + 1} \\
\alpha_s(V) &= \frac{-0.032(V - V_T - 15)}{e^{-(V - V_T - 15)/5} - 1} \\
\beta_n(V) &= 0.5e^{-(V - V_T - 10)/40}
\end{align*}
\]

I\textsubscript{Ms} and I\textsubscript{Mf} currents of HVC\textsubscript{RA} neurons

\[
\begin{align*}
\alpha_p(V) &= \frac{-10^{-4}(V + 33)}{e^{-(V + 33)/0.9} - 1} \\
\beta_p(V) &= \frac{10^{-4}(V + 33)}{e^{(V + 33)/0.9} - 1} \\
\alpha_q(V) &= \frac{-2\times10^{-7}(V + 33)}{e^{-(V + 33)/0.9} - 1} \\
\beta_q(V) &= \frac{2\times10^{-7}(V + 33)}{e^{(V + 33)/0.9} - 1} + \frac{0.2(V + 68)}{e^{(V + 68)/0.9} - 1}
\end{align*}
\]

I\textsubscript{h} current of HVC\textsubscript{I} neurons

\[
\begin{align*}
\tau_r(V) &= \frac{1}{e^{(V + 75)/5.5} + 1} \\
\tau_r(V) &= \frac{195 \text{ ms}}{e^{(V + 71.9)/14.27} + e^{-(89.3 + V)/11.65}}
\end{align*}
\]

Modeling HVC\textsubscript{RA} neurons

We assume that the HVC\textsubscript{RA} neurons that burst sparsely during singing are of the “tonic” type, which spike multiple times in response to depolarizing current injection, as described by Kubota and Taniguchi (1998). This type has been identified with the short dendrite (SD) morphological class. There is also a “phasic” HVC\textsubscript{RA}
type, which has been identified with the furry dendrite class (FD; Shea 2004; Nixdorf et al. 1989; Fortune and Margoliash 1995; see Discussion). To model Kubota and Taniguchi’s HVC\textsubscript{RA} neurons, we added two voltage-gated K\textsuperscript{+} currents to the basic spiking model described above and modified parameters from those used by Destexhe et al. (1998a) for cortical pyramidal neurons. To better match the in vitro data, we decreased the reversal potential, \( E_{L} \), of the leak current from -70 to -83 mV and slightly shifted the voltage-dependent rate functions for the Na\textsuperscript{+} and K\textsuperscript{+} channels (\( \alpha_{m}, \beta_{m}, \alpha_{h}, \beta_{h}, \alpha_{n} \), and \( \beta_{n} \); see Table 1.1 and the value of \( V_{T} \)) to raise the spike threshold.

The instantaneous spike frequency of these HVC\textsubscript{RA} neurons declines rapidly over the first few action potentials and then declines much more gradually thereafter during constant current injection (Kubota and Taniguchi 1998), which suggests that there are two timescales of spike-rate adaptation. Since the currents underlying this behavior have not been characterized in HVC\textsubscript{RA} neurons, we modified the voltage-gated K\textsuperscript{+} current \( I_{M} \), described by McCormick et al. (1993) and used by Destexhe et al. (1998a) in their model of cortical pyramidal neurons. The modified current \( I_{Ms} \) provides the slow component of adaptation, and the modified current \( I_{Mf} \) provides the fast component:

\[
I_{Ms} = g_{Ms} p(t)(V(t) - E_{K}) \quad \text{and} \quad I_{Mf} = g_{Mf} q(t)(V(t) - E_{K}),
\]

where \( g_{Ms} \) and \( g_{Mf} \) are the maximal conductances of \( I_{Ms} \) and \( I_{Mf} \). For both \( I_{Ms} \) and \( I_{Mf} \), we shifted the \( I_{M} \) rate functions by -3 mV and sharpened them so that small depolarizing current injections trigger very little spike-frequency adaptation, while
large injections trigger strong adaptation, in accord with the in vitro data. To generate
the rate functions of $I_{Mf}$, we multiplied the rate functions of $I_M$ by a constant. To
prevent a post-burst hyperpolarization that is not observed in the data, we modified $\beta_q$
so that $I_{Mf}$ rapidly deactivates below about -68 mV (see Table 1.1).

We adjusted the parameters to match the resting potential and the
instantaneous spike frequency as a function of time for different injected currents
described by Kubota and Taniguchi (1998; see Supplementary Fig. 1.S1). The
parameter values for the HVC<sub>RA</sub> neuron model were $g_{Na} = 50$ mS/cm$^2$, $E_{Na} = 45$ mV,
$g_K = 5$ mS/cm$^2$, $E_K = -88$ mV, $g_L = 0.1$ mS/cm$^2$, $E_L = -83$ mV, $C_M = 1$ $\mu$F/cm$^2$, $V_T = -53$ mV, $g_{Ms} = 0.3$ mS/cm$^2$, and $g_{Mf} = 0.8$ mS/cm$^2$.

**Modeling HVC<sub>I</sub> neurons**

To model HVC<sub>I</sub> neurons, we again built on the basic spiking model described
above. We modified parameters from those used by Destexhe et al. (1998a) to model
cortical interneurons, adjusting the parameters to match the resting potential and the
instantaneous spike frequency as a function of time for different injected currents
described by Kubota and Taniguchi (1998; see Supplementary Fig. 1.S1). The data of
Kubota and Taniguchi (1998) and Dutar et al. (1998) demonstrate that HVC<sub>I</sub> neurons
have a depolarizing sag in response to a hyperpolarizing current injection and show
little spike-rate adaptation. We represent this in the membrane voltage equation by
including $I_h$ but no special $K^+$ currents:

$$I_h = g_h r(t)(V(t) - E_h)$$

where $g_h$ and $E_h$ are the maximal conductance and reversal potential of $I_h$, and

$$\frac{dr(t)}{dt} = \frac{r_c(V(t)) - r(t)}{\tau_r(V(t))}$$

where $r_c(V)$ and $\tau_r(V)$ are given in Table 1.1, based on Huguenard and McCormick
(1994). This $\tau_r(V)$ is 20 times smaller than in the model on which it is based. We made
this modification solely to improve the match of the model to the data of Kubota and
Taniguchi (1998) and Dutar et al. (1998) by speeding up the sag. This modification
can be justified by the broad range of $I_h$ time constants reported in different cortical
and subcortical neurons, which depend on the subunit composition of $I_h$ channels
(Aponte et al. 2006). The parameter values of the HVC1 neuron model were $g_{Na} = 50$
mS/cm$^2$, $E_{Na} = 45$ mV, $g_K = 10$ mS/cm$^2$, $E_K = -85$ mV, $g_L = 0.15$ mS/cm$^2$, $E_L = -64$
mV, $C_M = 1 \mu F/cm^2$, $V_T = -63.4$ mV, $g_h = 0.07$ mS/cm$^2$, and $E_h = -40$ mV.

Once fit to the in vitro data, the parameters of the neuron models were held
fixed; only synaptic strengths and network connectivity were adjusted to achieve the
desired network behavior.
Modeling synaptic currents

We modeled both excitatory and inhibitory synaptic currents with the following equations (Destexhe et al. 1994; Destexhe and Sejnowski 2001):

\[
T(t) = \frac{T_{\text{max}}}{1 + \exp\left(-(V_{\text{pre}}(t) - V_p) / K_p\right)}
\]

\[
\frac{dr(t)}{dt} = \alpha T(t)(1 - r(t)) - \beta r(t)
\]

\[
I_{\text{syn}}(t) = g_{\text{syn}} r(t)(V_{\text{post}}(t) - E_{\text{rev}}),
\]

where \(T(t)\) is the concentration of neurotransmitter in the synaptic cleft, \(T_{\text{max}}\) is the maximal neurotransmitter concentration, \(V_{\text{pre}}(t)\) and \(V_{\text{post}}(t)\) are the membrane potentials of the presynaptic and postsynaptic neurons, \(r(t)\) is the fraction of the receptors in the open state, \(g_{\text{syn}}\) (\(g_{\text{AMPA}}\) or \(g_{\text{GABA}_A}\)) is the maximal synaptic conductance, and \(E_{\text{rev}}\) (\(E_{\text{AMPA}}\) or \(E_{\text{GABA}_A}\)) is the synaptic reversal potential.

The rate constants that we used are based on the time constants of AMPA- and GABA\(_A\)-mediated currents recorded in mammalian neocortical neurons and interneurons in vitro (Hestrin 1993; Destexhe et al. 1994; Destexhe and Sejnowski 2001). AMPA receptors at excitatory synapses onto interneurons are about twice as fast as those onto pyramidal neurons (Hestrin 1993; Destexhe and Sejnowski 2001).

For all synapses in our model, \(T_{\text{max}} = 1.5 \text{ mM}, V_p = 2 \text{ mV},\) and \(K_p = 5 \text{ mV}.\) For excitatory synapses, \(E_{\text{rev}} = 0 \text{ mV}.\) For excitatory synapses onto HVC\(_1\) neurons, \(\alpha = 2.2.\)
mM$^{-1}$ ms$^{-1}$ and $\beta = 0.38$ ms$^{-1}$ (decay time constant $1/\beta = 2.6$ ms). For excitatory synapses onto all other neuron types, $\alpha = 1.1$ mM$^{-1}$ ms$^{-1}$ and $\beta = 0.19$ ms$^{-1}$ ($1/\beta = 5.3$ ms). For inhibitory synapses, $\alpha = 5.0$ mM$^{-1}$ ms$^{-1}$ and $\beta = 0.18$ ms$^{-1}$ ($1/\beta = 5.6$ ms). In the reduced cluster model and the chain models based on it, $E_{rev} = -83$ mV for inhibitory synapses. In the models with synapses of physiological strength (see below), $E_{rev} = -88$ mV in order to give inhibitory postsynaptic potentials (IPSPs) a physiologically correct, nonzero amplitude near the HVC$_{RA}$ resting potential of -83 mV. Mooney and Prather (2005) did not measure $E_{rev}$ experimentally; our assumed value of -88 mV is similar to, and slightly less negative than, the mean GABA$A$ reversal potential measured in neurons of the medial portion of the dorsolateral thalamic nucleus (DLM; Person and Perkel 2005). This assumed value is necessarily an informed guess, since Mooney and Prather did not report the resting potentials of the neurons in which they measured IPSP amplitudes. Moreover, in two of the HVC$_I$ $\rightarrow$ HVC$_{RA}$ connections that they reported, the PSP was depolarizing rather than hyperpolarizing. However, we do not expect the precise value of $E_{rev}$ to be critical to the behavior of the model, as long as it is below the spiking threshold.

**Modeling temperature dependence of neurons and synapses**

In simulations of in vivo activity, as a first approximation of the temperature dependence of neurons and synapses, we scaled all rate functions by a factor of

$$\phi(T_i) = Q_{10}^{(T_i - T_0)/10},$$

assuming a $Q_{10}$ of 3 for both neuronal and synaptic rates (Hodgkin
and Huxley 1952; Collingridge et al. 1984). Here, $T_2$ is the brain temperature in vivo (assumed to be 40°C) and $T_1$ is the approximate temperature at which the measurements were made in vitro. $T_1$ is 32°C for neurons (Kubota and Taniguchi 1998), 31°C for AMPA synapses (Xiang et al. 1992; Destexhe et al. 1998b; Destexhe and Sejnowski 2001), and 34°C for GABA$_A$ synapses (Otis and Mody 1992a,b; Destexhe et al. 1998b; Destexhe et al. 2001).

**Synapses of physiological strength**

In some simulations, we used synapses of physiological strength, based on the measurements of Mooney and Prather (2005). The values of $\alpha$ and $\beta$ for these synapses followed the rules described above. We list the other parameter values and postsynaptic potential (PSP) peak amplitudes at 40°C here, together with the peak amplitudes measured by Mooney and Prather (abbreviated MP). HVC$_I$ → HVC$_{RA}$: $g_{GABA_A} = 0.11$ mS/cm$^2$, $E_{GABA_A} = -88$ mV, IPSP amplitude -0.9 mV (MP: -0.9 ± 0.2 mV). HVC$_{RA}$ → HVC$_I$: $g_{AMPA} = 0.034$ mS/cm$^2$, $E_{AMPA} = 0$ mV, EPSP amplitude 2.0 mV (MP: 2.0 ± 0.4 mV). HVC$_{RA}$ → HVC$_{RA}$: $g_{AMPA} = 0.018$ mS/cm$^2$, $E_{AMPA} = 0$ mV. EPSP amplitude 2.2 mV (MP: 2.2 ± 1.1 mV).
Poisson synapses

In some simulations, we included Poisson synapses in order to generate spiking of a particular frequency or to mimic background synaptic activity in vivo in a manner similar to that described by Destexhe et al. (1998a). Each Poisson synapse was an AMPA or GABA_A synapse receiving 1 ms, 1 mM neurotransmitter pulses as Poisson random events. The values of $\alpha$ and $\beta$ for these synapses again followed the rules described above. To mimic background synaptic activity, each HVC_I neuron received 20 Poisson AMPA synapses with a mean rate of 10 Hz and a maximal conductance of 0.02 mS/cm$^2$. This resulted in 8.8 Hz spiking (measured over 100 s), which is consistent with the 8 ± 6 Hz spontaneous firing rate measured by Kozhevnikov and Fee (2007). Similarly, each HVC_RA neuron received 20 Poisson AMPA (10 Hz, $g_{\text{AMPA}} = 0.02$ mS/cm$^2$) and 20 Poisson GABA_A synapses (10 Hz, $g_{\text{GABA}} = 0.1$ mS/cm$^2$). This resulted in a standard deviation of the membrane potential of 2.8 mV (measured over 100 s). This pattern of Poisson synapses—a combination of AMPA and GABA_A for HVC_RA neurons, and just AMPA for HVC_I neurons—mirrors the connectivity of our model: HVC_RA $\rightarrow$ HVC_RA, HVC_I $\rightarrow$ HVC_RA, HVC_RA $\rightarrow$ HVC_I, but no HVC_I $\rightarrow$ HVC_I synapses. We acknowledge that there may also be a significant number of HVC_I $\rightarrow$ HVC_I connections, but Mooney and Prather (2005) observed only one example. With appropriate parameter adjustments, we would expect to see similar results if Poisson GABA_A synapses were included on HVC_I neurons.
**Sparseness index and spike time**

We defined the sparseness of a spike train as \( S = 1 - D \), where \( D \) is the fraction of 10 ms bins containing one or more spikes. We defined spike time as the time of the peak depolarization following a crossing of a \(-15\) mV threshold in a positive direction.

Throughout the paper, we report values as mean ± SD.

**Results**

*Initial model: Dynamical behavior of a single bistable cluster*

Our current model has evolved from the simple idea that clusters of \( \text{HVC}_{\text{RA}} \) neurons could act as bistable units in generating the sparse bursting in HVC. The connectivity of our model clusters (Fig. 1.1C) is based on the HVC microcircuit described by Mooney and Prather (2005), in particular their observations of depolarizing \( \text{HVC}_{\text{RA}} \rightarrow \text{HVC}_{\text{RA}} \) and \( \text{HVC}_{\text{RA}} \rightarrow \text{HVC}_1 \) and hyperpolarizing \( \text{HVC}_1 \rightarrow \text{HVC}_{\text{RA}} \) connections. The recurrent (feedback) excitation of the \( \text{HVC}_{\text{RA}} \) neurons is the
basis for the bistability: the HVC\textsubscript{RA} cluster has two stable states, quiescent and persistently spiking. These states are a fixed point and a stable limit cycle, respectively.

Despite the existence of synaptic connections from HVC\textsubscript{X} to HVC\textsubscript{RA} neurons (Mooney and Prather 2005), we did not give HVC\textsubscript{X} neurons a role in HVC\textsubscript{RA} sparse burst generation in our model. The observation that adult song production is not affected by targeted ablation of HVC\textsubscript{X} neurons, while it is often disrupted by ablation of HVC\textsubscript{RA} neurons (Scharff et al. 2000), suggests that HVC\textsubscript{X} neurons are a less critical part of the premotor pattern generating circuit (or possibly that there is much greater redundancy in that part of the circuit with respect to premotor pattern generation).

We created clusters in which each HVC\textsubscript{RA} neuron sends excitatory synapses of physiological strength (see Methods) to a randomly selected set of 50\% of the other HVC\textsubscript{RA} neurons within the same cluster. We chose this degree of connectivity to provide a high level of mutual excitation without implying an excessively large number of reciprocally connected HVC\textsubscript{RA} neurons within each cluster, since reciprocally connected HVC\textsubscript{RA} neurons were not observed by Mooney and Prather (2005). We prohibited each HVC\textsubscript{RA} neuron from making a synapse onto itself or more than one synapse onto the same postsynaptic neuron.
FIG 1.1. Large clusters of HVC neurons with synapses of physiological strength. 

A: Duration of spiking in response to a 3 ms, 40 µA/cm² current pulse injected into 50% of the neurons in clusters of HVC_RA neurons with synaptic noise (green), no synaptic noise (blue), and no adaptation currents or noise (red). Spiking is truncated at 1 s by the end of the simulations. Each data point is the mean spiking duration of all the neurons in three separate simulations. We show error bars only for points at which SD > 20 ms.

B: Bistability of a 160-HVC_RA cluster without noise. Membrane potential of a representative HVC_RA neuron. We shifted the cluster into its persistently spiking state at t = 10 ms by exciting 50% of the HVC_RA neurons with a 3 ms, 40 µA/cm² DC current pulse (green arrow). We shifted the cluster back into its quiescent state at t = 110 ms by inhibiting the same 50% of the HVC_RA neurons with a 3 ms, -40 µA/cm² DC current pulse (red arrow).

C: Comparison of two distinct modes of inhibition for burst generation in 160-HVC_RA, 80-HVC_I clusters. Left: In the inhibitory buildup mechanism, the HVC_RA burst is terminated by relatively weak feedback inhibition throughout the burst. Right: In the inhibitory pause mechanism, HVC_RA neurons are disinhibited during the burst, and a strong onset of inhibition terminates the burst abruptly. Top: Schematics of the two mechanisms. Arrowheads, excitatory connections; dots, inhibitory connections. Middle: Representative HVC_RA voltage traces. Bottom: Spike time raster plot of 50% of the HVC_I neurons.
160-HVC\textsubscript{RA} cluster:

A

B

C

inhibitory buildup
burst terminated by feedback inhibition

inhibitory pause
burst terminated by background inhibition

HVC\textsubscript{RA}

HVC\textsubscript{i}

Poisson excitatory synapses
In Fig. 1.1A, we show the duration of spiking activity evoked by a 3 ms, 40 \( \mu \text{A/cm}^2 \) DC current pulse injected into 50% of the HVC\textsubscript{RA} neurons in clusters without inhibitory HVC\textsubscript{I} neurons, plotted as a function of HVC\textsubscript{RA} cluster size. The mean spiking duration increases as a function of the number of HVC\textsubscript{RA} neurons. For intermediate cluster sizes, with the normal adaptation currents (I\textsubscript{Ms} and I\textsubscript{Mi}) present (blue and green traces), the repetitively spiking state of the cluster is only transiently stable. The spiking duration reaches at least 1 s at a cluster size of 160 HVC\textsubscript{RA} neurons in the absence of synaptic noise (Fig. 1.1A, blue trace), 190 HVC\textsubscript{RA} neurons in the presence of synaptic noise (see Methods; Fig. 1.1A, green trace), and 60 HVC\textsubscript{RA} neurons in the absence of the adaptation currents and noise (Fig. 1.1A, red trace). These sizes are comparable to the number of HVC\textsubscript{RA} neurons, ~200, that Fee et al. (2004) estimated are coactive at each time in the song, but we will return to this point in the Discussion. Additionally, the spiking duration also increases as a function of the percent connectivity of the cluster (not shown).

In Fig. 1.1B, we demonstrate the bistability of a 160-HVC\textsubscript{RA} cluster without inhibitory HVC\textsubscript{I} neurons or synaptic noise. At \( t = 10 \) ms (green arrow), we shift the cluster into its persistently spiking state by exciting 50% of the HVC\textsubscript{RA} neurons with a 3 ms, 40 \( \mu \text{A/cm}^2 \) current pulse. This state persists for at least 1 s in the absence of further inputs (not shown). In Fig. 1.1B, we shift the cluster back into its quiescent state with a 3 ms, -40 \( \mu \text{A/cm}^2 \) current pulse into the same 50% of the HVC\textsubscript{RA} neurons at \( t = 110 \) ms (red arrow).
Mechanisms of inhibition

Among the possible mechanisms by which inhibition could contribute to sculpting HVC<sub>RA</sub> activity into sparse bursts, two stand out as extremes along a spectrum: At one end of the spectrum (an “inhibitory buildup” mechanism), relatively weak feedback inhibition of HVC<sub>RA</sub> neurons could exert a hyperpolarizing influence throughout the burst, leading to a reduced spiking frequency followed by a failure of spiking and a termination of the burst after several milliseconds. In this mechanism, relatively small changes in the strength and timescale of the inhibition strongly influence the duration of the burst. At the other end of the spectrum (an “inhibitory pause” mechanism), HVC<sub>RA</sub> neurons could be completely disinhibited during the burst, and a strong onset of inhibition after several milliseconds could terminate the burst abruptly. In this mechanism, small changes in the strength and timescale of the inhibition are inconsequential, but the delay between the onset of the burst and the onset of the inhibition controls the duration of the burst. Between these extremes is a range of intermediate mechanisms in which the onset of inhibition is delayed but relatively weak, so that a combination of inhibitory strength and inhibitory pause duration controls the duration of the burst.

A second dichotomy between inhibitory mechanisms is that between “local” inhibition, in which each HVC<sub>T</sub> neuron connects to a set of HVC<sub>RA</sub> neurons in only
one part of the HVC\textsubscript{RA} network, and “global” inhibition, in which each HVC\textsubscript{1} neuron connects to a set of HVC\textsubscript{RA} neurons throughout the HVC\textsubscript{RA} network.

In the present paper, we argue that if inhibition does indeed play a role in HVC\textsubscript{RA} burst termination, then global inhibition utilizing the inhibitory pause mechanism is a favored candidate (although the true mechanism may be somewhere between the extremes of inhibitory pause and inhibitory buildup). We make this argument along two main lines: First, in this section we show that a version of the inhibitory pause mechanism is more robust than a version of the inhibitory buildup mechanism to a variety of parameter changes. Second, in the following two sections, we show that (1) a chain network utilizing local inhibition and an inhibitory buildup mechanism is sensitive to parameter changes and also, critically, incompatible with the experimentally observed pattern of HVC\textsubscript{1} activity during singing; (2) a chain network utilizing global inhibition and an inhibitory buildup mechanism (i.e. global inhibition without any constraints on HVC\textsubscript{1}-HVC\textsubscript{RA} connectivity) is unable to generate bursts with a consistent, correct number of spikes; and (3) by contrast, a chain network utilizing global inhibition and an inhibitory pause mechanism (i.e. global inhibition with a specific constraint on HVC\textsubscript{1}-HVC\textsubscript{RA} connectivity) is able to generate appropriate bursts. Our main goals here are to demonstrate that HVC\textsubscript{1} neurons could participate in the HVC\textsubscript{RA} burst mechanism, to show a possible mechanism by which they could accomplish this, and to make experimental predictions on the basis of this mechanism.
Although estimates of the proportions of HVC neuron classes vary substantially (e.g. Alvarez-Buylla and Nottebohm 1988; Kirn et al. 1999), one estimate is that 50% of HVC neurons are RA-projecting, 25% are X-projecting, and 25% are interneurons (Nottebohm et al. 1990). Consistent with these proportions, we created a 160-HVC<sub>RA</sub>, 80-HVC<sub>I</sub> cluster. To illustrate the inhibitory buildup and inhibitory pause mechanisms of inhibition described above, we created two versions of this large cluster model (Fig. 1.1C). To verify the robustness of both of these models, we included synaptic noise in the HVC<sub>RA</sub> and HVC<sub>I</sub> neurons as described in Methods.

In our implementation of the inhibitory buildup mechanism (Fig. 1.1C, left), each HVC<sub>RA</sub> neuron in the bistable cluster sends a single synapse to each of 9 HVC<sub>I</sub> neurons, and each HVC<sub>I</sub> neuron sends a single synapse to each of 4 HVC<sub>RA</sub> neurons. We initiated the burst with a 3 ms, 40 mS/cm<sup>2</sup> DC current pulse into 50% of the HVC<sub>RA</sub> neurons beginning at t = 20 ms (the simulation began at t = -30 ms). For 10 trials with identical parameters but different random connectivity, the number of spikes per burst was 3.6 ± 1.1 and the burst duration was 6.7 ± 2.3 ms.

In our implementation of the inhibitory pause mechanism (Fig. 1.1C, right), each HVC<sub>I</sub> neuron sends a single synapse to each of 70 HVC<sub>RA</sub> neurons but does not receive synapses from any HVC<sub>RA</sub> neurons. We excite the bistable HVC<sub>RA</sub> cluster by a
brief current pulse. It is then inhibited by HVC\textsubscript{I} neurons that we begin exciting 6.4 msec later. This inhibition terminates the burst. In order to relate this model to a network model that we introduce later, we also excite the HVC\textsubscript{I} neurons until 7 ms before initiating the HVC\textsubscript{RA} burst; thus the HVC\textsubscript{RA} burst occurs largely during a pause in the high-frequency presynaptic HVC\textsubscript{I} spiking. We set the pause to begin 7 ms before burst initiation in order to give the inhibition sufficient time to decay so that it does not interfere with the burst. As in the inhibitory buildup mechanism, we initiated the burst with a 3 ms, 40 mS/cm\textsuperscript{2} current pulse into 50\% of the HVC\textsubscript{RA} neurons beginning at t = 20 ms (again, the simulation began at t = -30 ms). We elicited the relatively high-frequency spiking of each HVC\textsubscript{I} neuron before t = 13 ms and after t = 26.4 ms with a 100-Hz Poisson AMPA synapse with g\textsubscript{AMPA} = 0.2 mS/cm\textsuperscript{2} (see Methods), together with the synaptic noise. This 89-Hz HVC\textsubscript{I} spiking frequency (measured over 100 s) is consistent with the 95 \pm 40 Hz firing rate of HVC\textsubscript{I} neurons recorded during singing (Kozhevnikov and Fee 2007). For 10 trials with identical parameters but different random connectivity, the number of spikes per burst was 4.0 \pm 0.8 and the burst duration was 5.4 \pm 1.4 ms. The HVC\textsubscript{I} \rightarrow HVC\textsubscript{RA} connections are sufficiently strong that in the absence of the pause in the high-frequency HVC\textsubscript{I} spiking, none of the HVC neurons burst (10 trials).

What could generate the pause in the inhibition of HVC\textsubscript{RA} neurons in the inhibitory pause mechanism? We will return to this question in the context of our model utilizing globally connected HVC\textsubscript{I} neurons, below (Fig. 1.4).
Our implementation of the inhibitory buildup mechanism was more sensitive than the inhibitory pause mechanism to a variety of parameter changes. In the inhibitory buildup mechanism, longer bursts tended to be associated with weaker or more rapidly decaying inhibition (Fig. 1.2A,B,D), consistent with a termination of bursting by the hyperpolarizing influence of inhibition during the burst. The burst duration decreased with the inhibitory synaptic decay time constant, $1/\beta$ (Fig. 1.2A; larger values imply longer inhibitory postsynaptic current decay times). Similarly, the burst duration decreased as the number of inhibitory synapses sent to HVC$_{RA}$ neurons by each HVC$_{I}$ neuron increased, either with the normal adaptation currents present ($I_{Mg}$ and $I_{Mi}$; Fig. 1.2B) or with these currents absent (Fig. 1.2D). In the absence of the adaptation currents, there was an abrupt transition from long to short bursts (Fig. 1.2D), suggesting an important contribution of these currents to burst termination. Consistent with this observation, the burst duration decreased with increasing strength of the adaptation currents (Fig. 1.2C). Additionally, the burst duration increased with the number of excitatory HVC$_{RA}$-HVC$_{RA}$ synapses per HVC$_{RA}$ neuron (Fig. 1.2E), consistent with a competition between inhibition and excitation in determining the burst duration.
FIG 1.2. Burst duration for the inhibitory buildup mechanism of Fig. 1.1C as a function of various parameters. Dark gray lines: mean ± SD. Light gray regions: HVC\textsubscript{RA} burst duration (mean ± SD) measured by Kozhevnikov and Fee 2007. A: Inhibitory synaptic decay time constant, 1/\(\beta\) (normal value: 5.6 ms). B: Inhibitory synapses per HVC\textsubscript{I}, normal adaptation currents present (normal number: 9). C: Scale factor by which the maximal conductances of both adaptation currents are multiplied (normal value: 1). D: Inhibitory synapses per HVC\textsubscript{I}, adaptation currents absent. E: Excitatory synapses per HVC\textsubscript{RA} (normal number: 80).
For our implementation of the inhibitory buildup mechanism, the mean burst durations fell within one standard deviation of the mean recorded in vivo (Fig 1.2, light gray regions; Kozhevnikov and Fee 2007) only in a narrow range of values (1 to 4 data points). By contrast, in our implementation of the inhibitory delay mechanism, the mean burst durations fell within this range over a much wider range of values (more than 8 data points in every case, for the same spacing; Supplementary Fig. 1.S2). This suggests that the inhibitory pause mechanism is the more robust of the two mechanisms.

A model of HVC\textsubscript{RA} sparse bursting: the limitations of local inhibition

In order to explore a larger network of sparsely bursting HVC\textsubscript{RA} neurons in a more computationally efficient manner, we created a reduced cluster model that captures the recurrent excitation and feedback inhibition of the inhibitory buildup model of the previous section (Fig. 1.1C). This reduced model contains three HVC\textsubscript{RA} neurons connected in a ring by excitatory synapses (Supplementary Fig. 1.S3). Neuron 1 receives DC current input or input from another cluster, neuron 2 sends output to the next cluster, and neuron 3 completes the circuit back to neuron 1. Each of these HVC\textsubscript{RA} neurons makes an excitatory synapse on a local interneuron, which in turn makes inhibitory synapses on the three HVC\textsubscript{RA} neurons. The behavior of this reduced cluster model is qualitatively similar to that of the inhibitory buildup model of the previous section.
Such bistable clusters can be arranged in long chains so that they successively excite each other to generate a burst sequence; we illustrate this in Fig. 1.3 for the reduced cluster model. To initiate the wave of bursting activity, we set $I_{DC} = 30 \mu A/cm^2$ in the first neuron of the first chain for 5 ms beginning at $t = 0$ ms. This “begin song” command may correspond to synaptic input from an afferent nucleus or the end of another chain within HVC. The activity then propagates from cluster to cluster until it reaches the end of the chain. Activity in each cluster is evoked by excitatory input to its HVC$_{RA}$ neuron 1 from the HVC$_{RA}$ neuron 2 of the previous cluster, and terminated by inhibitory input from the local HVC$_I$.

Fig. 1.3B is a raster plot of the spike times of a subset of HVC$_I$ neurons from a total of 250 reduced clusters (3 HVC$_{RA}$ neurons each) linked in series. For HVC$_{RA} \rightarrow$ HVC$_{RA}$ synapses within each cluster, $g_{AMPA} = 1.95$ mS/cm$^2$. For those between clusters, $g_{AMPA} = 1.5$ mS/cm$^2$. We made the latter weaker to prevent driving the first neuron of each cluster too strongly, as it receives a synapse not only from within the cluster but also from the previous cluster (this is an artifact of our reduced model and is not meant to reflect differences in connectivity within HVC). For HVC$_{RA} \rightarrow$ HVC$_I$ synapses, $g_{AMPA} = 0.2$ mS/cm$^2$. For HVC$_I \rightarrow$ HVC$_{RA}$ synapses, $g_{GABA} = 1.45$ mS/cm$^2$. The burst duration was $5.6 \pm 1.5$ ms, with $3.3 \pm 0.9$ spikes per burst. We injected a $0.5 \mu A/cm^2$ DC current into the HVC$_I$ neurons so that they spiked spontaneously at $\sim 8$ Hz, consistent with their spontaneous firing rate in awake, non-
singing zebra finches. The HVC\textsubscript{1} neurons had identical initial conditions and DC current; this accounts for the identical timing of the first spike of most of these neurons.

**FIG 1.3.** Chains of clusters with local HVC\textsubscript{1} neurons produce sparse bursting in HVC\textsubscript{RA} neurons but do not produce sustained activity in HVC\textsubscript{1} neurons beyond their low-frequency spontaneous spiking. **A:** Schematic of model. Clusters consist of 3 recurrently excitatory HVC\textsubscript{RA} neurons and 1 local inhibitory HVC\textsubscript{1} neuron. Activity of the chain is initiated in the first HVC\textsubscript{RA} neuron of the first cluster by a DC current pulse, representing a synaptic input from an afferent nucleus or the end of another chain within HVC. Sparse bursting in HVC\textsubscript{RA} neurons is generated as a wave of activity propagates along the network, each cluster bursting briefly and then falling silent again as in Fig. 1.1C. **B:** Raster plot of the spike times of a subset of HVC\textsubscript{RA} and HVC\textsubscript{1} neurons in a chain of 250 clusters. Each row represents the spike times of one neuron. Activity of the chain was initiated in the first HVC\textsubscript{RA} neuron of the first cluster (not shown) by a 5 ms, 30 \( \mu \)A/cm\(^2\) current pulse beginning at \( t = 0 \) ms. Note that in this model, HVC\textsubscript{1} neurons also burst sparsely, which is inconsistent with the data of Hahnloser et al. (2002) and Kozhevnikov and Fee (2007).

However, this model required fine parameter tuning and was sensitive to changes in the HVC\textsubscript{1} \( \rightarrow \) HVC\textsubscript{RA} inhibitory synaptic strength. For example, a decrease from 1.45 mS/cm\(^2\) to 1.40 mS/cm\(^2\) destabilized the burst sequence, resulting in a mean burst duration of 27.0 ± 20.9 ms, with 8.9 ± 6.1 spikes per burst.
Significantly, in this model, each HVC\(_1\) bursts only once as activity propagates along the chain. By contrast, the HVC\(_1\) neurons described by Hahnloser et al. (2002) and Kozhevnikov and Fee (2007) show an elevated level of spiking and bursting throughout the song. To correct this discrepancy, we developed the model of global inhibition described in the next section.

*A model of HVC\(_{RA}\) sparse bursting utilizing global inhibition*

Our current model of sparse bursting is depicted in Fig. 1.4. As in the previous model, HVC\(_{RA}\) neurons are organized into a chain of bistable clusters. However, HVC\(_1\) neurons are no longer functionally localized to these clusters; rather, they are permitted to receive excitation from, and send inhibition to, any part of the chain. We initiate the burst sequence by setting \(I_{DC} = 20 \mu A/cm^2\) in the first neuron of the first chain for 5 ms beginning at 0 ms. Again, excitation travels through the network via HVC\(_{RA}\) → HVC\(_{RA}\) synapses. The local excitatory synapses between HVC\(_{RA}\) neurons keep each cluster of neurons in an excited state until a subpopulation of global HVC\(_1\) neurons terminates its activity.
FIG. 1.4. Chains of clusters with global HVC_I neurons produce sparse bursting if and only if each HVC_I neuron is constrained not to inhibit HVC_RA neurons in its own cluster or within some number of clusters downstream of one from which it receives excitation. A: Schematic of model. Each chain consists of a series of recurrently excitatory HVC_RA clusters; clusters do not contain local HVC_I neurons. Each HVC_I (dark blue circle) receives excitatory synapses from (light blue lines with arrowheads) and sends inhibitory synapses to (dark blue lines with dots), many HVC_RA neurons (light blue circles) throughout the chain. Activity in the chain is initiated in the first HVC_RA by a 5 ms, 20 μA/cm² current pulse beginning at 0 ms. As the wave of excitation propagates along the HVC_RA network, global HVC_I neurons are excited by HVC_RA clusters; each HVC_RA burst is terminated by inhibition from HVC_I neurons. Connection marked “X” is not permitted because it tends to influence the burst propagation: if the postsynaptic neuron is in the same cluster that sends excitation to the HVC_I neuron or one cluster downstream, then the connection tends to terminate the sequence. If the postsynaptic neuron is slightly further downstream, then it tends to slow the burst propagation and increase the burst duration. As a result of their constrained connectivity, HVC_I neurons inhibit only in front of and well behind the propagating wave of HVC_RA excitation, which permits the wave to pass through the inhibition despite sustained activity of the HVC_I population. B: Raster plot of the spike times of a subset of HVC_RA and HVC_I neurons from a simulation of 300 HVC_I neurons and 200 clusters of 3 HVC_RA neurons. Each HVC_I receives excitation from, and sends inhibition to, 100 HVC_RA neurons. HVC_I neurons also receive additional excitatory synapses from HVC_RA neurons at the end of the chain (to terminate activity at the end of the chain), as described in the text. C: Portion of voltage trace of HVC_RA neuron 14 from the same simulation. D: Subset of HVC_RA neurons in another simulation, in which one of these neurons (magenta) was in a cluster that escaped inhibition to fire a longer burst (60 clusters of 2 HVC_RA neurons; 90 HVC_I neurons; each HVC_I sends and receives 30 synapses).
However, without any constraints on HVC₁-HVC₉₆ connectivity, it is not possible for the inhibition to sculpt the HVC₉₆ activity into a sequence of appropriate bursts (not shown). With weak inhibition (e.g. \( g_{\text{GABAA}} = 0 \) or \( g_{\text{GABAA}} = 0.01 \text{ mS/cm}^2 \)), HVC₉₆ bursts failed to terminate, entering a state of persistent activity. With intermediate inhibition (e.g. \( g_{\text{GABAA}} = 0.02 \) to \( 0.04 \text{ mS/cm}^2 \)), HVC₉₆ neurons showed some combination of variable-duration bursting, persistent activity, and premature termination of the burst sequence. With strong inhibition (\( g_{\text{GABAA}} = 0.05 \) to \( 3.0 \text{ mS/cm}^2 \)), the burst sequence terminated within two clusters of the beginning of the chain: a burst in one cluster caused inhibition in a downstream cluster before that cluster was excited. There was no value of \( g_{\text{GABAA}} \) for which HVC₉₆ neurons showed bursts with a consistent, correct number of spikes.

A solution to this problem is to constrain each HVC₁ neuron not to make inhibitory synapses onto any cluster from which it receives an excitatory synapse, or onto clusters within some number of clusters downstream of one from which it receives an excitatory synapse (Figs. 1.4A and 1.5A). We refer to these downstream clusters as an HVC₁ “downstream connectivity gap.” A downstream connectivity gap of at least one cluster is necessary to allow enough time for the inhibition on HVC₉₆ neurons to decay before excitation arrives from an upstream cluster. When the downstream connectivity gap is zero, only the first cluster spikes. In order to reduce the influence of residual inhibition on burst duration and propagation speed (see Fig. 1.5), we used a larger, 7-cluster downstream connectivity gap. The HVC₁ neuron is
constrained not to make excitatory synapses onto any cluster from which it receives an excitatory synapse, so that the HVC_{RA} neurons have time to burst before the inhibition arrives to terminate the bursts. We generated this synaptic connectivity pattern by the following algorithm: (1) Create a new HVC_{I} neuron. (2) Select a candidate HVC_{RA} neuron to make an excitatory synapse onto the HVC_{I} neuron. If there exists an inhibitory synapse from the HVC_{I} neuron onto an HVC_{RA} neuron in the candidate neuron’s cluster or onto an HVC_{RA} neuron that is within 7 clusters downstream of the candidate HVC_{RA} neuron, select a new candidate HVC_{RA} neuron. Repeat this until an appropriate HVC_{RA} neuron is found. (3) Select a candidate HVC_{RA} neuron to receive an inhibitory synapse from the HVC_{I} neuron. If there exists a synapse onto the HVC_{I} neuron from an HVC_{RA} neuron in the candidate neuron’s cluster or from an HVC_{RA} neuron that is within 7 clusters downstream of the candidate HVC_{RA} neuron, select a new candidate HVC_{RA} neuron. Repeat this until an appropriate HVC_{RA} neuron is found. (4) Repeat steps 2-3 until all of the synapses onto and from the HVC_{I} have been created. (5) Repeat steps 1-4 until all of the HVC_{I} neurons have been created.

As a result of this constraint on connectivity, the wave of excitation is preceded and followed by inhibition but not interrupted by it, despite the sustained activity of the HVC_{I} population. The HVC_{I} neurons presynaptic to a given HVC_{RA} neuron are all silent at about the time of the HVC_{RA} burst and shortly before; this is functionally equivalent to the pause in HVC_{I} activity in our implementation of the
inhibitory pause mechanism in Fig. 1.1C. Thus this is an answer to the question posed earlier, namely what mechanism could generate such a pause.

In addition, we found that neurons in the last cluster of the chain remained persistently active, since there are no clusters downstream of these clusters to inhibit them via the HVC<sub>1</sub> neurons. In order to prevent this persistent activity, we added additional connections from the last two HVC<sub>RA</sub> clusters to the HVC<sub>I</sub> population.

Fig. 1.4B shows the spike times of a subset of HVC<sub>RA</sub> and HVC<sub>I</sub> neurons in a simulation containing a total of 300 HVC<sub>I</sub> neurons and 200 clusters of 3 HVC<sub>RA</sub> neurons (i.e. 33% HVC<sub>I</sub> and 67% HVC<sub>RA</sub> neurons) with this connectivity pattern. We obtained similar results when the numbers of HVC<sub>RA</sub> and HVC<sub>I</sub> neurons were the same. We also obtained similar results with 2 or 4 neurons per cluster. HVC<sub>RA2</sub> of each cluster sends an excitatory synapse to HVC<sub>RA1</sub> of the next. Each HVC<sub>I</sub> neuron sends 100 synapses to, and receives 100 synapses from, HVC<sub>RA</sub> neurons. For HVC<sub>RA</sub> → HVC<sub>RA</sub> synapses within each cluster, \( g_{\text{AMPA}} = 1.0 \text{ mS/cm}^2 \). For those between clusters, \( g_{\text{AMPA}} = 0.5 \text{ mS/cm}^2 \). We made the latter weaker for the reason described in the previous section. For HVC<sub>RA</sub> → HVC<sub>I</sub> synapses, \( g_{\text{AMPA}} = 0.1 \text{ mS/cm}^2 \). For HVC<sub>I</sub> → HVC<sub>RA</sub> synapses, \( g_{\text{GABA}} = 3.0 \text{ mS/cm}^2 \).

To prevent persistent activity at the end of the chain, randomly selected HVC<sub>RA</sub> neurons from the last two clusters make a total of 300 additional synapses
(g_{AMPA} = 0.1 \text{ mS/cm}^2) onto randomly selected HVC_1 neurons. The average burst duration was 4.3 ± 1.4 ms, with 3.2 ± 0.6 spikes per burst. Fig. 1.4C shows a portion of the voltage trace of one of the HVC_{RA} neurons in this simulation.

Because of the element of randomness in generating HVC_1 \rightarrow HVC_{RA} connectivity in the model, in some simulations the spiking in one or more HVC_{RA} clusters was not as rapidly terminated by inhibition. In Fig. 1.4D, we show an example neuron from such a cluster in a simulation containing 90 HVC_1 neurons and 60 clusters of 2 HVC_{RA} neurons, with 30 synapses to and from each HVC_1 neuron. The single HVC_{RA} neuron that spikes over an unusually long duration in the experiments of Hahnloser et al. (2002; their Fig. 2B) and Kozhevnikov and Fee (2007; their Fig. 2A) has a lower spiking frequency than this. However, it is sufficiently reminiscent that we suggest the possibility that both cases involve a failure of inhibition.

In this model, the duration of an HVC_{RA} burst is strongly influenced by the timing of inhibition. Most straightforwardly, a larger HVC_1 upstream connectivity gap (i.e. the number of clusters that an HVC_1 is not permitted to inhibit upstream of one from which it receives excitation; Fig. 1.5A) leads to a longer delay before a burst is terminated and therefore a longer burst (Fig. 1.5B). Longer burst durations also result from smaller downstream connectivity gaps (Fig. 1.5D). This may be related to the fact that smaller downstream connectivity gaps cause slower burst propagation (Fig. 1.5E): if the downstream cluster bursts later that usual, then its inhibition of the
upstream cluster via HVC₁ neurons should occur later than usual. The slower burst propagation may be due to residual inhibition delaying the downstream cluster in reaching its spike threshold. Larger upstream connectivity gaps also result in slower burst propagation (Fig. 1.5C). This may be due to the redistribution of the HVC₁ → HVC_RA synapses: when there are fewer HVC₁ → upstream HVC_RA synapses, there are more HVC₁ → HVC_RA synapses to clusters just downstream of the downstream connectivity gap. This may result in more residual inhibition, which may delay the downstream clusters in reaching their spike threshold.
FIG. 1.5. Effects of HVC_I-HVC_RA connectivity on burst duration and propagation speed. A: Schematic illustrating the constraint on HVC_I-HVC_RA connectivity that permits normal burst propagation. Because the representative HVC_I receives an excitatory synapse from the cluster shown, it is not permitted to make inhibitory synapses onto the clusters shown in white. The diagram assumes that the wave of activity propagates from left to right along the HVC_RA network. Burst duration increases (B) and burst propagation speed decreases (C) as a function of the HVC_I upstream connectivity gap. Burst duration decreases (D) and burst propagation speed increases (E) as a function of the HVC_I downstream connectivity gap. Burst propagation terminated prematurely (293 ms) for the simulation with a downstream connectivity gap of 4.
Sparse bursting without clusters or unidirectional HVC<sub>RA</sub> chains

The model does not fundamentally rely on a cluster organization nor on the unidirectionality of the HVC<sub>RA</sub> chain to produce a sequence of sparse bursts. For example, in one set of simulations (not shown), we reduced the clusters in our model to two reciprocally connected HVC<sub>RA</sub> neurons and connected these clusters bidirectionally \( (g_{\text{AMPA}} = 0.75 \text{ mS/cm}^2 \text{ for all synapses}) \), effectively creating a chain of 400 bidirectionally connected HVC<sub>RA</sub> neurons while retaining the HVC<sub>RA</sub>-HVC<sub>I</sub> connectivity. Stimulating the first cluster continued to produce a sequence of bursts (burst duration 3.9 ± 1.3 ms, spikes per burst 3.0 ± 0.7). Unidirectionality of burst propagation was maintained by the HVC<sub>RA</sub>-HVC<sub>I</sub> connectivity: stimulating the last neuron of the chain produced a burst only in the last 2 neurons (not shown). This is expected, since there is no gap in the reverse direction to allow the inhibition of the HVC<sub>RA</sub> neurons to decay before excitation arrives from an upstream cluster. Thus we expect this unidirectionality of burst propagation to depend on the asymmetry of the upstream and downstream connectivity gaps (Fig. 1.5A).

Similarly, Fig. 1.6 shows the activity of a short chain of 20 clusters with physiological synaptic strengths, in which the separate identity of the clusters is all but lost by their interconnectivity. Each cluster contains 80 HVC<sub>RA</sub> neurons, each of which sends AMPA synapses to a set of 40 other HVC<sub>RA</sub> neurons in its cluster, 40 in the previous cluster, and 40 in the next cluster. Thus the connectivity along the chain
has no directionality, and the connectivity is less one of a chain of clusters and more one of a network of HVC\textsubscript{RA} neurons with local random connections. Each of the 1600 HVC\textsubscript{I} neurons sends GABA\textsubscript{A} synapses to a set of 80 HVC\textsubscript{RA} neurons and receives AMPA synapses from a set of 20 HVC\textsubscript{RA} neurons. The upstream connectivity gap is 1 cluster, and the downstream connectivity gap is 10 clusters. To prevent persistent activity at the end of the chain, each HVC\textsubscript{RA} neuron in the last cluster sends AMPA synapses to a set of 30 HVC\textsubscript{I} neurons. In every case, we prohibited a neuron from making a synapse onto itself or more than one synapse onto the same postsynaptic neuron. We initiated activity in the first cluster with a 4 ms, 40 $\mu$A/cm\textsuperscript{2} current pulse in 50% of the HVC\textsubscript{RA} neurons, beginning at $t = 0$ ms. The HVC neurons spiked $4.3 \pm 0.9$ times in $5.3 \pm 1.3$ ms.

\textit{Experimental predictions of the sparse bursting model}

In Fig. 1.7, we show experimental predictions of the sparse bursting model. Since GABA\textsubscript{A} synapses play a critical role in the model, the model predicts that manipulations that influence GABA\textsubscript{A} synaptic transmission can profoundly affect HVC’s behavior. When we blocked inhibitory synapses (i.e. set $g\textsubscript{GABA} = 0$), the bursts of HVC\textsubscript{RA} neurons began normally but failed to terminate, entering a state of persistent activity. The prediction of normal burst onset will only hold if blocking GABA\textsubscript{A} synapses does not in itself lead to runaway excitation in HVC before the onset of singing.
FIG. 1.6. Raster plot of the spike times in a chain of 20 clusters with physiological synaptic strengths. A cluster contains 80 HVC\textsubscript{RA} neurons, each of which sends a single AMPA synapse to each of 40 other HVC\textsubscript{RA} neurons in its cluster, 40 in the previous cluster, and 40 in the next cluster. Each of the 1600 HVC\textsubscript{I} neurons receives an AMPA synapse from each of 20 HVC\textsubscript{RA} neurons and sends a GABA\textsubscript{A} synapse to each of 80 HVC\textsubscript{RA} neurons, and is constrained not to send inhibition within 10 clusters downstream, or 1 cluster upstream, of one from which it receives excitation. We initiated spiking in the first cluster with a 4 ms, 40 $\mu$A/cm\textsuperscript{2} DC current pulse in 50% of the HVC\textsubscript{RA} neurons, beginning at $t = 0$ ms.
Fig. 1.7A shows the effect of varying $g_{\text{GABA}_A}$ on the sparseness of spiking in a version of the model containing 90 HVC$_I$ neurons and 60 clusters of 3 HVC$_{RA}$ neurons, illustrated with the burst duration, spikes per burst, and the sparseness index defined in Methods. We calculated burst duration and spikes per burst over the time interval from the beginning of current injection into the first neuron (0 ms) to approximately the end of the last burst in the chain in the normal case (230 ms). For
very low values of $g_{GABA_A}$, HVC$_{RA}$ neurons entered a state of persistent activity. However, HVC$_{RA}$ neurons showed relatively short, consistent bursts over a wide range of $g_{GABA_A}$ values from about 0.1 to at least 3.0 mS/cm$^2$. This robustness is due to the HVC$_1$-HVC$_{RA}$ connectivity pattern described above, which results in an inhibitory pause during which bursting can occur.

We also found that the interspike interval (ISI) distribution may contain a signature of the HVC$_{RA}$ → HVC$_1$ connectivity pattern, if sufficiently many HVC$_1$ spikes are recorded. Since each HVC$_1$ is constrained not to receive excitation from any cluster to which it sends inhibition, or from clusters within 7 clusters upstream of one to which it sends inhibition, we would expect ISIs corresponding to this approximately 8-cluster gap in connectivity, and less, to be underrepresented in the ISI distribution. Since the propagation speed along the chain is 0.26 clusters/ms, we would expect a dip in the ISI distribution below ~31 ms. Fig. 1.7B, top, shows the interspike interval (ISI) distribution for all spike times of all HVC$_1$ neurons in the simulation illustrated in Fig. 1.4B-C. Figure 1.7B, bottom, shows the ISI distribution of another set of HVC$_1$ neurons. Each HVC$_1$ neuron in this second set was driven by exactly the same set of HVC$_{RA}$ bursts and received exactly the same number of HVC$_{RA}$ → HVC$_1$ synapses, but the HVC$_{RA}$ → HVC$_1$ connectivity pattern was completely random. These HVC$_1$ neurons did not send synapses to HVC$_{RA}$ neurons. Figure 1.7C shows the same ISI distributions with altered axis limits, to accentuate their differences. Unlike the random case (bottom), the constrained case (top) resulted in a histogram with a
prominent dip in ISIs below 30 to 35 ms, corresponding to the gap in HVC_{RA} \rightarrow HVC_{I} connectivity. Experimentally, such a histogram should only include ISIs from the period corresponding to singing and not from periods of spontaneous activity. The model predicts that both the size of the connectivity gap and the propagation speed of the waves will determine which ISIs are underrepresented in the histogram.

The role of inhibition in the sparse bursting model is similar to that in the inhibitory pause mechanism of Fig. 1.1C. If sleep replay or bird’s own song playback evoked activity in HVC_{RA} neurons is the result of the same mechanism as singing-related bursting (see Discussion), then the inhibitory pause mechanism makes two predictions that could be tested in head-fixed or anesthetized birds. First, under voltage clamp at the AMPA reversal potential (0 mV), an HVC_{RA} neuron will have a background of outward, hyperpolarizing current during the fictive singing, with a dip shortly before and during the time of the burst (Fig. 1.8A). Second, chloride loading should result in a background of spiking during the fictive singing by making the GABA_{A} synapses functionally excitatory (Fig. 1.8B).
Discussion

We have presented a model of sparse bursting based on inhibition, recurrent excitation, and bistability. This model builds on the observation by Hahnloser et al. (2002) and Kozhevnikov and Fee (2007) of sparse bursting in HVC\textsubscript{RA} neurons; and on their hypothesis that HVC\textsubscript{RA} neurons form a chain-like organization in which neuronal ensembles burst in sequence at every moment of the song, driving RA neurons to burst in sequence (Fee et al. 2004).

The chain-like organization of our bistable clusters is reminiscent of synfire chains, which have been discussed elsewhere (Abeles 1982, 1991; Hermann et al. 1995; Diesmann et al. 1999). Recently, chain models have been proposed for HVC (Fiete et al. 2005; Li and Greenside 2006; Jin et al. 2007), also building on the work of Fee et al. (2004). Although our model includes chain-like networks, we have focused on the role of bistability and inhibition rather than chains. Unlike other chain-like models, our model postulates a central role for inhibitory interneurons in sparsely bursting telencephalic premotor networks, HVC in particular. This mechanism is related to those of central pattern generators and cortical networks, which make use of inhibition, recurrent excitation, and bistability (Yuste et al. 2005; Shu et al. 2003; McCormick 2005).
FIG. 1.8. Experimental predictions from the inhibitory pause mechanism of Fig. 1.1C. A: Current in a representative HVC_{RA} neuron under voltage clamp at the AMPA reversal potential (0 mV). The neuron showed a background of outward, hyperpolarizing current, with a dip during and before the time of the burst. B: Membrane potential of a representative HVC_{RA} neuron from a different simulation, when we set the GABA_{A} reversal potential to -21 mV to simulate chloride loading. This resulted in spiking outside the time of the burst by making the GABA_{A} synapses functionally excitatory. In both A and B, the HVC_{I} pause lasted from \( t = 23 \) to 36.4 ms, the depolarizing DC current pulse into HVC_{RA} neurons lasted from \( t = 30 \) to 33 ms, and the simulation began at \( t = -20 \) ms.
Inhibitory interneurons also played a key role in Drew and Abbott’s (2003) model of song selectivity and sequence generation in HVC, but this role was not in terminating sparse HVC\(_{RA}\) bursts. Their model was limited by its dependence on a long afterhyperpolarization in HVC\(_{RA}\) neurons and by the fact that its HVC\(_{RA}\) neurons were only active during extrinsically generated excitatory pulses applied every 75 to 100 ms.

Our sparse bursting model is distinguished from other chain-like models by a number of key assumptions and predictions:

*First*, our model assumes that a large proportion of HVC\(_{RA}\) neurons participate in a local connectivity of recurrent excitation with other HVC\(_{RA}\) neurons that are nearby in the network; this is the basis of the bistability.

*Second*, our model assumes that each HVC\(_I\) neuron is constrained not to make inhibitory synapses onto HVC\(_{RA}\) neurons within some distance upstream and downstream of one from which it receives an excitatory synapse (Figs. 1.4A and 1.5A). This gives the HVC\(_{RA}\) neurons time to burst before the inhibition arrives and reduces the influence of residual inhibition on burst duration and propagation speed (Fig. 1.5).
Third, our model assumes that an additional mechanism activates $HVC_I$ neurons at the end of the $HVC_{RA}$ burst sequence to prevent persistent activity at the end of the chain. In this model, we added additional connections from $HVC_{RA}$ neurons at the end of the chain to the $HVC_I$ population. In the model presented in our companion paper (Gibb et al. 2008), which includes neural feedback via Uva, these connections are replaced by ones from Uva to $HVC_I$ neurons.

Fourth, as a result of the $HVC_I$-$HVC_{RA}$ connectivity, our model predicts that every $HVC_{RA}$ neuron that participates in sparse bursting receives a background of inhibition throughout the song, with a reduction shortly before and during the time that it bursts (Figs. 1.1C and 1.8A). If the mechanism of sparse bursting during sleep or BOS playback is sufficiently similar to that during singing (which is not certain, given NIf’s involvement in the former but not the latter; Cardin et al. 2005; Hahnloser and Fee 2007), then voltage clamp or chloride loading experiments can be used to test this prediction (Fig. 1.8). During fictive singing, an $HVC_{RA}$ neuron will have a background of outward current, with a dip shortly before and during the time of the burst (Fig. 1.8A), and chloride loading of an $HVC_{RA}$ neuron will result in a background of spiking (Fig. 1.8B).

Fifth, our model predicts that, if $GABA_A$ synapses are blocked, excited groups of $HVC_{RA}$ neurons will enter a state of persistent activity, whether in vitro or in vivo. How long this activity will last depends on the size of the groups (Fig. 1.1A), the
percent connectivity of the HVC\textsubscript{RA} neurons within groups, and the strength of voltage-dependent hyperpolarizing currents (Fig. 1.1A) and neurotransmitter-activated hyperpolarizing currents (e.g. GABA\textsubscript{B}; Dutar et al. 1998). However, this effect will only be seen if blocking GABA\textsubscript{A} synapses does not itself evoke a high level of song-independent activity. In order to evoke this persistent activity in vitro, it is necessary to excite a sufficiently large fraction of the HVC\textsubscript{RA} neurons in a cluster or the functional equivalent of a cluster. In vivo, our model predicts that song-related HVC\textsubscript{RA} bursts will be initiated normally but fail to be rapidly terminated.

Sixth, our model predicts that HVC\textsubscript{I} interspike intervals (ISIs) during song will have a distribution resembling Fig. 1.7C, top, (with a dip in the frequency of occurrence of certain ISIs), more than Fig. 1.7C, bottom. Which ISIs will be underrepresented in the histogram depends on both the burst propagation speed and the size of the HVC\textsubscript{I} connectivity gap. Since spontaneous activity between song bouts may influence the ISI distribution, the ISIs for such a test should be taken from HVC\textsubscript{I} neurons only during the period corresponding to singing. Additionally, it is possible that a version of the model could be developed in which the HVC\textsubscript{I} $\rightarrow$ HVC\textsubscript{RA} connectivity pattern is constrained but the HVC\textsubscript{RA} $\rightarrow$ HVC\textsubscript{I} connectivity pattern is random. In such a model, HVC\textsubscript{I} neurons would not be expected to have this ISI distribution.
Which type of $HVC_{RA}$ neuron exhibits sparse premotor bursting?

Two physiologically and morphologically distinct types of $HVC_{RA}$ neuron have been characterized (Dutar and Perkel 1998, Kubota and Taniguchi 1998, Shea 2004, Mooney and Prather 2005). We assumed in the present work that the $HVC_{RA}$ neurons that burst sparsely during singing (Hahnloser et al. 2002; Kozhevnikov and Fee 2007) are of the same short dendrite class that burst sparsely during BOS playback (Mooney 2000), and (following Shea 2004) that these are the neurons that spike tonically in response to depolarizing current injection. We fit our model to the physiology described in detail by Kubota and Taniguchi (1998) for their Type IIa neurons. Although this physiology may not be identical to that of adult neurons, Kubota and Taniguchi (1998) provide the most complete description of the physiology of short dendrite neurons.

Potential role for a $Ca^{2+}$ current in $HVC_{RA}$ bursting

Under normal conditions, injection of depolarizing current into the soma of an $HVC_{RA}$ neuron does not evoke $Ca^{2+}$ spikes or any evidence of bursting (Dutar et al. 1998; Kubota and Taniguchi 1998; Shea 2004; Mooney and Prather 2005; Long and Fee 2006). Consequently we formulated a model in which bursting is not intrinsic to $HVC_{RA}$ neurons but instead arises in the microcircuitry of HVC. However, recent
preliminary work shows that Ca$^{2+}$ spikes, which appear to be of the L-type, are elicited by somatic depolarization of HVC$_{RA}$ neurons in the presence of a Na$^+$-channel blocker (Long and Fee 2006). The same study demonstrates that sleep bursts of HVC$_{RA}$ neurons are augmented by an agonist of the L-type Ca$^{2+}$ current. These results suggest that an L-type Ca$^{2+}$ current is present in HVC$_{RA}$ neurons and is capable of influencing bursting. If this current proves to be essential to the bursting mechanism of HVC$_{RA}$ neurons, it may play a complementary role to inhibition and recurrent excitation.

*Cluster size*

Fee et al. (2004) estimated that ~200 HVC$_{RA}$ neurons may be coactive at each time in the song motif, which is similar to the number of neurons in our large clusters with physiological synaptic strengths. However, this may be an overestimate, since the study on which they based it (Wang et al. 2002) did not use stereological methods for reconstructing total cell number (J. R. Kirn, personal communication; West 1999). HVC contains an average of about 40 to 50 thousand neurons per hemisphere (Nordeen and Nordeen 1988; Nordeen and Nordeen 1989; Burek et al. 1991; Burek et al. 1997; Ward et al. 1998).

Using the estimate that half of HVC neurons are RA-projecting (Nottebohm et al. 1990) and assuming that only half of the HVC$_{RA}$ neurons participate in syllable networks, we arrive at an estimate of 10,000 to 12,500 participating HVC$_{RA}$ neurons.
Taking the motif duration to be about 0.5 to 1 s (Immelman 1969; Price 1979; Fee et al. 2004) and the average burst duration to be 5 ms (Kozhevnikov and Fee 2007), we calculate that about 50 to 125 HVC\textsubscript{RA} neurons may be coactive at each time in the song motif. In a chain framework, the propagation speed implied by these figures is about 10 to 25 HVC\textsubscript{RA} neurons per ms. Thus if the number of HVC\textsubscript{RA} neurons per cluster is 160, cluster activations must be staggered by about 6 to 16 ms to achieve such slow propagation. The result presented in Fig. 1.5E suggests that a smaller HVC\textsubscript{1} downstream connectivity gap may promote slower propagation. Alternatively, clusters could be smaller and the synaptic response of HVC\textsubscript{RA} neurons could be enhanced by a dendritic Ca\textsuperscript{2+} current (Long and Fee 2006; Jin et al. 2007) to achieve an equivalent synaptic effect.

Additionally, the result shown in Fig. 1.6 suggests the model does not fundamentally rely on a cluster organization; the important elements are recurrent local excitation among HVC\textsubscript{RA} neurons and patterned inhibition from HVC\textsubscript{1} neurons.

\textit{A possible role for developmental plasticity in the establishment of HVC\textsubscript{1} \rightarrow HVC\textsubscript{RA} connectivity}

What developmental mechanism could generate the pattern of HVC\textsubscript{1} \rightarrow HVC\textsubscript{RA} connectivity shown in Figs. 1.4A and 1.5A? We suggest that a novel form of timing-dependent synaptic plasticity at inhibitory synapses could serve this role. In
this proposed model, early in development, $\text{HVC}_{\text{RA}} \rightarrow \text{HVC}_1$ and $\text{HVC}_1 \rightarrow \text{HVC}_{\text{RA}}$ synapses are weak or absent, and sustained (non-sparse) spiking activity propagates along chains of recurrently excitatory $\text{HVC}_{\text{RA}}$ neurons, terminated by slow hyperpolarization. $\text{HVC}_{\text{RA}} \rightarrow \text{HVC}_1$ synapses are gradually added and/or strengthened with time. As they strengthen, subsets of them become strong enough that concurrent spiking in the presynaptic $\text{HVC}_{\text{RA}}$ neurons can elicit spiking in the postsynaptic $\text{HVC}_1$ neuron.

Additionally, $\text{HVC}_1 \rightarrow \text{HVC}_{\text{RA}}$ synapses begin weak and follow a specific timing-dependent synaptic plasticity rule: if the presynaptic $\text{HVC}_1$ neuron spikes within a few tens of ms before or a few ms after the postsynaptic $\text{HVC}_{\text{RA}}$ neuron, the inhibitory synapse is weakened. If the $\text{HVC}_1$ neuron spikes outside this time window, the synapse is strengthened. For some period of time after the induction of plasticity, the synapse is resistant to further induction, so that only the first spikes of the propagating activity are involved in triggering plasticity. We suggest that, over many bouts of propagation, a plasticity mechanism like this could generate the $\text{HVC}_1 \rightarrow \text{HVC}_{\text{RA}}$ connectivity required by our model. Our proposed plasticity rule is similar to, though distinct from, various forms of timing-dependent plasticity that have been observed at excitatory and inhibitory synapses (Holmgren and Zilberter 2001; Woodin et al. 2003; Haas et al. 2006; Dan and Poo 2006).
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Supplementary Methods

The behavior of the single-neuron HVC\textsubscript{RA} model in response to depolarizing and hyperpolarizing current pulses is shown in Fig. 1.S1A-C (cf. Kubota and Taniguchi 1998, Fig. 2A1,C,D). The behavior of the HVC\textsubscript{I} model is shown in Fig. 1.S1D-F (cf. Kubota and Taniguchi 1998, Fig. 3A,C,D; and Dutar et al. 1998, Fig. 1C).
FIG. 1.S1. Intrinsic properties of HVC<sub>RA</sub> and HVC<sub>I</sub> model neurons at 32°C. A: Plots of spiking frequency vs. time at different current amplitudes for our model HVC<sub>RA</sub> neuron (cf. Kubota and Taniguchi 1998, Fig. 2D). B: Response of the HVC<sub>RA</sub> neuron to a 300 ms, 0.09 nA depolarizing current pulse (cf. Kubota and Taniguchi 1998, Fig. 2C). C: Response of the HVC<sub>RA</sub> neuron to a 300 ms, -0.06 nA hyperpolarizing current pulse (cf. Kubota and Taniguchi 1998, Fig. 2A1). D: Plots of spiking frequency vs. time at different current amplitudes for our model HVC<sub>I</sub> neuron (cf. Kubota and Taniguchi 1998, Fig. 3D). E: Response of the HVC<sub>I</sub> neuron to a 300 ms, 0.70 nA depolarizing pulse (top) (cf. Kubota and Taniguchi 1998, Fig. 3C and Dutar et al. 1998, Fig. 1C1). F: Response of the HVC<sub>I</sub> neuron to a 300 ms, -0.50 nA hyperpolarizing pulse (bottom) (cf. Kubota and Taniguchi 1998, Fig. 3A and Dutar et al. 1998, Fig. 1C2).
In the simulations shown in Fig. 1.S1, we divided the injected current by an assumed effective membrane surface area. We adjusted the effective membrane surface area solely to adjust the input resistance and thereby scale the frequency-current relationships in this supplementary figure to match the data of Kubota and Taniguchi (1998). These effective membrane surface areas are not based on experimental data; however, they did not play a role in our network simulations because we expressed all ionic currents per unit surface area and adjusted maximal synaptic conductances to achieve the desired network behavior. In other words, a decrease in surface area can be compensated by an increase in synaptic strength, and vice versa. We assumed an effective membrane surface area of 2400 $\mu$m$^2$ for the HVC$_{RA}$ neuron and 12500 $\mu$m$^2$ for the HVC$_I$ neuron in this figure.
Supplementary Results

The connectivity of a reduced cluster model containing three HVC$_{RA}$ neurons is shown in Fig. 1.S3A. In Fig. 1.S3B, we demonstrate bistability in a 3-HVC$_{RA}$ cluster without an HVC$_I$ neuron and with $g_{AMPA} = 1.2$ mS/cm$^2$. In the absence of input, the cluster will remain in its quiescent state indefinitely. By exciting HVC$_{RA}$ neuron 1 of the cluster with a 3 ms, 50 µA/cm$^2$ DC current pulse beginning at $t = 10$ ms (green arrow), we shift the cluster into its persistently spiking state. We verified that this state persists for at least 1 s in the absence of further inputs (not shown). However, in Fig. 1.S3B, we shift the cluster back into its quiescent state with a 3 ms, -50 µA/cm$^2$ current pulse into the same neuron beginning at $t = 110$ ms (red arrow).

In our model, each HVC$_{RA}$ neuron sends an excitatory synapse to, and receives an inhibitory synapse from, the HVC$_I$ neuron (Fig. 1.S3A). In Fig. 1.S3C, we show the behavior of neurons in a cluster with $g_{AMPA} = 1.2$ for HVC$_{RA} \rightarrow$ HVC$_{RA}$ synapses, $g_{AMPA} = 0.1$ for HVC$_{RA} \rightarrow$ HVC$_I$ synapses, and $g_{GABA} = 0.8$ for HVC$_I \rightarrow$ HVC$_{RA}$ synapses. We again excite neuron 1 with a 3 ms, 50 µA/cm$^2$ current pulse beginning at $t = 5$ ms. The stimulated neuron (Fig. 1.S3C, top; blue trace) then excites the other two HVC$_{RA}$ neurons (Fig. 1.S3C, top; green and red traces) and the HVC$_I$ neuron (Fig. 1.S3C, bottom). The HVC$_I$ neuron in turn terminates the activity of the HVC$_{RA}$ neurons after each spikes 2 to 4 times, in $6.8 \pm 2.1$ ms.
FIG. 1.52. Burst duration for the inhibitory pause mechanism of Fig. 1.1C as a function of various parameters. Dark gray lines: mean ± SD. Light gray regions: HVC_{RA} burst duration (mean ± SD) measured by Kozhevnikov and Fee (2007). Note that this mechanism is less sensitive to parameter changes than the inhibitory buildup mechanism is (cf. Fig. 1.2). A: Inhibitory synaptic decay time constant, 1/β (normal value: 5.6 ms). B: Inhibitory synapses per HVC_{I}, normal adaptation currents present (normal number: 70). C: Scale factor by which the maximal conductances of both adaptation currents are multiplied (normal value: 1). D: Inhibitory synapses per HVC_{I}, adaptation currents absent. E: Excitatory synapses per HVC_{RA} (normal number: 80).
FIG. 1.S3. Reduced cluster model with a local HVC\textsubscript{1} neuron, used in the sparse bursting network of Fig. 1.3. A: Schematic of the model. Bursts are generated by recurrently excitatory HVC\textsubscript{RA} neurons inhibited by HVC\textsubscript{1} neurons. B: Bistability of the 3-HVC\textsubscript{RA} model without an HVC\textsubscript{1} neuron. We shifted the cluster into its persistently spiking state at t = 10 ms by exciting neuron 1 (membrane potential shown) with a 3 ms, 50 $\mu$A/cm\textsuperscript{2} DC current pulse (green arrow). We shifted the cluster back into its quiescent state at t = 110 ms by inhibiting neuron 1 with a 3 ms, -50 $\mu$A/cm\textsuperscript{2} DC current pulse (red arrow). C: When the HVC\textsubscript{1} $\rightarrow$ HVC\textsubscript{RA} synapses are of an appropriate strength, the HVC\textsubscript{RA} neurons show a brief burst of activity. Top: Response of a cluster of three HVC\textsubscript{RA} neurons to a 3 ms, 50 $\mu$A/cm\textsuperscript{2} current pulse injected into HVC\textsubscript{RA1} (blue trace), beginning at t = 5 ms. When HVC\textsubscript{RA1} is excited, it excites HVC\textsubscript{RA2}, (green), HVC\textsubscript{RA3} (red), and HVC\textsubscript{1} (bottom). The HVC\textsubscript{1} neuron terminates the spiking of the HVC\textsubscript{RA} neurons. D: Mean spiking duration of the three HVC\textsubscript{RA} neurons as a function of the inhibitory conductance, $g_{GABA}$, Because the simulations end at t = 110 ms, the maximum possible duration is 100 ms. For every point, SD < 2.1 ms.
3-HVC\textsubscript{RA} cluster:

- Bistable clusters
  - \( n \) HVC\textsubscript{RA} per cluster
  - \( m \) local HVC\textsubscript{I} per cluster

**A**

- Input from another cluster or Uva/MMAN/NIf
- Excitatory
- Inhibitory
- Output

**B**

Membrane potential (mV)

Time (ms)

**C**

Membrane potential (mV)

Time (ms)

**D**

Spiking duration (ms)

Inhibitory conductance (mS/cm\textsuperscript{2})
In Fig. 1.S3D, we show the mean spiking duration of the HVC\textsubscript{RA} neurons in this cluster as a function of $g_{\text{GABAA}}$. In this simple model, there is a narrow range of inhibitory coupling over which short bursts like those observed by Hahnloser et al. (2002) and Kozhevnikov and Fee (2007) are possible. At 0.6 mS/cm$^2$ or below, the HVC\textsubscript{RA} neurons showed persistent activity, while at 0.9 mS/cm$^2$ or above, the HVC\textsubscript{RA} neurons spiked only 0 to 2 times. In addition, we found that both 2- and 4-neuron clusters are also capable of generating brief bursts (not shown). The former may have a smaller parameter regime in which bistability is possible.

![Graph showing spiking activity of HVC\textsubscript{RA} and HVC\textsubscript{I} neurons](image)

**FIG. 1.S4.** Blocking GABA$\textsubscript{A}$ receptors reduces the sparseness of HVC\textsubscript{RA} bursting. Raster plot of spike times of a subset of HVC\textsubscript{RA} and HVC\textsubscript{I} neurons from a simulation of 90 HVC\textsubscript{I} neurons and 60 clusters of 3 HVC\textsubscript{RA} neurons, in which the inhibitory maximal conductance, $g_{\text{GABAA}}$, was set to zero. Each HVC\textsubscript{RA} burst began normally but failed to terminate, thus showing persistent activity. We set the threshold for determining spike time at -30 mV to catch low-amplitude spikes in the HVC\textsubscript{I} neurons.
Abstract

Uncovering the roles of neural feedback in the brain is an active area of experimental research. In songbirds, the telencephalic premotor nucleus HVC receives neural feedback from both forebrain and brainstem areas. Here we present a computational model of HVC and associated nuclei that builds on the model of sparse bursting presented in our preceding companion paper. Our model embodies the hypotheses that (1) different networks in HVC control different syllables or notes of birdsong, (2) interneurons in HVC not only participate in sparse bursting but also provide mutual inhibition between networks controlling syllables or notes, and (3) these syllable networks are stimulated by neural feedback via the brainstem and the afferent thalamic nucleus Uva, or a similar feedback pathway. We discuss the model’s ability to unify physiological, behavioral, and lesion results, and we use it to make novel predictions that can be tested experimentally.
Introduction

The song system of oscine songbirds is a collection of bilaterally coordinated brain structures, organized into both feedforward and feedback pathways (Fig. 2.1A). As a model system for vocal pattern generation and learning, it has been extensively studied using anatomical, lesion, and electrophysiological approaches, particularly in zebra finches.

The song of an adult zebra finch consists of a number of introductory notes followed by one or more motifs, each lasting about 0.5 to 1 s (Immelman 1969; Price 1979; Fee et al. 2004) and consisting of several syllables. Syllables are identified as the smallest units of song separated by silent intervals and are a few tens to a few hundreds of ms in duration (Zevin et al. 2004; Glaze and Troyer 2006). Syllables can be further subdivided into notes, which are delimited by sudden changes in the spectrogram (Ashmore et al. 2005).

In the preceding companion paper (Gibb et al. 2008), we presented a model of sparse bursting in the telencephalic premotor nucleus HVC (used as the proper name; Reiner et al. 2004) in which inhibitory interneurons play a key role. Here we incorporate HVC into a larger model of the song system. As Fig. 2.1A illustrates, HVC projects to another telencephalic premotor nucleus, RA (the robust nucleus of the arcopallium), which in turn projects to respiratory and vocal areas in the brainstem.
Following Ashmore et al. (2005), we use the terms dorsal RA (dRA) and ventral RA (vRA) to refer to the dorsal cap area of RA (Vicario 1991; Reinke and Wild 1998) and the rest of RA, respectively. Ventral RA projects to the brainstem nucleus nXIIIts (tracheosyringeal part of the hypoglossal nucleus), which controls the syrinx; while dorsal RA projects to a brainstem respiratory network consisting of DM (dorsomedial nucleus of the intercollicular complex), PAm (nucleus paraambigualis), and RAm (nucleus retroambigualis) (Fig. 2.1A; Nottebohm et al. 1976, 1982; Vicario 1991; Wild 1993a,b; Reinke and Wild 1998; Wild 1997; Suthers et al. 1999; Sturdy et al. 2003).

No direct bilateral connections exist between any of the telencephalic song nuclei, including HVC. However, two neural feedback pathways to HVC are known to contain bilateral connections, potentially helping to coordinate activity in the two hemispheres: HVC \(\rightarrow\) dRA \(\rightarrow\) brainstem \(\rightarrow\) Uva \(\rightarrow\) (NIf) \(\rightarrow\) HVC and HVC \(\rightarrow\) dRA \(\rightarrow\) DMP \(\rightarrow\) MMAN \(\rightarrow\) HVC (Fig. 2.1B,C; Foster et al. 1997; Vates et al. 1997; Wild 1997; Reinke and Wild 1998; Striedter and Vu 1998).
FIG. 2.1. A: Bilateral view of the song system. We focus on the generation of sparse burst sequences in HVC and the potential role of the bilateral neural feedback pathway through the brainstem (DM, PAm, and RAm) to Uva and back to HVC. Based on a figure from Schmidt, Ashmore, and Vu (2004) and Ashmore, Wild, and Schmidt (2005), with permission. Dotted lines indicate the weak projection from RA to DLM described by Wild (1993) and Vates et al. (1997) and the projection from dorsal RA to HVC described by Roberts et al. (2008). NIf, interfacial nucleus of the nidopallium; X, Area X of the medial striatum; DLM, dorsolateral nucleus of the medial thalamus; LMAN, lateral magnocellular nucleus of the anterior nidopallium; MMAN, medial magnocellular nucleus of the anterior nidopallium; dRA and vRA, dorsal and ventral regions of the robust nucleus of the arcopallium; DMP, dorsomedial nucleus of the posterior thalamus; Uva, uvaeform nucleus of the posterodorsal thalamus; RAm, nucleus retroambigualis; PAm, nucleus paraambigualis; DM, dorsomedial nucleus of the intercollicular complex; nXIIIts, tracheosyringeal part of the twelfth cranial nerve nucleus; INSP and EXP, inspiratory and expiratory motor neurons. B and C: HVC’s bilateral feedback can be divided into 2 pathways. Larger arrows indicate bilateral projections. B: HVC → dRA → brainstem → Uva → HVC pathway (which also has an additional circuit, Uva → NIf → HVC, not shown). C: HVC → dRA → DMP → MMAN → HVC pathway. We focus on pathway B while recognizing that pathway C may serve part of the function we propose for B, particularly in juveniles, decrystallized adults, and other birds with variable song sequences.
The focus of our model is the potential role of neural feedback to HVC in syllable sequencing. Although the stereotypy of adult zebra finch song motifs might superficially suggest that motifs are monolithic units with a fixed syllable sequence, there are a number of hints that individual syllables are more basic units, which can be rearranged, repeated, or skipped. Many of these hints come from cases of adult syllable sequence variability (Williams and Vicario 1993; Scharff and Nottebohm 1991; Thompson and Johnson 2006; Leonardo and Konishi 1999; Brainard and Doupe 2001). Two additional pieces of evidence for the idea that syllables are basic units are that syllables stretch and compress proportionally less than intersyllable gaps during changes in song tempo (Glaze and Troyer 2006) and birds tend to interrupt their songs during gaps, rather than syllables, when presented with light flashes (Cynx 1990; Franz and Goller 2002).

Schmidt (2003) showed that peaks in the interhemispheric synchronization of HVC lead the onsets of syllables and notes, suggesting a model in which HVC receives bilaterally synchronized timing pulses from an afferent nucleus shortly before the onsets of syllables and notes (Schmidt 2003; Coleman and Vu 2005). In the present work, we asked: What model of HVC input emerges if we make the reasonable assumption that the two HVCs remain coordinated during variable, as well as normal, song sequences? We suggest that if the syllable transitions in the left and right HVCs are to remain coordinated during variable song sequences, then these bilaterally synchronized pulses should specify not just the timing, but also the identity
of the next syllable. We refer to this as “syllable-specific input,” in contrast to “syllable-unspecific input.” As a thought experiment, if atypical syllable transitions were instead initiated independently in the two hemispheres, then the two HVCs could make transitions to different syllables. This would violate our assumption of interhemispheric coordination during variable sequences.

Coordination does not necessarily imply that the two hemispheres produce the same commands; it only implies that they are in agreement about what syllable is being sung, no matter what their relative contributions to the sound produced. A model of input to HVC in songbirds must be flexible enough to explain the bilateral coordination of HVC commands whether syllables are produced by one side of the syrinx, by both sides in concert, or by switching between sides (Nottebohm 1971; Nottebohm and Nottebohm 1976; Nottebohm 1977; Suthers 1990; Williams et al. 1992; Goller and Suthers 1995; Floody and Arnold 1997).

Because it participates in a bilaterally connected pathway and is necessary for normal song production in adult songbirds (Coleman and Vu 2005), Uva is a good candidate for a nucleus specifying the timing and identity of syllables in the left and right HVC. NIf is not necessary for normal adult song production (Cardin et al. 2005), and bilateral adult MMAN lesions affect song only slightly (Foster and Bottjer 2001).
The present study has the goal of integrating the model of HVC sparse bursting
developed in our companion paper into a larger song-system model including nuclei of
the HVC → dRA → brainstem → Uva → HVC feedback loop, to provide an
integrated picture of the dynamics of syllable sequencing in songbirds. In this model,
the feedback pathway to HVC via Uva sequentially activates networks controlling
different syllables in the model. HVC, dRA, DM/PAm, and Uva are all assumed to
contain syllable-specific populations of neurons, which could potentially be organized
as “syllabotopic” maps, i.e. topographic maps organized by syllable. Our model
embodies a set of hypotheses, and we use it to derive predictions to be tested
experimentally.

Given the assumption of interhemispheric coordination during variable
sequences, the syllable-specific input to HVC must at least be present during variable
sequences. We implement the simplest version of the model, in which the sequence in
HVC is controlled by syllable-specific input no matter how stereotyped the syllable
sequence is. In a more complex version of this model, HVC input could be syllable
unspecific when the sequence is stereotyped and syllable specific when it is variable.
Thus we acknowledge the possibility that, during states of sequence variability, a
bilaterally coordinated MMAN → HVC input (Fig. 2.1C) or NIf → HVC input could
play the central role in promoting syllable transitions.
A portion of this work has appeared previously in abstract form (Gibb and Abarbanel 2006).

Methods

We implemented all models in C++ using a neural simulation framework developed by T. Nowotny and extended by L. Gibb, using a Runge-Kutta 6(5) algorithm with a relative error of $10^{-6}$, and we performed analyses of model output in MATLAB.

Basic spiking model

As in our companion paper (Gibb et al. 2008), the membrane potential of the basic spiking model (Traub and Miles 1991; Destexhe et al. 1998a; Destexhe and Sejnowski 2001) follows the equation:

$$C_m \frac{dV(t)}{dt} = -g_{Na} m(t)^3 h(t)(V(t) - E_{Na}) - g_K n(t)^4 (V(t) - E_K) - g_L (V(t) - E_L) - I_{syn} + I_{DC},$$

where $V(t)$ is the membrane potential; $g_{Na}$, $g_K$, and $g_L$ are the maximal conductances of the Na⁺, K⁺, and leak currents; $E_{Na}$, $E_K$, and $E_L$ are the reversal potentials of the Na⁺,
$K^+$, and leak currents; $I_{\text{syn}}$ is the sum of synaptic currents; and $I_{\text{DC}}$ is the value of an injected current ($I_{\text{DC}} = 0$ unless otherwise noted). The gating variables $X(t) = \{m(t), h(t), n(t)\}$ are taken to satisfy the first-order kinetics

$$\frac{dX(t)}{dt} = \alpha_X(V(t))(1 - X(t)) - \beta_X(V(t))X(t),$$

where $\alpha_X(V)$ and $\beta_X(V)$ are given in Table 1.1 of our companion paper.

**Modeling HVC$_{RA}$ neurons**

As in our companion paper, to model fast and slow components of spike rate adaptation in HVC$_{RA}$ neurons (Kubota and Taniguchi 1998), we included two voltage-gated $K^+$ currents modified from the current $I_M$, described by McCormick et al. (1993) and used by Destexhe et al. (1998a) in their model of cortical pyramidal neurons:

$$I_{Mf} = g_{Mf} q(t)(V(t) - E_K),$$

where $g_{Ms}$ and $g_{Mf}$ are the maximal conductances of $I_{Ms}$ and $I_{Mf}$ ($\alpha_p(V)$, $\beta_p(V)$, $\alpha_q(V)$, and $\beta_q(V)$ are given in Table 1.1 of our companion paper).

The parameter values for the HVC$_{RA}$ neuron model were the same as in our companion paper: $g_{Na} = 50 \text{ mS/cm}^2$, $E_{Na} = 45 \text{ mV}$, $g_K = 5 \text{ mS/cm}^2$, $E_K = -88 \text{ mV}$, $g_L = 0.1 \text{ mS/cm}^2$, $E_L = -83 \text{ mV}$, $C_M = 1 \mu\text{F/cm}^2$, $V_T = -53 \text{ mV}$, $g_{Ms} = 0.3 \text{ mS/cm}^2$, and $g_{Mf} = 0.8 \text{ mS/cm}^2$. 
Modeling HVC\textsubscript{I} neurons

As in our companion paper, to model the depolarizing sag in HVC\textsubscript{I} neurons (Kubota and Taniguchi 1998; Dutar et al. 1998), we included the current $I_h$:

$$I_h = g_h r(t)(V(t) - E_h)$$

where $g_h$ and $E_h$ are the maximal conductance and reversal potential of $I_h$, and

$$\frac{dr(t)}{dt} = \frac{r_n(V(t)) - r(t)}{\tau_r(V(t))}$$

$$r_n(V) = \frac{1}{e^{(V+75)/5.5} + 1}$$

$$\tau_r(V) = \frac{195 \text{ ms}}{e^{(V+71.9)/14.27} + e^{-(89.3+V)/11.63}}$$

based on Huguenard and McCormick (1994). The parameter values of the HVC\textsubscript{I} neuron model were the same as in our companion paper: $g_{Na} = 50 \text{ mS/cm}^2$, $E_{Na} = 45 \text{ mV}$, $g_K = 10 \text{ mS/cm}^2$, $E_K = -85 \text{ mV}$, $g_L = 0.15 \text{ mS/cm}^2$, $E_L = -64 \text{ mV}$, $C_M = 1 \mu\text{F/cm}^2$, $V_T = -63.4 \text{ mV}$, $g_h = 0.07 \text{ mS/cm}^2$, and $E_h = -40 \text{ mV}$.

Once fit to the in vitro data, the parameters of the neuron models were held fixed; only synaptic strengths and network connectivity were adjusted to achieve the desired network behavior.
Modeling other neurons (RA, DM/PAm, Uva, RAm, nXIIIts)

We created a simple computational sketch of the nuclei of the feedback and
motor pathways using the basic spiking model described above. The parameter values
of these model neurons were $g_{\text{Na}} = 50 \text{ mS/cm}^2$, $E_{\text{Na}} = 45 \text{ mV}$, $g_{\text{K}} = 5 \text{ mS/cm}^2$, $E_{\text{K}} = -85 \text{ mV}$, $g_{\text{L}} = 0.1 \text{ mS/cm}^2$, $E_{\text{L}} = -70 \text{ mV}$, $C_M = 1 \mu \text{F/cm}^2$, and $V_T = -55 \text{ mV}$.

Modeling synaptic currents

We modeled both excitatory and inhibitory synaptic currents with the
following equations (Destexhe et al. 1994; Destexhe and Sejnowski 2001):

$$T(t) = \frac{T_{\text{max}}}{1 + \exp(-(V_{\text{pre}}(t) - V_p)/K_p)}$$

$$\frac{dr(t)}{dt} = \alpha T(t)(1 - r(t)) - \beta r(t)$$

$$I_{\text{syn}}(t) = g_{\text{syn}} r(t)(V_{\text{post}}(t) - E_{\text{rev}}),$$

where $T(t)$ is the concentration of neurotransmitter in the synaptic cleft, $T_{\text{max}}$ is the
maximal neurotransmitter concentration, $V_{\text{pre}}(t)$ and $V_{\text{post}}(t)$ are the membrane
potentials of the presynaptic and postsynaptic neurons, $r(t)$ is the fraction of the
receptors in the open state, $g_{\text{syn}}$ ($g_{\text{AMPA}}$ or $g_{\text{GABAA}}$) is the maximal synaptic
conductance, and $E_{\text{rev}}$ ($E_{\text{AMPA}}$ or $E_{\text{GABAA}}$) is the synaptic reversal potential. The basic
synapse model does not include transmission delays. Where noted, we inserted delays
in the feedback pathway by delaying the time of the synaptic current in the postsynaptic neuron.

For all synapses in our model, $T_{\text{max}} = 1.5 \, \text{mM}$, $V_p = 2 \, \text{mV}$, and $K_p = 5 \, \text{mV}$. For excitatory synapses, $E_{\text{rev}} = 0 \, \text{mV}$. For excitatory synapses onto HVC$_I$ neurons, $\alpha = 2.2 \, \text{mM}^{-1} \, \text{ms}^{-1}$ and $\beta = 0.38 \, \text{ms}^{-1}$. For excitatory synapses onto all other neuron types, $\alpha = 1.1 \, \text{mM}^{-1} \, \text{ms}^{-1}$ and $\beta = 0.19 \, \text{ms}^{-1}$. For inhibitory synapses, $\alpha = 5.0 \, \text{mM}^{-1} \, \text{ms}^{-1}$, $\beta = 0.18 \, \text{ms}^{-1}$, and $E_{\text{rev}} = -83 \, \text{mV}$.

**Modeling temperature dependence of neurons and synapses**

As in our companion paper, as a first approximation of the temperature dependence of neurons and synapses, we scaled all rate functions by a factor of

$$\phi(T_i) = Q_{10}^{(T_T - T_1)/10},$$

assuming a $Q_{10}$ of 3 for both neuronal and synaptic rates (Hodgkin and Huxley 1952; Collingridge et al. 1984). Here, $T_T$ is the brain temperature in vivo (assumed to be 40°C) and $T_1$ is the approximate temperature at which the measurements were made in vitro. $T_1$ is 32°C for neurons (Kubota and Taniguchi 1998), 31°C for AMPA synapses (Xiang et al. 1992; Destexhe et al. 1998b; Destexhe and Sejnowski 2001), and 34°C for GABA$_A$ synapses (Otis and Mody 1992a,b; Destexhe et al. 1998b; Destexhe et al. 2001). To produce a uniform slowing of HVC neurons and synapses in the cooling simulation, we scaled not only the rate functions but also the voltage equations of the neurons by a single constant.
Spike time

We defined spike time as the time of the peak depolarization following a crossing of a –15 mV threshold in a positive direction.

Analysis of stimulation results

For simplicity of analysis, in the stimulation results presented in Figs. 4 and 6, we classified a distortion as spiking activity of at least 100 Hz in HVC_RA neurons that lasted for more than 12 ms. We counted a given result as belonging to only one of the four categories: a syllable transition or song stop that was accompanied by distortion was counted simply as a syllable transition or song stop.

The syllable sequencing model contained 25% HVC_X neurons (not shown) in addition to 50% HVC_RA neurons and 25% HVC_I neurons (Nottebohm et al. 1990). These HVC_X neurons did not send synapses to any other neurons in the model, but they were included among the neurons stimulated in HVC. This reduced the probability of stimulating HVC_RA and HVC_I neurons.
Statistics

We made statistical comparisons using Liddell’s (1978) exact test (two tailed), because the sample sizes were small. Throughout the paper, we report values as mean ± SD.

Results

Brainstem feedback in a model of syllable sequencing

How can our model of sparse bursting, presented in the preceding companion paper (Gibb et al. 2008), be related to syllable sequencing? Figure 2.2A illustrates our current model of syllable sequencing via the brainstem and Uva. Since the differential contributions of the Uva-projecting brainstem structures DM and PAm are unknown, we grouped them together in the model. We suggest that each syllable is controlled by a dedicated network in HVC, which in our current implementation is a chain of bistable clusters like the one described in our companion paper (3 neurons per cluster, 20 clusters per chain). We frame our model in terms of syllables but suggest that both syllables and subsyllabic notes may be sequenced in a similar manner.
As in our companion paper, the HVC\textsubscript{I} neurons (90 in all) play the role of terminating HVC\textsubscript{RA} bursts. For the reasons described in that paper, each HVC\textsubscript{I} neuron is again constrained not to make inhibitory synapses onto any cluster from which it receives an excitatory synapse, or onto clusters within 7 clusters downstream of one from which it receives an excitatory synapse. Additionally, the HVC\textsubscript{I} neurons provide inhibition between syllable networks and mediate feedforward inhibition from Uva (i.e. Uva → HVC\textsubscript{I} → HVC\textsubscript{RA}). We included connections from Uva to HVC\textsubscript{I} neurons based on the observation that low-frequency electrical stimulation in Uva elicits EPSPs in HVC\textsubscript{I} neurons (Coleman et al. 2007) and the observation that thalamocortical feedforward inhibition is present in mammals (Gabernet et al. 2005). Since it is not clear that HVC\textsubscript{I} neurons receive direct connections from Uva, we also created a version of the model in which the feedforward inhibition is absent (see below). The feedback connections are organized so that HVC activity near the end of syllable network N stimulates a specific subpopulation of DM/PAm-projecting dRA (dRA\textsubscript{DM/PAm}), DM/PAm, and Uva neurons, which then stimulates syllable network N+1 in HVC, and so forth, until all the syllables of the motif are completed.

Table 2.1 summarizes the connectivity of the model. Neurons in the dRA\textsubscript{DM/PAm}-projecting region of each HVC\textsubscript{RA} syllable network (the third- and fourth-to-last clusters) synapse on a subpopulation of 10 dRA\textsubscript{DM/PAm} neurons, which synapse on a subpopulation of 10 DM/PAm neurons. The presynaptic and postsynaptic neurons are selected randomly from these sets. The neurons in this DM/PAm...
subpopulation synapse on a subpopulation of 10 Uva neurons, which synapse both on HVC\textsubscript{RA} neurons in the initiation region of the next syllable network (the first cluster) and on global HVC\textsubscript{I} neurons. At each step in this pathway, the postsynaptic neurons are selected randomly from the appropriate subpopulation.

To provide a release of inhibition from the beginnings of all syllable networks when a syllable ends, so that a new syllable network may be excited by input from Uva, we extended the constraint on HVC\textsubscript{I}-HVC\textsubscript{RA} connectivity as follows: for the purposes of the HVC\textsubscript{I}-HVC\textsubscript{RA} constraint rule, “7 clusters downstream” extends from the end of a syllable network to the beginnings of all syllable networks (Fig. 2.2B). For example, an HVC\textsubscript{I} neuron that receives excitation from the third cluster from the end of one syllable network cannot send inhibition to either the last three clusters of the same syllable network or the first five clusters of any syllable network.

Additionally, to prevent the feedforward inhibition from blocking the initiation of the new syllable network, we permit each subpopulation of Uva neurons to synapse only on those HVC\textsubscript{I} neurons that do not synapse on the first 8 clusters of the corresponding HVC syllable network (Fig. 2.2C). We chose this number of clusters to ensure that the feedforward inhibition has enough time to decay, just as the 7-cluster HVC\textsubscript{I} downstream connectivity gap ensures that the inhibition on HVC\textsubscript{RA} neurons has enough time to decay before excitation arrives from an upstream cluster (see our companion paper). However, we did not fine-tune this parameter.
**TABLE 2.1. Strengths and numbers of synapses in brainstem feedback model (E, excitatory; I, inhibitory)**

<table>
<thead>
<tr>
<th>Synapse</th>
<th>Type</th>
<th>Maximal conductance (mS/cm²)</th>
<th>Number of synapses</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVC&lt;sub&gt;RA&lt;/sub&gt; → HVC&lt;sub&gt;RA&lt;/sub&gt; within cluster</td>
<td>E</td>
<td>1.0</td>
<td>1 per HVC&lt;sub&gt;RA&lt;/sub&gt; neuron</td>
</tr>
<tr>
<td>HVC&lt;sub&gt;RA&lt;/sub&gt; → HVC&lt;sub&gt;RA&lt;/sub&gt; between clusters</td>
<td>E</td>
<td>0.5</td>
<td>1 per cluster (except last cluster)</td>
</tr>
<tr>
<td>HVC&lt;sub&gt;RA&lt;/sub&gt; → HVC&lt;sub&gt;1&lt;/sub&gt;</td>
<td>E</td>
<td>0.1</td>
<td>100 per HVC&lt;sub&gt;1&lt;/sub&gt; neuron</td>
</tr>
<tr>
<td>HVC&lt;sub&gt;1&lt;/sub&gt; → HVC&lt;sub&gt;RA&lt;/sub&gt;</td>
<td>I</td>
<td>3.0</td>
<td>100 per HVC&lt;sub&gt;1&lt;/sub&gt; neuron</td>
</tr>
<tr>
<td>HVC&lt;sub&gt;RA&lt;/sub&gt; → dRA&lt;sub&gt;DM&lt;/sub&gt;</td>
<td>E</td>
<td>0.004</td>
<td>1170 per syllable&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>HVC&lt;sub&gt;RA&lt;/sub&gt; → dRA&lt;sub&gt;RAm&lt;/sub&gt;</td>
<td>E</td>
<td>0.3</td>
<td>Variable (see text)</td>
</tr>
<tr>
<td>HVC&lt;sub&gt;RA&lt;/sub&gt; → vRA&lt;sub&gt;nXIIts&lt;/sub&gt;</td>
<td>E</td>
<td>0.3</td>
<td>Variable (see text)</td>
</tr>
<tr>
<td>dRA&lt;sub&gt;DM/PAm&lt;/sub&gt; → DM/PAm</td>
<td>E</td>
<td>0.075</td>
<td>5 per dRA&lt;sub&gt;DM&lt;/sub&gt; neuron</td>
</tr>
<tr>
<td>DM/PAm → Uva</td>
<td>E</td>
<td>0.075</td>
<td>5 per DM neuron</td>
</tr>
<tr>
<td>Uva → HVC&lt;sub&gt;RA&lt;/sub&gt;</td>
<td>E</td>
<td>0.04</td>
<td>3 per Uva neuron</td>
</tr>
<tr>
<td>Uva → HVC&lt;sub&gt;1&lt;/sub&gt;</td>
<td>E</td>
<td>0.0125</td>
<td>5 per Uva neuron</td>
</tr>
<tr>
<td>dRA&lt;sub&gt;RAm&lt;/sub&gt; → RAm</td>
<td>E</td>
<td>0.02</td>
<td>15 per dRA&lt;sub&gt;RAm&lt;/sub&gt; neuron</td>
</tr>
<tr>
<td>vRA&lt;sub&gt;nXIIts&lt;/sub&gt; → nXIIts</td>
<td>E</td>
<td>0.02</td>
<td>15 per vRA&lt;sub&gt;nXIIts&lt;/sub&gt; neuron</td>
</tr>
</tbody>
</table>

<sup>*</sup>150 synapses per syllable with a maximal conductance of 0.03 mS/cm², and other combinations, produce indistinguishable model spike patterns.
FIG. 2.2. Model of syllable sequencing via brainstem feedback. A: Schematic of model. The motif is initiated by a current pulse into the first subpopulation of Uva (“Init”), and the activity propagates through the network in a spiral in this diagram. Each syllable is controlled by a different network in HVC (dark gray rectangles); a syllable network is a chain of 20 clusters of 3 HVC_{RA} neurons. Each of 90 HVC_{I} neurons (black rectangle) receives excitation from, and sends inhibition to, 30 HVC_{RA} neurons. dRA_{DM/PAm} (the DM- and/or PAm-projecting region of dorsal RA), DM/PAm, and Uva are assumed to contain subpopulations dedicated to the transitions between individual syllables (which may or may not be spatially organized as “syllabotopic” maps); in the model, a subpopulation consists of 10 neurons. See text and Table 2.1 for details of connectivity. B: Schematic illustrating the constraint on HVC_{RA}-HVC_{I} connectivity that provides a release of inhibition from the beginnings of all syllable networks when a syllable ends (see text). Because the representative HVC_{I} neuron receives an excitatory synapse (arrow) from a cluster near the end of an HVC_{RA} syllable network, it is not permitted to make inhibitory synapses onto the parts of syllable networks shown in white. C: Schematic illustrating the constraint on Uva → HVC_{I} connectivity that prevents feedforward inhibition from blocking the initiation of the new syllable network. Because the representative HVC_{I} neuron does not make inhibitory synapses onto the first 8 clusters of HVC_{RA} syllable network 2 (white), it is permitted to receive an excitatory synapse (arrow) from Uva subpopulation 2. D: The first 14 clusters of each HVC_{RA} network control syllables, while the last 6 clusters control gaps. Each neuron in dRA_{Ram} and vRA_{nXIIIts} (not shown in A for simplicity) is twice as likely to receive a synapse from a syllable-controlling HVC_{RA} neuron as from a gap-controlling one. E: Spike times of all neurons in the simulation. The first subpopulation of Uva is excited by a current pulse at t = 0 ms, which excites HVC_{RA} neurons at the beginning of the first HVC syllable network and inhibits other HVC_{RA} neurons via the HVC_{I} neurons. Neurons near the end of each HVC syllable network excite a dRA_{DM} subpopulation, which excites a DM/PAm subpopulation, which excites the next Uva subpopulation, which in turn excites HVC_{RA} neurons at the beginning of the corresponding HVC syllable chain while inhibiting other HVC_{RA} neurons via the HVC_{I} neurons. The terminal DM/PAm subpopulation excites all Uva subpopulations, which causes the inhibitory effect of Uva on HVC_{RA} neurons to predominate and terminates activity in HVC. Without this inhibitory effect of Uva on HVC, HVC_{RA} neurons remain persistently active at the end of the last syllable network. Neurons of dRA_{Ram} and vRA_{nXIIIts} (30 neurons each) send connections to RA_{M} and nXIIIts (30 neurons in each nucleus).
HVC*RA (syllable 1)

HVC*RA (syllable 2)

HVC*RA (syllable 3)

DM/PAm

Uva

RAm

nXIts

Time (ms)

B

HVC*RA syllable networks

example HVC

example HVC

example HVC

D

HVC*RA syllable networks

14 clusters 6 clusters

example HVC subpop 1

example HVC subpop 2

example HVC subpop 3

HVC*RA syllable networks

controls syllable:
mORE connections TO
RA
RAm
and RA
nXIts
neurons

controls gap:
fEWer connections TO
RA
RAm
and RA
nXIts
neurons

E
To prevent persistent activity in the HVC<sub>RA</sub> clusters at the end of the last syllable network, and to account for the multiunit “superbursts” observed in Uva by Williams and Vicario (1993) and in HVC by Schmidt (2003), neurons in the final DM/PAm subpopulation in the model synapse on all of the Uva subpopulations (Fig. 2.2A). As discussed below, the simultaneous activation of all Uva subpopulations causes the feedforward inhibition via the HVC<sub>1</sub> population to predominate over the excitation of HVC<sub>RA</sub> neurons in the initiation regions of syllable networks.

The model assumes that both syllables and intersyllable gaps are controlled by parts of the HVC<sub>RA</sub> networks: there is no difference between the parts of HVC<sub>RA</sub> networks controlling syllables and networks aside from their downstream connectivity. In principle, gaps could be controlled by HVC<sub>RA</sub> neurons anywhere along the HVC<sub>RA</sub> network. However, given the evidence that syllables are units of song (see Introduction), it is reasonable to assume that gaps are controlled by the beginnings and/or ends of HVC<sub>RA</sub> networks, near HVC syllable transition points. In the present model, gaps are controlled by the ends of HVC<sub>RA</sub> networks (Fig. 2.2D).

In our model, syllable-controlling parts of HVC<sub>RA</sub> networks project more strongly to RAm- and nXIIIts-projecting regions of RA (dRA<sub>RAm</sub> and vRA<sub>nXIIIts</sub>), while gap-controlling parts of HVC<sub>RA</sub> networks project less strongly. The summed activity of these RA regions thus reaches a low at gaps. Since Leonardo and Fee (2005) reported that the fractions of RA neurons bursting during syllables and gaps are not
significantly different, we regard this aspect of our model as an oversimplification designed to convey that some portions of the chains control syllables, while others control gaps. The manner in which syllables and gaps are encoded in RA is likely to differ from our model, but this is beyond the scope of the present paper.

More specifically, the syllable-controlling part of each HVC\textsubscript{RA} network consists of the first 14 clusters, while the gap-controlling part consists of the last 6 clusters (Fig. 2.2D). The propagation time for the syllable-controlling part is $56.1 \pm 1.7$ ms ($n = 96$) from the first to the last spike; that for the gap-controlling part is $17.7 \pm 0.7$ ms ($n = 96$). The activity of the gap-controlling part is truncated by the arrival of input from Uva. The total propagation time for an HVC\textsubscript{RA} network of 20 clusters is $70.4 \pm 0.8$ ms ($n = 96$). Each neuron in dRA\textsubscript{Ram} and vRA\textsubscript{nXIIIts} is twice as likely ($p = 0.05$) to receive a synapse from a syllable-controlling HVC\textsubscript{RA} neuron as from a gap-controlling one ($p = 0.025$). The model nuclei dRA\textsubscript{Ram}, vRA\textsubscript{nXIIIts}, RA\textsubscript{m}, and nXIIIts contain 30 neurons each. Lows in the activity of RA\textsubscript{m} and nXIIIts correspond to gaps between syllables. The dRA\textsubscript{Ram} and vRA\textsubscript{nXIIIts} regions are not part of the neural feedback pathway to HVC.

Figure 2.2E shows the spiking activity of the model. A wave of HVC\textsubscript{RA} bursting ($5.1 \pm 2.0$ ms burst duration, $3.6 \pm 1.0$ spikes per burst; 32 trials) is initiated by a brief burst of spikes in the first Uva subpopulation (triggered in the model by a 5 ms, $20 \mu$A/cm$^2$ current pulse injected into all Uva neurons of this subpopulation at $t =$
0 ms). Once this wave of activity has propagated to the end of a given HVC syllable network, these neurons trigger a sequence of activations of the appropriate dRA_{DM/PAm}, DM/PAm, and Uva subpopulations. The Uva subpopulation then excites the next HVC syllable network and, via HVC{\textsubscript{1}} neurons, inhibits HVC{\textsubscript{RA}} neurons other than those in the first 8 clusters of the next syllable network, including those at the end of the previous syllable network. The last DM/PAm subpopulation excites all of the Uva subpopulations, which causes both excitation and feedforward inhibition of the first cluster of each chain. The parameters are set so that the inhibition predominates over the excitation and the activity in HVC ends.

This version of the model predicts that each dRA_{DM/PAm} neuron will be active only at times near the end of a specific syllable network’s activity. By contrast, the RA neurons that have been recorded during song generate an average of 12 bursts per motif (Leonardo and Fee 2005). However, it is possible that these recordings do not include dRA_{DM/PAm} neurons. Alternatively, it is possible that dRA_{DM/PAm} neurons do have many bursts per motif but that the summed activity of subpopulations of these neurons peaks at the ends of syllables.

If we omit synapses from the final DM/PAm subpopulation to all of the Uva subpopulations, then one or two clusters at the end of the last HVC{\textsubscript{RA}} syllable network enter a state of persistent activity (Fig. 2.3A). Even without the Uva \rightarrow HVC{\textsubscript{1}} connections, the model contains mechanisms for suppressing persistent activity at the
ends of syllables and motifs. Each syllable network inhibits the previous network, preventing persistent activity at the ends of syllables; and stimulation of the beginnings of all syllable networks by Uva at the end of a motif causes brief activity in all of them, which causes inhibition of all of them via the HVC neurons and terminates their activity.

*Variations on and perturbations of the syllable sequencing model*

We imagine that in real birds, the neural “tracks” that lead from one syllable representation to another are not strict: under certain circumstances, the bird can leap from one track to another, resulting in a stutter or other sequence variation. The model can be made to stutter in two very simple ways. In the first (Supplementary Fig. 2.S2), we rewired the HVC → dRA connections so that HVC syllable network 3 activates dRA subpopulation 2 instead of dRA subpopulation 3. Such a dramatic rewiring is unlikely to occur in real birds, but this simulation is suggestive of a more likely possibility, in which HVC syllable network 3 sends a weaker projection to dRA subpopulation 2 as well as the projection to dRA subpopulation 3. Variable excitation of dRA neurons, perhaps due to synaptic input from lateral LMAN (Johnson et al. 1995), would permit subpopulation 2 to be activated with some probability, resulting in a stutter.
FIG. 2.3. Variations on and perturbations of the syllable sequencing model. Green rectangles indicate stimulation times. All examples except for F are for versions of the model that include Uva → HVC connections. A: Spike times of the last HVC_{RA} syllable network when we omit the excitation of all Uva subpopulations by the terminal DM/PAm subpopulation. HVC_{RA} neurons at the end of the sequence remain persistently active. B: Example of truncation and “song stop,” i.e. termination of the HVC_{RA} burst sequence, caused by a 5 ms, 20 µA/cm² current pulse injected into all dRA<sub>DM/PAm</sub> neurons, beginning at t = 125 ms. C: Example of syllable transition, elicited by a 20 ms, 20 µA/cm² current pulse injected into dRA<sub>DM/PAm</sub> subpopulation 2, beginning at t = 50 ms. D: The model provides a basis for understanding sequence variations like stuttering. Stuttering of the second syllable, generated when a 20 ms, 20 µA/cm² current pulse is injected into dRA<sub>DM/PAm</sub> subpopulation 2, beginning at t = 150 ms. This could represent either electrical stimulation by an electrode or synaptic input from LMAN to RA. E: Example of distortion in the HVC_{RA} burst sequence caused by a 5 ms, 20 µA/cm² current pulse injected into dRA<sub>DM/PAm</sub> subpopulation 2, beginning at t = 175 ms. F: Example of distortion in the HVC_{RA} burst sequence caused by a 5 ms, 20 µA/cm² current pulse injected into dRA<sub>DM/PAm</sub> subpopulation 2, beginning at t = 25 ms. G: Motif restart generated after syllable truncation caused by a 5 ms, 20 µA/cm² current pulse injected into all DM/PAm neurons, beginning at t = 120 ms (green rectangle). In this version of the model, we stimulate the first neuron of the first syllable network in HVC with a 5 µA/cm² current lasting from t = 0 to t = 500 ms, which could represent continuous excitatory synaptic drive to the initiation region of the first syllable network (“intention” to sing).
In the second approach to inducing stuttering in the model (Fig. 2.3D), we stimulated all of the neurons in dRA_{DM/PAm} subpopulation 1 with a 20 ms, 20 µA/cm\(^2\) current pulse beginning at 150 ms, triggering a reactivation of HVC\(_{RA}\) syllable network 2 followed by a normal completion of the sequence. This current injection could represent the synaptic influence of LMAN on RA; it is possible that the lateral LMAN \(\rightarrow\) dRA input (Johnson et al. 1995) could alter song sequence by stimulating a dRA_{DM/PAm} subpopulation out of sequence.

As described above, the model normally runs through a set of syllables in a fixed sequence and then stops. However, a slight modification of the model generates repeated motifs. We suggest that a population of initiator neurons in HVC may be activated by input carrying an “intention” to sing from elsewhere in the brain. The model is neutral with regard to the source of this input, although it is plausible that the source is again Uva and the brainstem (perhaps DM). We performed 10 simulations (Supplementary Fig. 2.S1) in which we injected a constant current of 5 µA/cm\(^2\) into the first neuron of the first HVC\(_{RA}\) cluster from the start of the simulation to \(t = 500\) ms. In these simulations, we did not inject current into any other neuron, including Uva neurons, for the duration of the simulation. The current initiates each wave of activity in the syllable networks in the usual manner. While the syllable networks are active, they inhibit the initiator neuron via the same HVC\(_1\) neurons that are involved in sparse burst generation, preventing the initiator neuron from spiking. When the activity reaches the end of the last syllable network, this inhibition is released,
allowing the initiator neuron to reach threshold again and trigger a new motif. In one of the 10 simulations, the model also exhibited a stutter of syllable 1.

*The effects of stimulation on model behavior*

We performed 128 simulations with and 128 simulations without \( Uva \rightarrow HVC_1 \) connections, stimulating HVC, \( dRA_{DM/PAm} \), DM/PAm, and Uva with 20 \( \mu A/cm^2 \) current pulses of different durations and with greater or lesser numbers of neurons stimulated (Fig. 2.4; Table 2.2). Specifically, we stimulated with either 5 ms or 20 ms current pulses. In HVC, we stimulated either a single neuron or 10% of the total population. In \( dRA_{DM/PAm} \), DM/PAm, and Uva, we stimulated either a single subpopulation of 10 neurons (“1 subpop” in Fig. 2.4B) or the total population of 30 neurons (“all subpops” in Fig. 2.4B). For each simulation, the connectivity of the model was recalculated with new random numbers. For each stimulation type (i.e., each combination of nucleus stimulated, extent of stimulation, and duration of stimulation), we stimulated at \( t = 25, 50, 75, 100, 125, 150, 175, \) and 200 ms.
FIG. 2.4. Effects of stimulation on the syllable sequencing model.  

**A:** Effects of stimulating HVC.  
*Left:* Percentage of simulations showing HVC<sub>RA</sub> distortion (*distort*), truncation and song stop (*truncate & stop*), or syllable transition (*transition*) in response to stimulation of HVC neurons with 20 µA/cm<sup>2</sup> current pulses: either a single HVC neuron or 10% of the total HVC population, and either 5 ms or 20 ms current pulses. We grouped the data for the versions of the model with and without Uva → HVC<sub>I</sub> connections.  
*Right:* Schematic of the two spatial patterns of HVC stimulation. *White rectangles* represent HVC<sub>RA</sub> networks, *black squares* represent the population of HVC<sub>I</sub> neurons, *arrowheads* represent excitation, *dots* represent inhibition, and *small gray squares* represent single excited neurons.

**B:** Effects of stimulating in the neural feedback pathway of the model with Uva → HVC<sub>I</sub> connections intact.  
*Left:* Percentage of simulations showing HVC<sub>RA</sub> distortion, truncation and song stop, or syllable transition in response to stimulation of dRA<sub>DM/PAm</sub>, DM/PAm, or Uva neurons with 20 µA/cm<sup>2</sup> current pulses: either a single subpopulation of 10 neurons (*stimulate 1 subpop*) or the total population of 30 neurons (*stimulate all subpops*), and either 5 ms or 20 ms current pulses. We grouped the data for stimulation of dRA<sub>DM/PAm</sub>, DM/PAM, and Uva.  
*Right:* Schematic of the two spatial patterns of feedback pathway stimulation. *Numbered rectangles* represent syllable-specific subpopulations of neurons in either dRA<sub>DM/PAm</sub>, DM/PAM, or Uva. We show excited subpopulations in gray, unexcited ones in white.

**C:** Different effects of stimulating in the feedback pathway of models with or without Uva → HVC<sub>I</sub> connections.  
*Left:* Percentage of simulations showing distortion, truncation and song stop, syllable transition, or no effect. Without the Uva → HVC<sub>I</sub> connections, the number of truncations drops dramatically, and the number of simulations with no effect rises.  
*Right:* Schematic of the model with and without Uva → HVC<sub>I</sub> connections. The *white rectangles* and *black circles* represent the HVC<sub>RA</sub> and HVC<sub>I</sub> populations, respectively.
A  stimulate HVC

B  stimulate in feedback pathway (Uva → HVC, connections intact)

C  stimulate in feedback pathway (Uva → HVC, connections intact)

Uva → HVC connections intact:

no Uva → HVC connections:
We observed three effects of perturbing our model: truncation, syllable transition, and HVC$_{RA}$ distortion (Fig. 2.3B-F). Our focus here is on truncations and
transitions rather than HVC\textsubscript{RA} distortions. We define the latter narrowly as spiking activity of at least 100 Hz in HVC\textsubscript{RA} neurons that lasts for more than 12 ms. Figure 2.3E shows an HVC\textsubscript{RA} distortion caused by injecting a 5 ms, 20 \(\mu\)A/cm\(^2\) current pulse into all the neurons of dRA\textsubscript{DM/PAm} subpopulation 2, beginning at \(t = 175\) ms. Figure 2.3F shows an HVC\textsubscript{RA} distortion in the version of the model without Uva \(\rightarrow\) HVC\textsubscript{I} connections, caused by injecting a 5 ms, 20 \(\mu\)A/cm\(^2\) current pulse into all the neurons of dRA\textsubscript{DM/PAm} subpopulation 2 beginning at \(t = 25\) ms. The duration of HVC\textsubscript{RA} distortions in response to stimulation of HVC was 22.1 \(\pm\) 6.6 ms \((n = 16\) neurons; grouping data from the models with and without Uva \(\rightarrow\) HVC\textsubscript{I} connections). The duration of HVC\textsubscript{RA} distortions in response to stimulation in the feedback pathway was 25.1 \(\pm\) 8.2 ms \((n = 36\) neurons) for the model with Uva \(\rightarrow\) HVC\textsubscript{I} connections and 18.3 \(\pm\) 7.8 ms \((n = 23\) neurons) for the model without these connections. HVC\textsubscript{RA} distortions may be generated in three ways: excitation, which evokes spiking directly; inhibition, which may cause a brief pause in a cluster’s spiking, thus prolonging the total spiking duration and delaying the activation of the downstream cluster; or release of inhibition, i.e. inhibition of HVC\textsubscript{RA} neurons which would ordinarily inhibit other HVC\textsubscript{RA} neurons via HVC\textsubscript{I} neurons. In 19 of 22 trials containing distortions, the distortions were confined to neurons in just one cluster.

A truncation in the model was a premature termination of the HVC\textsubscript{RA} burst sequence. This corresponds to the syllable truncations observed by Ashmore et al. (2005). Figure 2.3B shows a syllable truncation and song stop of the HVC\textsubscript{RA} burst
sequence caused by injecting a 5 ms, 20 µA/cm² current pulse into all the neurons of all 3 subpopulations of dRA_{DM/PAm}, beginning at t = 125 ms. The truncation and song stop can be understood intuitively as the simple result of activating the HVC_I neurons in HVC. In addition, Ashmore et al. (2005) observed examples of motif restart following syllable truncation. Figure 2.3G shows that the version of the model in which a constant current into the initiator neuron is maintained produces a motif restart after truncation.

A syllable transition in the model was premature termination of the HVC_{RA} burst sequence followed by a continuation of the burst sequence from the beginning of a different syllable network. In addition to the specific type of syllable transition (stutter) shown in Fig. 2.3D, Fig. 2.3C shows an example of a syllable transition evoked by stimulation of the neurons in RA_{DM/PAm} subpopulation 2 with a 20 ms, 20 µA/cm² current pulse beginning at 50 ms. Syllable transitions in response to experimental stimulation of the feedback pathway have not been reported; we will return to this issue in the Discussion.

Figure 2.4A summarizes the combined truncation, transition, and HVC_{RA} distortion results of stimulating HVC both with and without Uva → HVC_I connections. The differences between the results with and without Uva → HVC_I connections were not significant (p > 0.1, Liddell’s exact test), but we note that for 5 ms, 10% stimulation, we observed no syllable transitions when Uva → HVC_I
connections were included and three when they were omitted. We observed
significantly fewer truncations for either 5 ms or 20 ms single-neuron stimulation than
for either 5 ms or 20 ms stimulation of 10% of HVC neurons (p < 0.001, Liddell’s
exact test). Figure 2.4B summarizes the combined results of stimulating dRA$_{DM/PAm}$,
DM/PAm, and Uva when the Uva $\rightarrow$ HVC$_1$ connections were intact. We observed
significantly fewer truncations (p < 10$^{-5}$, Liddell’s exact test) and significantly more
syllable transitions (p < 0.01, Liddell’s exact test) for 20 ms stimulation of a single
subpopulation than for the other three stimulation protocols.

A key finding was that longer (20 ms) stimulation of a single subpopulation
increased the likelihood of triggering a syllable transition, while shorter (5 ms)
stimulation tended to promote truncation and song stop (Fig. 2.4B). We investigated
the mechanism of this effect in the simulations summarized in Fig. 2.5. We stimulated
a single subpopulation of Uva neurons with durations from 5 to 20 ms (2.5 ms
increment; stimulation beginning at $t = 25$ ms in every case), and calculated the
integrated excitatory and inhibitory synaptic conductances at synapses onto HVC$_{RA}$
neurons of the initiation cluster of the corresponding, triggered syllable network (see
Fig. 2.5A). This initiation cluster receives excitatory input from the stimulated Uva
subpopulation and inhibition from other clusters via the HVC$_1$ population.
FIG 2.5. Mechanism of truncation vs. syllable transition in the syllable sequencing model. A: The HVC\textsubscript{RA} cluster in which we measured the excitatory and inhibitory conductance (circled; first cluster of syllable network 2 in B-D, first cluster of syllable network 3 in E) receives excitatory input from the stimulated Uva subpopulation and inhibition from other clusters via the HVC\textsubscript{I} population. B: Stimulation of a single Uva subpopulation causing truncation (black dots) tended to be associated with higher integrated inhibitory conductance and lower integrated excitatory conductance in the neurons of the first cluster of the corresponding syllable network, while stimulation causing transition (red dots) tended to be associated with the converse. Integrated conductance is the mean of 3 neurons, integrated over the time of stimulation. C and D: The normal onset time of syllable 2 is 79.2 ± 0.4 ms. C: Representative time courses of inhibitory and excitatory conductances, normalized, for a short (5 ms) stimulation of Uva causing truncation (mean of 3 neurons). D: Representative time courses of inhibitory and excitatory conductances, normalized, for a long (20 ms) stimulation of Uva causing syllable transition (mean of 3 neurons). E: When we modified the HVC\textsubscript{I}-HVC\textsubscript{RA} connectivity to release inhibition from the first cluster at the time of stimulation, a 5 ms stimulation caused transition rather than truncation. This is consistent with the idea that stimulation-induced truncation is related to the inhibition of initiation clusters. E1: Schematic illustrating the modified connectivity providing the release of inhibition (cf. Fig. 2.2B). Because the representative HVC\textsubscript{I} neuron receives an excitatory synapse (arrow) from a cluster that spikes near what would be the truncation time, it is not permitted to make inhibitory synapses onto the parts of syllable networks shown in white. E2: Representative time courses of inhibitory and excitatory conductances, normalized, for such a simulation (mean of 3 neurons). The modified connectivity permits the inhibitory conductance to decay before the excitatory input arrives from Uva. The normal onset time of syllable 3 is 154.1 ± 0.7 ms.
excitation and inhibition of 1st (initiation) cluster of HVCRA syllable network 2

Integrated inhibitory conductance (mS cm⁻²)

Integrated excitatory conductance (mS cm⁻²)

HVCRA syllable networks

HVC₁ population

Uva sub-pop 2 (stim)

excitation and inhibition of 1st (initiation) cluster of HVCRA syllable network 2
The excitatory conductance is the AMPA conductance of the Uva → HVC$_{RA}$ synapses onto the three HVC$_{RA}$ neurons in the first cluster of the syllable network (triggered by Uva stimulation), averaged over the three neurons. The inhibitory conductance is the GABA$_A$ conductance of the HVC$_{I}$ → HVC$_{RA}$ synapses onto the same three HVC$_{RA}$ neurons, averaged over the three neurons. The integrated conductance is the excitatory or inhibitory conductance integrated over the stimulation interval.

As Fig. 2.5B shows, truncation tended to be associated with higher inhibitory conductance and lower excitatory conductance values at the initiation cluster of the syllable network, while transition tended to be associated with the converse. There is, however, overlap between the two clusters: the integrated excitatory and inhibitory conductances did not completely predict transition and truncation. It seems reasonable to suppose that the specific time courses of the excitatory and inhibitory conductances, in interaction with the voltage-dependent currents, are important in determining whether excitation or inhibition predominates. The integrated conductances do not capture these details, which may account for the overlap. To illustrate these two classes of stimulation effect, we show the time course of the mean excitatory and inhibitory conductance of the three neurons of the initiation cluster for stimulation producing truncation (Fig. 2.5C) and transition (Fig. 2.5D).
If, as these results suggest, truncation is related to the inhibition of initiation clusters, then artificially removing this inhibition at about the time of stimulation should change truncations into transitions. This is indeed what we find (Fig. 2.5E). Normally, when we stimulate at $t = 125$ ms, the twelfth cluster in the second syllable network is the second-to-last cluster to spike before the truncation. To artificially release the inhibition of the third syllable network’s initiation cluster at the appropriate time, we treated the twelfth cluster in the second syllable network like the end of a syllable network: the “7 clusters downstream” rule extended from this cluster to the beginnings of all syllable networks (Fig. 2.5E1). Fig. 2.5E2 shows that this modification permits the inhibitory conductance to decay before the excitatory input arrives from Uva. With this modification, 10 of 10 trials with 5 ms stimulation showed transition, whereas in the normal case, 10 of 10 trials with 5 ms stimulation showed truncation.

Truncation in the model depends not only on the predominance of inhibition at the initiation cluster but also on the termination of the burst sequence of the previous syllable network. Fig. 2.4C shows the number of distortions, truncations, transitions, and simulations with no effect for dRA$_{DM/PAm}$, DM/PAm, and Uva stimulation either with (black) or without (white) the Uva $\rightarrow$ HVC$_I$ connections. There was a large decrease in the number of truncations (from 60% to 14%) and a large increase in the number of simulations with no effect (from 5% to 56%) without the Uva $\rightarrow$ HVC$_I$ connections ($p < 10^{-10}$ for both, Liddell’s exact test).
In the 128 stimulation trials that we performed with Uva → HVC₁ connections, 8 showed a transition to another syllable at the end of the motif rather than a stop at the end of the motif, within the 350 ms simulation. In the 128 stimulation trials that we performed without Uva → HVC₁ connections, 26 showed such a transition. The reason for this difference is unknown. In 30 unstimulated trials with Uva → HVC₁ connections, only 1 showed such a transition, while in 30 unstimulated trials without Uva → HVC₁ connections, only 2 showed such a transition.

Timing dependence of stimulation effects

Given the nonuniform functional connectivity of the model, one might expect to observe different effects depending on the timing of the stimulation relative to syllable onset. As Fig. 2.6 suggests, we did see evidence for such timing dependence, and we observed different timing effects depending on whether we included Uva → HVC₁ connections or not.

In Fig. 2.6, the “corrected stimulation time” is the approximate time of the stimulation’s effect on HVC relative to the onset of the current syllable. More specifically, it is the time of stimulation, plus the mean delay to HVC in the model (9.9 ms for RA, 8.1 ms for DM, and 5.6 ms for Uva), minus the mean onset time of
the most recent \( \text{HVC}_{\text{RA}} \) syllable. Because we only stimulated at 25, 50, 75, 100, 125, 150, 175, and 200 ms relative to the start of the simulation, all corrected stimulation times for the feedback pathway fell within one of three 6 ms bins: 0 to 6 ms, 25 to 31 ms, or 50 to 56 ms.

FIG. 2.6. Timing of stimulation in the feedback pathway (same data as for Fig. 2.4). The percentage of different effects evoked in each of three time bins is shown. *Corrected stimulation time* is the approximate time of the stimulation’s effect on HVC relative to the onset of the current syllable (see text). Percentage of simulations showing HVC\(_{\text{RA}}\) distortions (*distort*), truncation and song stop (*truncate & stop*), or syllable transition (*transition*) for corrected stimulation times in three 6 ms bins. *A:* Model with Uva \( \rightarrow \) HVC\(_{\text{i}}\) connections. *B:* Model without Uva \( \rightarrow \) HVC\(_{\text{i}}\) connections.
Fig. 2.6 shows the grouped effects for all trials in which we stimulated the feedback pathway. In the model with Uva → HVC connections (Fig. 2.6A), we observed no distortions when the corrected stimulation time was in the 50 to 56 ms bin, significantly fewer than the number in the other two time bins (p < 0.05, Liddell’s exact test). We also observed significantly fewer transitions in the 0 to 6 ms time bin than in the other two bins (p < 0.05, Liddell’s exact test).

In the model without Uva → HVC connections (Fig. 2.6B), there were no significant differences among the numbers of observed distortions at the three stimulation times (p > 0.1, Liddell’s exact test). However, stimulation of the feedback pathway at a corrected time of 50 to 56 ms evoked significantly more transitions than did stimulation at 0 to 6 ms (p = 4.3×10^{-2}, Liddell’s exact test) or 25 to 31 ms (p = 3.8×10^{-6}, Liddell’s exact test), and stimulation at 0 to 6 ms evoked significantly more transitions than stimulation at 25 to 31 ms (p = 6.7×10^{-3}, Liddell’s exact test; the latter did not evoke any transitions). The reason for these differences is unknown; further analysis of this is beyond the scope of the present paper.

Feedback delay and HVC cooling

The HVC → dRA_{DM/PAm} → DM/PAm → Uva → HVC delay in our current model, from HVC spike to HVC spike, is 14.3 ± 0.5 ms (n = 10). In our model, the
signal to dRA_{DM/PAm} is sent by the third- and fourth-to-last clusters of each syllable network, so this delay does not result in a pause in HVC_{RA} activity. This arrangement permits the generation of intersyllable gaps which are shorter than the feedback delay. The brief pause in HVC activity at transitions in our model (4.6 ± 1.0 ms; n = 10) can probably be accounted for by the difference between the time it takes the excitation from Uva to bring the HVC_{RA} initiation neurons of the next syllable network to spiking threshold and the time it takes the feedforward inhibition from Uva to suppress the previous syllable network. The time from the beginning of the depolarization to the first spike peak in the HVC_{RA} initiation neurons is 5.9 ± 0.7 ms (n = 10). In the version of our model without Uva → HVC_{I} connections, the pause is absent: the first spike of an HVC network occurs 1.0 ± 0.5 ms before the last spike of the previous HVC network (n = 10).

The HVC → dRA → brainstem → Uva → HVC feedback delay has not been measured directly in birds. Based on experimental measurements, the HVC → RA delay is ~4.5 ms (Hahnloser et al. 2002, consistent with Kimpo et al. 2003), and the delay between Uva stimulation and the onset of postsynaptic potentials in HVC is ~5 ms (Coleman et al. 2007). If the other two connections in the feedback loop have similar delays, then the total delay is ~20 ms. Consistent with this estimate, Vu et al. (1998) found that the shortest delay between stimulation of HVC in one hemisphere and full suppression of the other was 24 ms (mean = 36.1 ms). This provides an upper limit on the feedback delay.
In ten simulations, we increased the HVC-to-HVC delay to $36.2 \pm 0.2$ ms by inserting a total of 24 ms of delays in the pathway from HVC to Uva. We found that having the ninth- and tenth-to-last clusters, rather than the third- and fourth-to-last clusters, project to the feedback pathway compensated for this increased delay.

This solution to the problem of feedback delay may have important experimental consequences. In a recent abstract, Long and Fee (2007) report that selectively cooling HVC causes a uniform expansion of syllables and gaps. However, since these data are preliminary, it may be premature to assume that gaps and syllables scale uniformly. This type of experiment could provide an important test of our model because, as we demonstrate below, our model predicts that syllables and gaps will show differences in expansion. Depending on the size of the feedback delay and on whether the gaps are controlled by the beginnings or ends of syllables, the differences may be subtle.

Our syllable sequencing model assumes that both syllables and gaps are controlled by segments of HVC syllable networks. These segments differ only in their locations in the HVC networks and in their projections to RA. Gap-controlling segments could be located at either the ends (as in the model shown in Fig. 2.2) or the beginnings of HVC syllable networks, i.e. either before or after syllable transition points.
In Fig. 2.7 (top), we show a schematic diagram of our feedback delay model with gaps controlled by the beginnings of syllable networks. In Fig. 2.7 (bottom), we show the effect of slowing HVC activity, assuming that input from Uva truncates the previous syllable network while activating the next syllable network.

**FIG 2.7.** A consequence of the brainstem feedback delay in the syllable sequencing model. *Top, normal:* A region of a syllable network sends a signal to the feedback pathway. This feedback-related region is well before the end of the network, in order to compensate for the feedback delay: the second syllable network is activated just as the first one is terminates, rather than after a delay. *Bottom, HVC cooled:* when the activity of HVC is slowed, the feedback delay remains approximately constant while the HVC activity expands in time. Consequently, the end of the syllable network’s activity is suppressed by Uva input and/or activation of the next syllable network (gray X). This truncation has different effects depending on whether the gap is controlled by the beginning or the end of the syllable network (see text).
We set the feedback delay to $23.4 \pm 0.4$ ms (n = 5) and made the fifth- and sixth-to-last clusters project to the feedback pathway. We implemented the slowing in a phenomenological manner by multiplying all of the neuronal and synaptic differential equations in HVC by a constant, 0.67, to produce a uniform 50% slowing of activity within each syllable network in HVC. This is meant to model the functional but not the physiological effects of cooling. This approach is appropriate to the goal of this section, which is to show that our model’s feedback architecture itself predicts nonuniformities in the expansion of syllables and gaps, irrespective of physiological details.

As a result of truncation, more clusters were silent at the end of the syllable network in the slowed case (the third-to-last cluster is the last to exhibit spiking) than in the normal case (the last cluster is the last to exhibit spiking). What effect will this truncation have on syllable and gap durations? If the last 6 clusters of each syllable network control the gap, then we calculate from these simulations that gaps lengthen by $14.7 \pm 9.6\%$ (n = 5) and syllables by $51.1 \pm 2.5\%$ (n = 5). If the first 6 clusters control the gap, then gaps lengthen by $51.6 \pm 2.3\%$ (n = 5) and syllables lengthen by $36.6 \pm 4.7\%$ (n = 5).

Slowing HVC increased the measured feedback delay (to $27.3 \pm 1.4$; n = 5), which can partially be accounted for by the increase in time from the onset of
depolarization to the first spike in the HVC\textsubscript{RA} initiation neurons (7.4 ± 0.9 vs. 5.1 ± 0.5; n = 5).

We can analytically calculate the degree of expansion of syllables and gaps during cooling in an idealized version of this syllable sequencing model. In this idealized model, let us assume that a syllable network in HVC sends a brief signal to the feedback loop at a time $t_{\text{delay}}$ before the end of the syllable network. After a delay of $t_{\text{delay}}$, this signal activates the next syllable network. To accommodate the possibility of a slight change in this delay with HVC cooling, we distinguish the original and final delay, $t_{\text{delay1}}$ and $t_{\text{delay2}}$, respectively. If the previous syllable network is still active at this time, it is instantaneously suppressed. Now suppose that HVC activity is stretched in time by the factor $f_{\text{slow}}$ ($f_{\text{slow}} = 1.5$ implies a 50% lengthening). Let $t_{\text{syllable1}}$ and $t_{\text{syllable2}}$ be the original and final duration, respectively, of the network activity controlling a syllable, and $t_{\text{gap1}}$ and $t_{\text{gap2}}$ be the original and final duration, respectively, of the network activity controlling a gap. For simplicity, let us consider syllables and gaps that are not at the beginning or end of the motif, and let us assume for now that gaps and syllables are consistently controlled by either the beginnings or the ends of syllable networks throughout the motif.

If gaps are controlled by the ends of syllable networks and $t_{\text{gap1}} \geq t_{\text{delay1}} - t_{\text{delay2}}/f_{\text{slow}}$, then when HVC is cooled, the activity controlling a gap will be partially truncated and the activity controlling a syllable will not be truncated at all. In this case,
\[ t_{gap2} = f_{\text{slow}}(t_{gap1} - t_{\text{delay1}}) + t_{\text{delay2}} \text{ and } t_{\text{syllable2}} = f_{\text{slow}}t_{\text{syllable1}}. \] This implies a uniform expansion of the syllable and a competition between expansion and truncation of the gap during cooling. For our simulations, described above (using the mean values \( t_{gap1} = 24.4 \text{ ms}, t_{\text{delay1}} = 23.4 \text{ ms}, \) and \( t_{\text{delay2}} = 27.3 \text{ ms} \)), this predicts an 18% expansion of the gaps and a 50% expansion of the syllables, which are similar to the values calculated above from our model.

If gaps are controlled by the beginnings of the syllable networks, then if, as is reasonable to assume, \( t_{\text{syllable1}} \geq t_{\text{delay1}} - t_{\text{delay2}}/f_{\text{slow}} \), the activity controlling the first gap will not be truncated at all and the activity controlling the first syllable will be partially truncated. In this case, \( t_{gap2} = f_{\text{slow}}t_{gap1} \) and \( t_{\text{syllable2}} = f_{\text{slow}}(t_{\text{syllable1}} - t_{\text{delay1}}) + t_{\text{delay2}}. \) This implies a uniform expansion of the gap and a competition between expansion and truncation of the syllable during cooling. For our simulations, described above (using the mean values \( t_{\text{syllable1}} = 55.5, t_{\text{delay1}} = 23.4 \text{ ms}, \) and \( t_{\text{delay2}} = 27.3 \text{ ms} \)), these equations predict a 50% expansion of the gaps and a 36% expansion of the syllables, which are similar to the values calculated above from our model. This case implies a more uniform expansion of syllables and gaps (Long and Fee 2007) and also may be related to Glaze and Troyer’s (2006) finding that gaps are more elastic than syllables.

These effects may be small. For example, suppose that the syllable duration is 150 ms, the gap duration is 30 ms, the feedback delay is 24 ms, and \( t_{\text{delay1}} = t_{\text{delay2}}. \) If HVC activity is slowed by 50%, and if the gap is controlled by the beginning of the
syllable network, then the new gap duration will be 45 ms (a 50% expansion), while
the new syllable duration will be 213 ms (a 42% expansion). These considerations
predict that the shorter the feedback delay, the more similar the expansion of gaps and
syllables will be.

Discussion

We have presented a model of syllable sequencing involving syllable-specific
populations of neurons in the feedback pathway, HVC → dRA → DM/PAM → Uva
→ HVC. Our model further extends the framework of sparsely bursting networks of
HVC$_{RA}$ neurons presented in the preceding companion paper (Gibb et al. 2008) by
proposing that each syllable is controlled by such a network, and that such networks
are activated in sequence by feedback via the brainstem and Uva.

This model makes a number of assumptions and predictions:

First, our model predicts that either: 1. individual Uva, DM/PAm, or dorsal
RA neurons are active only near offsets of particular syllables or notes; or 2. the
summed activity of subpopulations of Uva, DM/PAm, or dorsal RA neurons peaks
only near the offsets of particular syllables or notes. If the signal sent through this
neural feedback loop is triggered N ms before the end of the HVC chain (Fig. 2.7), then the peak activity of these nuclei will be shifted N ms earlier. For all but the first and last syllable in a motif, the syllable offset also necessarily corresponds to a syllable onset. In the version of our model in which excitation of the first subpopulation of Uva neurons initiates a motif (Fig. 2.2 and others), Uva is also active preceding the onset of the first syllable.

Second, our model of repeated motif generation predicts that HVC\textsubscript{RA} and HVC\textsubscript{I} activity reaches a low between motifs. A pause between motifs corresponds to a pause in HVC\textsubscript{RA} activity after the termination of the last HVC\textsubscript{RA} network. This contrasts with intersyllable gaps, which are controlled by the activation of segments of HVC\textsubscript{RA} networks.

Third, our model of syllable sequencing predicts that electrically stimulating Uva, DM/PAm, or dorsal RA neurons may promote syllable truncation, song stop, HVC\textsubscript{RA} distortion, or atypical syllable transitions, depending on stimulus parameters and number of neurons stimulated (Fig. 2.4B and C). We related the increased number of transitions and decreased number of truncations that we observed in response to longer-duration stimulation to the relative excitation and inhibition at syllable initiation zones in HVC (Fig. 2.5). Our model also predicts that stimulating HVC may promote syllable truncation, song stop, or HVC\textsubscript{RA} distortion (Fig. 2.4A). Our model is not designed to simulate the distortions observed by Ashmore et al. (2005), since it
does not yet include respiration, sound production, or projections either from DM/PAm to RAm and nXIIIts or from PAm and RAm to spinal motor neurons innervating respiratory muscles.

In addition to syllable truncation, song stop, and distortion, Ashmore et al. (2005) often observed song restarts in response to stimulating RA, PAm, and HVC. The modeling result in Fig. 2.3G suggests that song restart may occur after song stop if the HVC input representing an “intention” to sing continues after the stimulation interrupts the song. Our model does not, however, explain what determines whether this input continues or not.

Fourth, our solution to the problem of feedback delay (Fig. 2.7) results in the prediction that there will be deviations from uniform expansion of syllables and gaps when HVC is cooled as in the experiments of Long and Fee (2007). The size of these deviations will depend on the size of the feedback delay and on whether the gaps are controlled by the beginnings or ends of syllable chains. In our calculations, gaps expand less than syllables when they are controlled by the ends of syllable networks and more than syllables when they are controlled by the beginnings of syllable networks. The latter effect is similar to Glaze and Troyer’s (2006) observation that gaps are more elastic than syllables, but further work should be done to determine how far this similarity extends. In HVC syllable networks in real songbirds, the control of gaps could be shared by the end of one syllable network and the beginning of the next;
the asymmetry of this sharing could vary from gap to gap. In addition, these calculations can also be expanded to cases in which subsyllabic notes and/or gaps are controlled by discrete syllable networks activated by Uva input.

These first four predictions are closely tied to our model of syllable sequencing via brainstem and Uva feedback. An additional prediction, which is less specific to our model, is that a repetition of a syllable within a motif (e.g. ABBC) will be accompanied by a repetition of an HVC<sub>RA</sub> burst; each HVC<sub>RA</sub> burst recorded in singing birds is associated with a particular syllable type (A, B, C, etc.). This feature is not included explicitly in models that describe a single HVC chain (Abarbanel et al. 2004, Fiete et al. 2005; Li and Greenside 2006; Jin et al. 2007), nor in other models that assume a fixed sequence of bursts in HVC (Fiete et al. 2004; Fiete et al. 2007). As Hahnloser et al (2002) suggest, the assumption that that repeated syllables are associated with repeated bursts in HVC<sub>RA</sub> neurons is consistent with the fact that repetitions of a syllable type are associated with very similar ensemble burst patterns in RA (Leonardo and Fee 2005). Finally, our model assumes that the two hemispheres of HVC are coordinated even during variable sequences. Whenever syllable B is sung, HVC<sub>RA</sub> neurons associated with syllable B will burst in both hemispheres.

Atypical syllable transitions in our model, such as the stutter in Fig. 2.3D, can be induced by stimulating a syllable-specific subpopulation of dRA<sub>DM/PAm</sub> neurons out of sequence. In real songbirds, input from lateral LMAN to dRA (Johnson et al. 1995)
could play this role. This would predict that neural activity corresponding to an atypical syllable transition would begin in lateral LMAN and spread to dRA, the feedback pathway, HVC, and vRA, in that order. This input could also potentially play a role in sequence learning. In the model of Troyer and Doupe (2000), input from LMAN to RA biases sequence transitions and plays a role sequence learning. However, their model does not include the HVC neural feedback pathway and differs fundamentally from ours in the manner in which it represents sequences.

Central to our model’s behavior is the assumed connectivity of inhibitory interneurons in HVC. First, as in our companion paper, the interneurons are able to participate in the generation of sparse bursting because of a specific constraint on their connectivity that results in a release of inhibition in HVC$_{RA}$ neurons at the time of their bursts. Second, we extended the constraint on their connectivity in a manner that provides a release of inhibition from the beginnings of all syllable chains at the time of syllable transitions, so that short bursts in Uva can reliably evoke syllable transitions. Without this latter constraint, only long bursts in Uva (like those evoked by 20 ms stimulation in the simulations shown in Fig. 2.3C-D, Fig. 2.4B, and Fig. 2.5D) would evoke syllable transitions with high probability. Third, in the version of our model that includes Uva $\rightarrow$ HVC$_1$ connections, we constrained the connectivity in a manner that ensures that the HVC$_{RA}$ neurons in the first segment of a newly initiated syllable network are not inhibited by Uva $\rightarrow$ HVC$_1$ feedforward inhibition.
The results of our stimulation experiments (Fig. 2.4) are closely tied to the inhibitory connectivity in HVC. As shown in Fig. 2.5B, syllable truncations elicited by stimulation of the feedback pathway tended to be associated with higher inhibitory conductance and lower excitatory conductance values at the first cluster of a syllable network, while syllable transitions tended to be associated with the converse. Intuitively, strong, sustained excitation from Uva is necessary to overcome inhibition of the first HVC\textsubscript{RA} cluster by HVC\textsubscript{I} neurons and initiate a new syllable. If the excitation from Uva is insufficient to initiate a syllable transition, then a syllable truncation may occur instead. In this circumstance, truncation is far more likely to occur if the Uva $\rightarrow$ HVC\textsubscript{I} connections are present than if they are not (Fig. 2.4C). Intuitively, the Uva $\rightarrow$ HVC\textsubscript{I} $\rightarrow$ HVC\textsubscript{RA} inhibition terminates activity in the current syllable network. If this termination is accompanied by initiation of a new syllable (as described above) then syllable transition occurs. Otherwise, truncation occurs. Additionally, the nonuniform connectivity of the model is reflected in the timing dependence of stimulation effects (Fig. 2.6).

*Absence of syllable transitions in experimental data*

Importantly, although stimulation of our model can produce jumps to syllables out of sequence (i.e. syllable transitions), such transitions have not been reported in response to experimental stimulation in the feedback pathway or HVC of songbirds. Syllable truncations with song stop, however, have been frequently observed
(Ashmore et al. 2005). It is possible that the stimulation protocols that have been reported experimentally correspond functionally to only a subset of our stimulation protocols.

For example, the 5 ms stimulation of all the subpopulations in our model produces only a very small number of syllable transitions (Fig. 2.4B, “stimulate all subpops”; only 1 of 24 simulations showed syllable transition), and in this respect is similar to the data of Ashmore at al. (2005). This stimulation may be functionally similar to their experimental stimulation, although theirs consisted of a somewhat longer, 12 ms series of 400 µs biphasic extracellular current pulses. As with our “all subpops” stimulation, the experimental stimulation may excite neurons broadly throughout a nucleus, rather than within a single putative syllable-specific subpopulation. Experimentally, selective stimulation of a single subpopulation is likely to be very difficult, unless such subpopulations are arranged topographically.

Although our model stimulation is a crude representation of real stimulation, our results suggest that, at a minimum, it would be worthwhile to explore the experimental stimulus parameter space more extensively to see if syllable transitions can be evoked in singing birds. Can longer stimulus trains (perhaps also at a different frequency or with modified pulse characteristics) evoke syllable transitions? Based on the possibility that syllable-specific subpopulations are in fact arranged “syllabotopically,” can more focal stimulation in the feedback pathway evoke syllable
transitions? For example, perhaps a bipolar stimulating electrode with very closely spaced tips, and an appropriate stimulus amplitude, could stimulate neurons primarily within a single subpopulation and thereby evoke a syllable transition.

The model results suggest that only by both using a long stimulus and stimulating a single subpopulation can one evoke a large fraction of syllable transitions rather than truncations and song stops. Additionally, it is possible that our model is only partly correct—that some of its predictions are accurate, while others are the result of perturbing the model beyond its range of applicability. The model will have served its purpose if it prompts researchers to look at the system in a new way and to devise and perform new experiments. Future experiments will enable the refinement of the model and the generation of new experimental predictions which are both more accurate and more precise.

Since MMAN may play part of the role we have proposed for Uva (see Introduction and Fig. 2.1C), it is possible that stimulating MMAN or its afferent thalamic nucleus, DMP, may evoke the syllable transitions that our model predicts for stimulation of the brainstem feedback pathway. For MMAN, too, our model results suggest that syllable transitions are much more likely to be evoked with longer stimulation of specific subpopulations.
Empirical influence of Uva on HVC

In recently reported work, Coleman et al. (2007) stimulated Uva and recorded from HVC in anesthetized zebra finches. Future work will need to match this new experimental data to the syllable sequencing model. Our model is broadly consistent with their finding that low-frequency Uva stimulation elicits EPSPs in HVC\textsubscript{I} neurons and projection neurons, but three issues in particular will need to be addressed. First, all of the HVC\textsubscript{RA} neurons that they recorded from while stimulating Uva at low frequency showed short-latency EPSPs, whereas in our model, only the HVC\textsubscript{RA} neurons at the beginnings of syllable networks receive input directly from Uva. However, these data are consistent with a minor modification of our model in which many or all HVC\textsubscript{RA} neurons receive input from Uva but the input is only large enough to initiate a burst sequence at the beginning of a syllable network. Alternatively, it may be that a large proportion of the HVC\textsubscript{RA} neurons recorded by Coleman et al. (2007) are of the class that does not participate in sparse bursting during singing.

Second, the mean delay from Uva stimulation to EPSP onset was somewhat longer in HVC\textsubscript{I} neurons than in projection neurons, suggesting the possibility that the HVC\textsubscript{I} EPSPs are elicited only indirectly via the projection neurons. By contrast, in the main version of our model, Uva makes direct excitatory connections onto the HVC\textsubscript{I} neurons, in addition to the indirect ones via HVC\textsubscript{RA} neurons. As we indicated in Results, the model continues to function even in the absence of the direct Uva →
HVC connections, but the stimulation results of Fig. 2.4 are substantially different depending on the presence or absence of these connections. Third, Coleman et al. indicate that high-frequency stimulation of Uva can cause a long-lasting (~2 s) suppression of auditory responses in HVC. Further work is needed to ensure that the inclusion of a mechanism in the model to reproduce this result does not interfere with sequence generation.

*Which brainstem nucleus (if any) is involved in syllable sequencing?*

In an earlier version of this model (Gibb and Abarbanel 2006), we gave PAm the key role of providing syllable-specific feedback to HVC via Uva. Given PAm’s role in inspiration (Reinke and Wild 1998), it is reasonable to suppose that its respiratory neurons show activity corresponding to the inspiratory “minibreaths” between syllables (Calder 1970; Hartley and Suthers 1989; Wild et al. 1998). Additionally, recent work shows that bursting in a type of nonrespiratory neuron in PAm is correlated with bursting in the contralateral RA (Ashmore et al. 2008). However, as a medullary nucleus, PAm may convey simple timing signals to Uva, rather than relatively high-level signals concerning syllable identity.

As a midbrain nucleus, DM may be a reasonable candidate for carrying syllable-specific information; other avian midbrain nuclei have been shown to be relatively complex, adaptive, topographically mapped processing centers (Knudsen
2002). However, DM is commonly believed to be involved in the production of calls rather than song (Simpson and Vicario 1990; Wild 1997). Only simple, call-like sounds have been observed in response to electrical stimulation of DM (Vicario and Simpson 1995; Wild et al. 1997), while stimulating HVC and RA can elicit more complex vocalizations (Vicario and Simpson 1995). Nottebohm et al. (1976) reported small effects of unilateral intercollicular nucleus lesions, including loss of syllables and what they described as “instability.” Vicario and Simpson (1990) found that DM lesions affect the temporal characteristics of song but do not abolish it (Vicario 1991). However, these lesions may have been incomplete (D. S. Vicario, personal communication), so it is possible that a small part of DM is essential for song production.

As we discussed in the Introduction, it is possible that the dRA → DM/PAm → Uva → HVC feedback loop provides only syllable-unspecific timing pulses and that bilateral coordination during variable sequences is achieved via another mechanism such as MMAN → HVC input.

*Thalamic gating and interruption of song at syllable boundaries*

Birds tend to interrupt their songs in the silent intervals between syllables, rather than within syllables, when presented with light flashes (Cynx 1990; Franz and Goller 2002). This can be explained within the framework of our model. If a light
flash prevents the activation of the next syllable network in HVC, the wave of bursting in the current syllable network must still complete its propagation; thus the current syllable is completed. Uva receives not only visual but also somatosensory, auditory, and neuromodulatory inputs (Wild 1994; Akutagawa and Konishi 2005; Coleman et al. 2007). Our proposed role for Uva makes it an ideal site for rapid thalamic gating of the song on the basis of sensory input, arousal, and attention.

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Supplementary Figures

FIG. 2.S1. Repeated neural “motifs” can be generated by continuous stimulation of the beginning of the first HVC$_{RA}$ syllable network. We stimulate the first neuron of the first syllable network in HVC (HVC$_{RA}$ Neuron 1; voltage trace shown above raster plot) with a 5 µA/cm$^2$ current lasting from t = 0 to t = 500 ms, which could represent continuous excitatory synaptic drive to the initiation region of the first syllable network (“intention” to sing). HVC$_{I}$ neurons inhibit this neuron as activity propagates along the three syllable networks (“Motif 1”). When this activity ceases, the constant current causes the first neuron to reach threshold again and initiate a new neural motif (“Motif 2”). The neural patterns of the two motifs are the same.
FIG. 2.S2. Stuttering of the last syllable, generated when RA subpopulation 2 is more strongly excited than RA subpopulation 3 by neurons near the end of HVC syllable network 3. In this simple example, all of the synapses from neurons near the end of HVC syllable network 3 excite RA subpopulation 2.
FIG. 2.S3. A consequence of the brainstem feedback delay in the syllable sequencing model. 

A: Activity of all HVC<sub>RA</sub> neurons in a normal simulation of the type shown in Fig. 2.2. The last cluster is the last to exhibit spiking. B: Activity of all HVC<sub>RA</sub> neurons in a simulation in which HVC activity has been slowed. Now the third-to-last cluster of each syllable network is the last to exhibit spiking, as a result of the suppressive effect.
Appendix

Chapter 1

Extended summary of HVC neuron types

There are two classes of HVC_{RA} neuron (Shea 2004) and at least two morphological classes of HVC_{X} neuron (Fortune and Margoliash 1995). HVC_{I} neurons have been described physiologically and morphologically (Dutar et al. 1998; Kubota and Taniguchi 1998; Mooney 2000; Rauske et al. 2003; Shea 2004; Mooney and Prather 2005; Wild et al. 2005). On the basis of in vivo (Mooney 2000; Rauske et al. 2003) and in vitro observations (Solis and Perkel 2005), there may be at least two distinct physiological types of HVC_{I} neuron. Two distinct HVC_{I} morphologies have also been described (Mooney 2000), and HVC_{I} neurons can be further classified by their differential expression of calcium-binding proteins (Wild et al. 2005).

In vitro, one type of HVC_{RA} neuron (“phasic”) usually spikes one to three times in response to constant depolarizing current injection (Dutar et al. 1998, Shea 2004, Mooney and Prather 2005). A phasic, putative HVC_{RA} neuron described by Kubota and Taniguchi (1998) shows longer bursts of spikes (up to 13 spikes in about
150 ms) in response to current injection. Despite this difference, it may represent the same type (S. D. Shea, personal communication), especially given that they used young (37-54 day old) birds, in which some neurons may have an immature physiology. A second type of HVC<sub>RA</sub> neuron ("tonic") spikes multiple times, with significant but relatively weak spike frequency adaptation, in response to constant depolarizing current injection (Kubota and Taniguchi 1998, Shea 2004; Mooney and Prather 2005). At first glance, the phasic type seems well suited for sparse bursting. However, the one to three spikes that it produces in response to current injection are fewer than the 4.3 ± 1.3 spikes per song-associated HVC<sub>RA</sub> burst.

While the HVC<sub>RA</sub> neurons that burst sparsely during singing have not been characterized morphologically, the HVC<sub>RA</sub> neurons that burst sparsely during BOS playback in anesthetized birds appear to be of the short dendrite class (Mooney 2000; Nixdorf et al. 1989; Fortune and Margoliash 1995). It is plausible that these represent the same type as the HVC<sub>RA</sub> neurons that burst sparsely during singing. It would be a worthwhile, though difficult, experiment to verify that the HVC<sub>RA</sub> neurons that burst sparsely during singing, sleep, and BOS playback are of the same class by holding single HVC<sub>RA</sub> units across the different states.

The most complete analysis of HVC<sub>RA</sub> types in vitro appears to be that of Shea (2004), who identified tonic HVC<sub>RA</sub> neurons with the short dendrite class, and phasic HVC<sub>RA</sub> neurons with the furry dendrite class (Nixdorf et al. 1989; Fortune and
Margoliash 1995). This analysis conflicts with the classification by Dutar et al. (1998) of their phasic HVC$_{RA}$ neurons as having the short dendrite morphology. In agreement with Shea (2004), Kubota and Taniguchi (1998) found tonically spiking neurons of what appeared to be the short dendrite class of HVC$_{RA}$ neurons. While they also found tonically spiking neurons that they believed were of the furry dendrite HVC$_{RA}$ class, they did not verify that any of these projected in the direction of RA. These latter neurons may actually be HVC$_X$ neurons (S. D. Shea, personal communication).

Roles of other neurons and synaptic connections in the HVC microcircuit

Mooney and Prather (2005) analyzed the synaptic connectivity among the three general classes of HVC neuron using paired intracellular recordings and antidromic stimulation in slices. We summarize their results, together with observations of Schmidt and Perkel (1998), Dutar et al. (1999), and Dutar et al. (2000), in Fig. A1.
FIG. A1. Synaptic connections between the 3 broad neuronal classes in HVC. The weight of each line connecting neurons reflects, approximately, the number of connected pairs observed by Mooney and Prather (2005). Due to sampling bias, this number does not necessarily reflect the true proportion of each connection type in HVC. Arrowheads represent depolarizing connections and dots represent hyperpolarizing (inhibitory) connections. The weight of both lines in the HVC_{RA} -> HVC_{I} -> HVC_{X} circuit reflects the number of inhibitory HVC_{RA} -> HVC_{X} pairs observed, and the weight of the HVC_{X} -> HVC_{I} line reflects the number of inhibitory HVC_{X} -> HVC_{X} pairs observed. In addition to the direct connections shown and the indirect connections mentioned above, the observations include a reciprocally connected HVC_{RA}-HVC_{I} pair in which the HVC_{RA} neuron excited the HVC_{I} neuron, a reciprocally connected HVC_{RA}-HVC_{I} pair in which the HVC_{RA} neuron inhibited the HVC_{I} neuron, and a HVC_{RA} -> HVC_{RA} inhibitory connection. mGluR and GABA_B refer to the slow inhibitory synapses onto HVC_{X} neurons investigated by Schmidt and Perkel (1998), Dutar et al. (1999), and Dutar et al. (2000). Slow inhibition was also evoked by Mooney and Prather (2005) by antidromic stimulation of HVC_{RA} fibers during picrotoxin application.
Figure A1 invites speculation as to the roles of synaptic connections we have not included in our current model. For example, the HVC_{RA} \rightarrow HVC_{I} \rightarrow HVC_{I} \rightarrow HVC_{RA} circuit could potentially disinhibit HVC_{RA} neurons just in front of the propagating sparse burst wave.

What is the role of HVC_{X} \rightarrow HVC_{RA} synapses, given the finding of Scharff et al. (2000) that targeted ablation of HVC_{X} neurons did not cause deterioration of song? It is possible that these synapses are only onto the phasic (furry dendrite) HVC_{RA} type, rather than the tonic (short dendrite) type that we assume is involved in sparse bursting, especially given that Mooney and Prather’s (2005) HVC_{RA} exemplar was of the phasic class. Alternatively, it is possible that they play a redundant role in premotor pattern generation, perhaps increasing the robustness of HVC_{RA} bursting.

What is the role of phasic HVC_{RA} neurons? It is likely that these neurons, like similar neurons in the cochlear nucleus, carry precise timing information (Manis and Marx 1991; Oertel 1983; Wu and Oertel 1994; Dutar et al. 1998). This timing information may be either auditory or premotor. Cholinergic agonists increase evoked spiking in phasic HVC_{RA} neurons and decrease evoked spiking in tonic HVC_{RA} neurons (Shea 2004). Given the evidence that acetylcholine modulates HVC towards an awake-like state (Shea and Margoliash 2003), this suggests the intriguing possibility that the two classes of HVC_{RA} neuron exchange roles in a waking vs. a sleeping and/or anesthetized state.
**Anatomical clusters in HVC**

Do our bistable clusters map onto the clusters that have been described anatomically (Burd and Nottebohm 1985; Kirn et al. 1999; Alexander et al. 2004)? The cluster type most commonly encountered by Alexander et al. (2004) contains either one HVC$_{RA}$ and one HVC$_{I}$ or one HVC$_{RA}$ and one HVC$_{X}$ neuron. Thus if our bistable clusters exist, they may consist of superclusters of such smaller clusters. However, the model does not fundamentally rely on a cluster organization.

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**Chapter 2**

**Extended summary of inputs and feedback pathways to HVC**

HVC receives inputs from: (1) the telencephalic song system nucleus NIf (interfacial nucleus of the nidopallium) through which it receives most or all of its auditory information (Cardin and Schmidt 2004; Coleman and Mooney 2004; Cardin et al. 2005); (2) the thalamic nucleus Uva (uvaeformis; Nottebohm et al. 1982; Bottjer et al. 1989; Fortune and Margoliash 1995; Foster and Bottjer 1998); (3) the anterior
forebrain nucleus MMAN (medial magnocellular nucleus of the anterior nidopallium; Foster et al. 1997; Vates et al. 1997; Foster and Bottjer 1998); (4) dorsal RA (dRA; a projection recently described by Roberts et al. 2008); (5) neuromodulatory areas of the basal forebrain (Li and Sakaguchi 1997; Shea and Margoliash 2003) and brainstem (Appeltants et al. 2000); and (6) possibly the primary auditory telencephalon, Field L (most likely indirectly via the HVC shelf; Fortune and Margoliash 1995; Vates et al. 1996) (Fig. 2.1A).

Of the known inputs to HVC, four (Uva, NIf, MMAN, and dRA) participate in anatomically defined feedback pathways, potentially conveying information from dRA and the brainstem to influence HVC’s premotor program. Uva receives input from PAm and DM (Reinke and Wild 1998; Striedter and Vu 1998). NIf receives input from Uva and thus superficially appears to be connected to the same feedback pathway (Nottebohm et al. 1982; Wild 1994). However, the NIf-projecting part of Uva does not appear to receive the projection from PAm (J. M. Wild, personal communication), and thus may be functionally distinct. MMAN receives input from dRA via the thalamic nucleus DMP (Foster et al. 1997, Vates et al. 1997).

Extended summary of birdsong sequence variability

Adult zebra finches that sustained partial Uva lesions as adults or juveniles (Williams and Vicario 1993) or Area X lesions as juveniles (Scharff and Nottebohm
1991) sing a more variable syllable sequence. This is also true of adult zebra finches that have received bilateral HVC microlesions (Thompson and Johnson 2006). Long-term perturbation of auditory feedback or deafening can also cause syllable sequence abnormalities (Leonardo and Konishi 1999; Brainard and Doupe 2001). In some of these cases, abnormal repetition of syllables (stuttering) is observed (Leonardo and Konishi 1999; Williams and Vicario 1993). Adult zebra finches that repeat a syllable at the end of a motif can be induced to stutter the syllable many times by Area X lesions (Kubikova et al. 2006). Variable syllable sequence is typical of juvenile zebra finches (Olveczky et al. 2005) and of normal adult birds in some other species, like Bengalese finches (Okanoya 2004; Sakata and Brainard 2006).

*Extended discussion of the nature and function of bilateral input to HVC*

A simple conceptual model that accounts for a number of experimental facts is one in which Uva sends synchronizing synaptic pulses to HVC neurons shortly before the onsets of syllables (Schmidt 2003; Coleman and Vu 2005). This synaptic input could be excitatory, inhibitory, or both. In this simple model, Uva does not need to communicate the specific identity of each syllable; its input to HVC is “syllable unspecific.” The Uva in each hemisphere conveys just the onset time of each syllable to the ipsilateral HVC, and each HVC determines which syllable is next in the sequence, independently of the other HVC.
This simple model is appropriate when there is a high degree of sequence stereotypy. However, the situation is not so straightforward when the sequence is variable (Scharff and Nottebohm 1991; Williams and Vicario 1993; Leonardo and Konishi 1999; Brainard and Doupe 2001; Okanoya 2004; Olveczky et al. 2005; Kubikova et al. 2006; Sakata and Brainard 2006; Thompson and Johnson 2006). If the sequence in each HVC is variable and independent of the other, then the two HVCs will sometimes have different sequences.

If the two HVCs are variable and independent in their sequences, then two possible effects on the syrinx are evident: (1) the two halves of the syrinx will produce muscle patterns corresponding to different syllables under the command of the two HVCs; or (2) the two HVCs will have different sequences, but the command from one HVC will predominate over the other in controlling the syrinx. In case 1, it is not clear how the two sides of the brainstem respiratory network would resolve the discrepancy between the commands from the HVCs (e.g. should there be a long or a short expiration if one HVC specifies a long syllable and the other a short one?). In alternative versions of this conceptual model, one HVC may be suppressed when the two HVCs are not in accord, or the bird may stutter a syllable, terminate the motif, or terminate the song bout when the two HVCs are not well synchronized. It is also possible that a combination of these effects occurs.
The stuttering observed by Leonardo and Konishi (1999), Williams and Vicario (1993) and Kubikova et al. (2006) may be the result of either the same mechanism as other types of sequence variability, or a different one. If the bird stutters syllable “B” a variable number of times before advancing to syllable “C,” then either: (1) both HVCs receive a syllable-specific signal that triggers either “B” or “C”; or (2) both HVCs receive a signal to stutter or advance that is not syllable specific (for example, a weak synaptic input from Uva to HVC may trigger a stutter whereas a strong input may cause HVC to advance to the next syllable); or (3) one hemisphere alone determines the syllable sequence while the other is suppressed.

Birdsong is lateralized in some species: denervating one side of the syrinx produces greater song deficits than denervating the other (Nottebohm 1971, Nottebohm and Nottebohm 1976; Nottebohm 1977). In other species, both sides of the syrinx participate in generating most syllables (Suthers 1990). In zebra finches, both right and left syringeal denervation and right and left HVC lesion degrade the song, but the effect is said to be greater for damage on the right side (Williams et al. 1992; Floody and Arnold 1997). Goller and Suthers (1995) found in brown thrashers that muscles on both sides of the syrinx are active during lateralized syllable production but airflow through one side is gated, a finding that may apply more generally. A model of input to HVC in songbirds must be flexible enough to explain the bilateral coordination of HVC commands whether syllables are produced by one side of the syrinx, by both sides in concert, or by switching between sides (Suthers 1990).
The model that emerges from the assumption that the two HVCs remain coordinated during variable, as well as normal, song sequences is one in which one of HVC’s afferent nuclei provides an input to HVC that specifies the identity of the next syllable to be sung (i.e. a syllable-specific input). This is because syllable-unspecific timing pulses given to two variable-sequence HVCs would often lead to activity patterns corresponding to different syllables in each HVC. Coordination does not necessarily imply that the two hemispheres produce the same commands; it only implies that they are in agreement about what syllable is being sung, no matter what their relative contributions to the sound produced. Given the assumption of interhemispheric coordination during variable sequences, the syllable-specific input to HVC must at least be present during variable sequences. Thus, at least two main versions of the syllable-specific model are possible. In the simplest version, the sequence in HVC is controlled by syllable-specific input no matter how stereotyped the syllable sequence is. This is the model that we have implemented computationally. In a more complex version, the sequence in HVC is influenced by syllable-specific input only when the sequence is variable, and otherwise the sequence is determined by connections intrinsic to HVC or unilateral inputs to HVC (and interhemispherically synchronized by syllable-unspecific timing pulses).

What characteristics would such a syllable-specific afferent nucleus or nuclei have? In the simpler model described above, in which the input to HVC is always
syllable specific, the nucleus must meet three criteria: (1) it must be necessary for normal song production in adult songbirds (complete bilateral lesions should eliminate normal syllable production), and (2) it must be capable of passing a bilaterally synchronized signal to HVC, which implies that it must participate in a pathway containing one or more bilateral projections. Of the known afferent nuclei to HVC, only Uva has been shown to meet these first two criteria fully. Only MMAN, Uva, and NIf participate in bilaterally connected pathways. As stated previously, NIf is not required for normal adult song production (Cardin et al. 2005). Bilateral MMAN lesions appear to affect adult song too slightly to be a candidate for this role (Foster and Bottjer 2001). By contrast, extensive bilateral Uva lesions result in severe impairment of adult song involving complete loss of normal syllables (but continued production of vocalizations resembling distorted introductory notes; Coleman and Vu 2005). Additionally, the nucleus should either: (3a) contain subpopulations of neurons that activate for only one syllable type during singing; or (3b) contain subpopulations of neurons whose summed activity during singing peaks for only one syllable type. This activity could be in the form of a brief pulse or a more prolonged input up to approximately the duration of the syllable. This syllable-specific pattern of activity has neither been demonstrated nor ruled out for Uva (or MMAN). It is consistent with the bursts of multiunit activity in Uva observed by Williams and Vicario (1993) during song. They showed, in a small number of birds, that bursts of multiunit Uva activity lead the onsets of calls and introductory notes and occur throughout the song, although
their analysis did not permit them to associate bursts with the onsets of individual song syllables.

In the more complex model described above, HVC input is syllable-unspecific when the sequence is stereotyped and syllable specific when it is variable. In this case, if the input to HVC is provided by a single nucleus, then in addition to 1 and 2, above, the nucleus should contain neurons that activate unspecifically for each syllable when the sequence is stereotyped, and should also contain neurons that activate specifically for each syllable when the sequence is variable. Alternatively, if the role were played by two nuclei, then one nucleus could contain a syllable-unspecific population, and the other (important only during variable sequence production) could contain a syllable-specific population. The syllable-unspecific nucleus would only be necessary for variable adult song production, not for stereotyped adult song production. For example, Uva could play the role of the syllable-unspecific nucleus, providing a synchronizing timing pulse near the onset of each syllable, and MMAN could play the role of the syllable-specific nucleus, directing the onset of specific syllables when the sequence is variable (e.g. in juveniles and decrystallized adults). Consistent with this latter idea, MMAN-lesioned juveniles never develop normal adult song (Foster and Bottjer 2001). This idea is related to the suggestion of Coleman and Vu (2005) that MMAN may be involved in recovery from unilateral Uva lesions. During stereotyped song, the sequence could be determined by connections intrinsic to HVC.
Rhythm generation by HVC in vitro

Solis and Perkel (2005) observed repetitive postsynaptic potentials in all three neuronal classes in HVC following high-frequency extracellular stimulation in slices. These PSPs in vitro had a frequency and number of repetitions similar to the frequency and number of repetitions of syllables during song. Although the results of Coleman and Vu (2005) suggest that Uva input is critical to HVC’s pattern generation, it is possible that endogenous rhythmic properties of the HVC network also contribute to its function.

Extended discussion of Uva as a center for polysensory and neuromodulatory influences on the song system

Uva receives input from the dorsal part of the superior reticular nucleus of the thalamus and the cholinergic medial habenular nucleus, which have been suggested to play a role in auditory gating (Akutagawa and Konishi 2005). Uva also receives a visual input from the optic tectum, a somatosensory input from the dorsal column nuclei, and an auditory input from the ventral nucleus of the lateral lemniscus; and it has corresponding visual, somatosensory, and auditory responses (Wild 1994; Coleman et al. 2007). Uva responds to the onsets and offsets of light pulses (Coleman et al. 2007). These observations point to functions for Uva beyond those we have described in our model. However, they are consistent with our hypothesis that one role
of Uva is to provide a syllable-specific input to HVC. Indeed, as stated in the Discussion of Chapter 2, this proposed role for Uva makes it an ideal site for rapid thalamic gating of the song on the basis of sensory input, arousal, and attention.

Hypothetical evolution of DM’s role in song

As stated in the Discussion of Chapter 2, DM is commonly believed to be involved in the production of calls rather than song (Simpson and Vicario 1990; Wild 1997). Long calls in males have learned features that depend on Uva, HVC and RA (Simpson and Vicario 1990; Coleman and Vu 2005) and may depend on the DM \( \rightarrow \) Uva \( \rightarrow \) HVC pathway. A role for DM in song would be a natural evolutionary outgrowth of its role in producing calls. It is possible that, in songbirds, evolution has augmented DM’s command circuitry for the production of calls by making use of forebrain circuitry that is well suited to learning. This idea is related to the suggestion that songs may have evolved from sequences of unlearned calls (Zann 1993). Given that it is a bilaterally connected structure and sends bilateral connections to Uva, DM is well situated to resolve mismatches between the two hemispheres.
“Syllabotopy” in the dRA → DM → Uva pathway

Our model does not assume that the syllable-specific neuronal populations in dRA, DM, or Uva are topographically mapped. However, given the topographic organization of other avian forebrain (Wild et al. 1993; Johnson et al. 1995; Luo et al. 2001), midbrain (Knudsen 2002), and thalamic nuclei (Johnson et al. 1995; Luo et al. 2001), it is at least conceivable that the nuclei in this pathway could contain topographic maps organized by syllable. It is known that vRA has a coarse topography in its projections to nXIIIts (Vicario 1991), and in adult birds, LMAN projects topographically onto RA (Johnson et al. 1995). The lack of topography in the projections to HVC (Fortune and Margoliash 1995; Foster and Bottjer 1998) does not rule out this organization for the dRA → DM → Uva pathway.

Modeling sequence variability

As discussed above, our main reason for assuming that the input to HVC is syllable-specific is the need for interhemispheric coordination during variable sequences. Given that the anterior forebrain pathway (AFP) is implicated in a number of cases of sequence variability (Scharff and Nottebohm 1991; Brainard and Doupe 2001; Olveczky et al. 2005; Thompson and Johnson 2006), and the anterior forebrain nucleus LMAN shows either variable or relatively stereotyped spike patterns in an age
and behavioral context-dependent manner (Hessler and Doupe 1999a,b; Kao et al. 2005; Olveczky et al. 2005), a reasonable approach to modeling sequence variability is to assume that it is the result of variable activity in the AFP. If, as we have assumed, the sequence of syllable representations in HVC is determined (or influenced) by the HVC $\rightarrow$ dRA $\rightarrow$ DM $\rightarrow$ Uva $\rightarrow$ HVC feedback pathway, then the lateral LMAN $\rightarrow$ dRA projection (Johnson et al. 1995) is a candidate for influencing the sequence. Indeed, given the role of the AFP in song plasticity (Bottjer et al. 1984; Sohrabji et al. 1990; Scharff and Nottebohm 1991; Brainard and Doupe 2000), this projection could potentially promote the formation of a new HVC $\rightarrow$ dRA connectivity pattern defining a new syllable sequence.

Alternatively, the MMAN $\rightarrow$ HVC connection potentially provides a more direct influence on sequence. Given the similarities between MMAN and LMAN, both in terms of location and in terms of the thalamocortical pathways in which they participate (RA $\rightarrow$ DMP $\rightarrow$ MMAN $\rightarrow$ HVC vs. RA $\rightarrow$ DLM $\rightarrow$ LMAN $\rightarrow$ RA; Fig. 2.1A; Wild 1993, Foster et al. 1997, Vates et al. 1997; Okuhata and Saito 1987; Bottjer et al. 1989; Williams 1989; Foster and Bottjer 2001), it would be interesting to know if MMAN also participates in a basal ganglia pathway. It would also be interesting to know if it shows variable spike patterns under the same conditions that cause sequence variability.
The relationship between premotor, sleep and BOS-evoked activity in HVC

We are developing a model of sparse bursting during sleep and BOS playback, part of which has been supported by recent experimental work (Hahnloser and Fee 2007). It is clear that in the long run, any model that purports to explain sparse premotor bursting in HVC should also be able to account for sparse bursting during these other states, and we will describe a computational implementation of this model in a future report.

HVC\textsubscript{RA}, HVC\textsubscript{X} and HVC\textsubscript{I} show premotor-like activity during experimental playback of recordings of the bird’s own song (BOS) to anesthetized zebra finches: both projection neuron classes burst sparsely and HVC\textsubscript{I} neurons exhibit more sustained spiking activity (Mooney 2000). This BOS-responsive activity requires an intact NIf, as demonstrated by the lesion experiments of Cardin et al. (2005) and inactivation experiments of Coleman and Mooney (2004). This is in contrast to song production, which does not require an intact NIf (Cardin et al. 2005). The timing of the bursts in HVC\textsubscript{RA} neurons relative to each other has not been demonstrated to be the same during BOS playback as during singing. However, the burst sequences in RA neurons during sleep, both in response to BOS playback and in spontaneous episodes, are very similar to those in the same neurons during singing (Dave and Margoliash 2000). Given the evidence that a large fraction of RA bursts are driven directly by HVC\textsubscript{RA} bursts (Hahnloser et al. 2002; Fee et al. 2004; Hahnloser et al. 2006), it is
likely that the timing relationships between \( \text{HVC}_{RA} \) bursts are also similar across states.

Why would it be useful for auditory activity to drive HVC in a premotor-like pattern in anesthetized or sleeping birds? Because birds normally never hear their own song while sleeping, BOS-playback activity is likely to be an epiphenomenon of a normal function of the song system.

What is the mechanism by which sleep replay sequences are generated in RA and HVC? An obvious hypothesis is that these sequences are the result of exactly the same mechanism as the generation of premotor sequences in the singing bird. However, given that BOS playback also evokes premotor-like activity in the song system, an equally valid hypothesis is that sleep replay activity is driven by spontaneous auditory-like activity in auditory areas. Since one of these mechanisms depends on NIf and the other does not, they are not identical.

Based on the similarities between spontaneous replay during sleep and responses to BOS, we propose the following set of hypotheses: (1) Premotor-like activity in HVC and other song system nuclei during sleep replay episodes is driven by replay of auditory-like activity in NIf or an upstream nucleus. (2) Synaptic plasticity between “auditory” neurons (e.g. NIf) and concurrently active “premotor” neurons (e.g. HVC) during song production generates a mapping between auditory and
premotor representations that enables the former to drive the latter during sleep. Given that BOS responses in HVC stabilize in adulthood (Nick and Konishi 2005), this plasticity is likely to occur during song development. (3) During BOS playback, the replay of the premotor pattern is driven by a real auditory input, rather than by the replay of an auditory memory; the rest of the mechanism is the same. These hypotheses are a focus of our current work.

According to these hypotheses, replay of premotor-like activity during sleep occurs, not as a simple reactivation of premotor mechanisms like the ones we have proposed in the present work, but rather as the result of the replay of an auditory memory in NIf or an upstream nucleus. This may be a mechanism for replaying the combination of auditory and premotor activity, in an appropriate temporal relationship, during a plastic sleep state. This could form the basis of a mechanism of song plasticity during sleep (Deregnaucourt et al. 2005; Margoliash 2005), which would very likely also involve the anterior forebrain pathway. The premotor-like response to BOS playback is a natural outcome of such a model.

The proposed auditory-replay nucleus may be identified by the following two characteristics: (1) Its spontaneous sleep activity matches its auditory activity during song production; and (2) It lacks an afferent nucleus exhibiting characteristic 1. If NIf activity during singing is auditory, then it may be the auditory-replay nucleus. In this case, synaptic plasticity in the NIf → HVC synapses may generate the sensorimotor
mapping. If NIf activity during singing is premotor (McCasland 1987; Hahnloser and Fee 2003), then the auditory-replay nucleus may be upstream of NIf in the auditory pathway (e.g. CLM).

In support of these ideas, Hahnloser and Fee (2007) recently found that NIf is necessary for spontaneous sleep activity in HVC.

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