How do songbirds sing birdsongs?

Liora Las

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Outline:

1) Introduction to songbirds as a model.
2) Neuronal circuits underlying mature song production (motor system).
3) Neuronal circuits underlying early stages of singing (learning system).
Is there an innate natural language?

In the 13th century the Holy Roman Emperor *Frederick II* carried out a deprivation language experiment on young infants raised without human interaction in an attempt to determine if there was an innate natural language that they might demonstrate once their voices matured.

It is claimed he was seeking to discover what language would have been imparted unto Adam and Eve by God.

https://www.historyanswers.co.uk/kings-queens/crusader-queen-sibylla-of-jerusalem-sacrificed-the-holy-city-for-love/
Vocal learning

To get the brain mechanisms that underline vocal learning it will be very useful to have an animal model system.

Vocal learning is not very common in the animal kingdom: Lots of animals are able to vocalize but they are not necessarily have to hear other or themselves in order to do so.

**Mammals:**
- Humans
- Other primates???
- Dolphins/ Whales
- Sea lions/ Seals
- Elephants
- Bats

**Birds:**
- Parrots
- Hummingbirds
- **Songbirds**
  Over 5000 different species of songbirds

A Modular Approach to Vocal Learning: Disentangling the Diversity of a Complex Behavioral Trait
What do songbirds and humans have in common?

Both humans and songbirds learn their motor behavior (e.g. vocal) early in life.
Both learn to communicate by listening to their parents.
They must be able to hear their own vocalization in order to learn to sing/speak.
Both humans and songbirds have evolved a complex hierarchy of specialized brain areas essential for vocal control.
Birds sing for two main reasons:

1. To attract a mate.
2. To establish and hold a territory.

Singing is mostly a male activity.

*Male’s* brains are specialized in *singing*, *female’s* brains are specialized in *evaluating* the song.
There are more than 5000 species of songbirds

Zebra finch - the lab “rat” of the vocal learning field

1) Small and cheep
2) We have lots of knowledge
3) Relatively short time-window of learning
4) Simple song that is super stereotype!
Songbirds sing! Adult song is highly stereotyped

Zebra finch

There are two modes of singing:
1) Directed singing
2) Undirected singing

Ofer Tchernichovski’s Lab (CUNY)
Songbirds learn to sing by imitating their tutor

- The song is learned through **trail-and-error process**
- If you deafen a bird, the song does not evolve properly → the importance of auditory feedback
Reinforcement learning model for song acquisition

Song evaluation

Auditory feedback

Auditory Memory

Reinforcement signal
simple evaluation signal: good/bad

Song motor system

Exploratory variability

By trial and error learning the bird can update his song to get a better match to his template.

Konishi 1965; Marler 1970
The brain areas that are devoted to the Song system

Many brain nuclei in avian telencephalon are derived from the pallial layer of embryos, which also gives rise to mammalian cortex.

The motor pathway - if you lesion any of these song nuclei you disrupt the output of the song

The learning pathway is not necessary for adult song production, but is required for learning  (Bottjer, 1984, Scharff and Nottebohm, 1991, Goldberg @ Fee , 2010)
No laminar structure found in avian brain
Instead there are Nuclei
Motor pathway

- **HVC** (*high vocal center*, originally was named *hyperstriatum ventrale, pars caudalis (HVc)*)
- **RA** primary motor “cortex”
- Brainstem motor areas
  - Muscles of the syrinx
  - Respiratory muscles

The brain areas that are involved in song production have being outline mostly by *Frenando Nottebohm’s* lab

Nottebohm et al., *(J Comp Neurol, 1976)*
• Question: How do these circuits work to produce a song?

**Record** from brain areas and see what are the firing patterns of these neurons during singing.
Multi unit recording in HVC showed increase activity during singing.

Recording from HVC showed a massive neuronal signal that was throughout the song - that was not well correlated with the song pattern.
Neuroanatomy suggested that there are three types of neurons → may be there are also 3 distinct neuronal responses???

3 types of neurons in HVC –
1) HVC-RA projecting neurons
2) HVC-X projecting neurons
3) interneurons
How to identify neurons you are recording from?

- Antidromic electrical stimulation

Hahnloser et al. (Nature, 2002)
Using antidromic stimulation to identify cell types within HVC

- Antidromic stimulation induce current in the axon terminals around the area of the stimulation-
  this results in AP that is generate and propagate on the axon backwards towards the soma.
How do we differentiate between these two options?
Collision test

refactory period

Recording electrode

Stimulating electrode

HVC

Evoked spike

Spontaneous spike

Stimulus artefact

Stimulus artefact
How to identify neurons you are recording from?

- Antidromic stimulation (electrical or optogenetic)

- Labeling (genetically or injections)
Using antidromic stimulation to identify cell types within HVC

Hahnloser et al. (Nature, 2002)
Activity of HVC-RA neurons during singing

Hahnloser et al. (Nature, 2002)
This suggest a *sparse* sequence of activity that propagates through the song.

Hahnloser et al. (Nature, 2002)
HVC bursting produces continuous coverage of time in the song

The population of HVC projection neurons is *continuously active* during singing.

These extremely sparse and precise patterns of activity suggest the entire ensemble of HVC\textsubscript{RA} neurons could function to specify the timing of syllables, notes, and even the intervening silent “gaps.”
The sparse activity of HVC neurons and its location makes it perfect for two-photon calcium imaging.
The song

Recordings from RA neuron during the song
RA activity during singing

HVC-RA neurons

Leonardo & Fee (J Neurosci, 2005)
Simple sequence generation circuit

Bursting activity propagates through a chain of synaptically connected $HVC_{RA}$ neurons (like falling dominoes), creating a timing signal that spans the entire motif.

Leonardo and Fee, 2005
The output of HVC cell goes to more than one RA neuron $\rightarrow$ each RA neuron will be activated more than just once in a motif. The summation of RA neurons converge onto to the mn in the brainstem.
Part 1: Summary

• HVC exhibits sparse bursts during singing.
• RA transforms the sparse code into multiple bursts which then drive motoneurons.
• BUT: Where are these patterns of activity coming from? What is driving HVC to fire at a particular moment?
What and where is the mechanism that determines tempo? Are the slow dynamics generated within HVC?

If song tempo is determined by the activity of the HVC local network, then song should slow as HVC is cooled.

Theoretical predictions

Fee & Long (Curr Opin Neurobiol, 2011)
Local manipulation of brain temperature

Long & Fee (Nature, 2008)
Cooling of HVC causes slowing of song...

3% per 1 degree

Long & Fee (*Nature*, 2008)
Stretching of song is highly uniform

Control: $T=40 \, ^\circ C$

Cooled: $T=30 \, ^\circ C$

Control: image stretched
Cooling of HVC: Stretching of song is highly uniform! Song tempo slowed similarly across all timescales:  
1) individual notes (~10 ms), 
2) entire motif (~1s) 
3) the silent gaps

Long & Fee (*Nature*, 2008)
Are the **short dynamics (within one syllable)** generated within HVC? **YES!!!**

Fee & Long (*Curr Opin Neurobiol*, 2011)
Where are the long timescale (switching between syllables) dynamics?

(b) Dynamics within HVC

Syllable modules in HVC

Fee & Long (Curr Opin Neurobiol, 2011)
Maybe it's within HVC?
one chain model for all song dynamics

A continuous-time model

Li and Greenside, 2006
Jin et al, 2007
Long, Jin, Fee, 2010
OR: The chain might have links outside of HVC

Hamaguchi, Tanaka, Mooney, 2016
Cooling RA has no effect on song timing at any timescale, including song speed or the structure of the notes. Song timing is not controlled by dynamics within RA (from bilateral cooling in RA).

In contrast to the huge effect cooling in HVC.

Long & Fee (Nature, 2008)
Multiunit activity in Uva shows syllable-onset-related bursts

A

Smoothed, rectified neural activity

Danish, Aronov, Fee, 2017
Uva likely triggers short chains in HVC

Modified from Fee MCN2017 lecture
Part 1: Summary – A simple model of vocal sequence generation in adult birds

**HVC:**

- HVC exhibits sparse bursts during singing.
- Song timing is controlled within HVC.
- HVC contains multiple syllable-modules that can be activated by midbrain/thalamic circuitry.

**RA:**

- RA transforms the sparse code into multiple bursts which then drive motoneurons.
- The configuration of the vocal organ (muscle activity) is determined by the convergent input from RA neurons on short time scale (~10 to 20 ms).
15 min break
RA also gets input from LMAN!

What is the role of the learning pathway?

The motor pathway - if you lesion any of these song nuclei you disrupt the output of the song

The learning pathway is not necessary for adult song production, but is required for learning  (Bottjer, 1984, Scharff and Nottebohm, 1991, Goldberg @ Fee , 2010)
Subsong ("babbling") – i.e., the highly variable song in very young juveniles
• Question:
What are the mechanisms that produce subsong ("babbling") – i.e., the highly variable song in very young juveniles?

Does the motor pathway activity during subsong is similar to the activity during adulthood?
Do we need HVC for producing subsong?
HVC-lesioned birds could still produce subsong!

Control

Subsong stage (37 dph)

Plastic song stage (50 dph)

Adult

no HVC

Subsong does not require HVC

Aronov et.al. (2008) Science
What drives *subsong* production?

Babbling requires LMAN

- **Control**: N=57/57
- **no HVC**: N=26/27
- **no RA**: N=0/5
- **no HVC no LMAN**: N=0/12
- **no LMAN (38-44 dph)**: N=0/6

*plastic song*  
*crystallized song*

**Age (days)**

- Hatch
- 20d
- 40d
- 60d
- 80d
- 100d

**Frequency of vocalizations (sec/day)**

- N=57/57
- N=26/27
- N=0/5
- N=0/12
- N=0/6

**Muscles**

- HVC
- RA
- LMAN
- nXIIIts

**What drives *subsong* production?**
LMAN drives subsong

LMAN-RA projecting neurons exhibit activity primarily prior to subsong syllable onsets

Aronov et al. (2008) Science

this suggest that babbling is not a result of immature motor pathway but it is actually deriving by this learning circuit
LMAN drives subsong
LMAN neurons exhibit activity also prior to subsong syllable offsets

Aronov et al. (2008) Science
• AFP (anterior forebrain pathway) is necessary for producing subsong, suggesting that this circuit is important for vocal variability.

• Which part of AFP is necessary for producing babbling?

The basal ganglia (area X) are not necessary for subsong or vocal variability. DLM is necessary for the production of subsong. LMAN → RA pathway cannot generate subsong like vocalizations independent of DLM.

motor thalamus plays a key role in the expression of exploratory juvenile behaviors during learning.
• **Question:**
  What is the role of LMAN in older juveniles?
What drives plastic song production?

Aronov et al. (2008) Science

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Frequency of vocalizations (sec/day)</th>
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<tbody>
<tr>
<td>Hatch</td>
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</tr>
<tr>
<td>100d</td>
<td>no LMAN noLMAN (45-67 dph) 6/7</td>
</tr>
</tbody>
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Subsong plastic crystallized song

N=57/57

Muscles

HVC

RA

LMAN

nXIIIts

Sensory only

Sensory-motor
What drives plastic song production?

Subsong/plastic song requires LMAN

Babbling requires LMAN → LMAN give rise to a high variability sounds, whereas HVC is slowly taking over to give structure into the song
Role of LMAN in older juveniles

Role of LMAN in older juveniles

LMAN is a generator of variability

Separate premotor pathways for stereotyped song and babbling

Sequence
Stereotypy
Precision

Randomness
Variability
Exploration

Uva
HVC

DLM
LMAN

Adult song
Subsong

Motor Output
Reduced variability in pitch after LMAN inactivation during crystalized song

LMAN involves in injecting stochastic noise into the naive behavior so to have more variation on which to select the better performance.

LMAN activity in older juveniles

The crystallized songs of male zebra finches display different amounts of acoustic variability depending on social context. Directed singing may serve an essential role in song learning by driving variability in all stages: in subsong, plastic song, and even in adult song.

Kao et al. (Journal of Neuroscience, 2008)
Role of LMAN in learning

• Question
  – Is variability purely random or is it biased?

• Difficulty
  – Song learning is a slow process.

• Strategy
  – Use real-time feedback to induce error in the song artificially (using young adult- (1) sing a lot, (2) still show high variability)

The AFP does more than generate variability!
Conditional auditory feedback

Tumer & Brainard (Nature, 2007)
Andalman & Fee (PNAS, 2009)
This means that the noise injected by the AFP into RA has to be biased $\rightarrow$ it doesn’t change the signal around an average but pushes the pitch away from the noise in a more directional way.

Andalman & Fee (PNAS, 2009)
Two possibilities

Where does the learning take place?

• **Hypothesis 1:** motor pathway
  → LMAN inactivation will not change the pitch.

• **Hypothesis 2:** learning pathway
  → LMAN inactivation will change the pitch to go back.

![Diagram showing the motor pathway from RA to HVC, and the learning pathway from HVC to LMAN.](Image)
Motor pathway consolidation

This variability produced by the learning pathway is not purely random, but instead biased. This bias is consolidated in the motor pathway after one day delay.

Learning pathway

Andalman & Fee (PNAS, 2009)
LMAN is the essential premotor nucleus for the earliest babbling vocalizations (Aronov and Fee, 2008).

LMAN may serve an essential role in song learning by driving variability in all stages: in subsong, plastic song, and even in adult song (Kao et al, 2005).

LMAN adds variability to enable exploration. This variability produced by the learning pathway is not purely random, but instead biased.

This bias is consolidated in the motor pathway after one day delay.
Separate premotor pathways for stereotyped song and babbling

Sequence
Stereotypy
Precision

Adult song

Randomness
Variability
Exploration

Babbling

Motor Output
Innate versus learned

• Is there any component in the vocal learning that is innate?

https://www.youtube.com/watch?v=fO1M1_xOSro

A comic relief
Chomsky’s linguistic theory
Noam Chomsky proposed in the 60s that the principles underlying the *structure of language* (syntax) are biologically determined and hence *genetically transmitted*. He argued that all humans share the same underlying linguistic structure, irrespective of socio-cultural difference. *Universal grammar constraints syntactic diversity in humans* → the structure of language is common among different socio-cultural groups.

In other words: genes constrain language diversity.

Ofer Tchernichovski:
Do genes constrain song diversity?

The City University of New York
Song culture in birds

• There are more than 5000 species of songbirds

• Some songbirds provide biologically tractable models of culture: geographically separated groups have *local song dialects*- just like humans.

• But *the variety is not infinite*: different species exhibit distinct song cultures, suggesting genetic constraints.
What happens when you isolate a bird from his father before the sensory period?
Can we rise a colony from isolates?

The experiment: to determine whether normal wild-type song culture might emerge over multiple generations in an isolated colony founded by isolates.

Konishi 1965; Marler 1970
Fehe´r et al. Nature 2009
The lab of Ofer Tchernichovski
Culture in the lab: development of song culture in the zebra finch

Fehe´r et al. Nature 2009
The lab of Ofer Tchernichovski
Song evolved towards the wild-type in three to four generations. Thus, species-typical song culture can appear *de novo*.

Fehe´r et al. Nature 2009
The lab of Ofer Tchernichovski
Juvenile zebra finches fostered by Bengalese finch learned Bengalese finch syllable morphology but not temporal gap timing.

Temporal gap coding is innate, whereas syllable coding is experience dependent.

Mind the gap: Neural coding of species identity in birdsong prosody
Makoto Araki, M. M. Bandi, Yoko Yazaki-Sugiyama
Cross fostered zebra finches have similar temporal structure to zebra finch song, but the spectral was of Bengalese.

Mind the gap: Neural coding of species identity in birdsong prosody
Makoto Araki, M. M. Bandi, Yoko Yazaki-Sugiyama
Summary

• Activity of the motor pathway is stereotyped.
• Activity of the learning pathway is variable.
• These two signals are combined at RA.
• Temporal gap coding is innate, whereas syllable coding is experience dependent.