How songbirds sing birdsongs?

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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).
The brain is a learning machine

• Many motor and cognitive skills are learned through trial-and-error process -> i.e. playing the piano or playing tennis.
• **Human speech learning** is a sensorimotor skill that needs auditory feedback; we have brain areas that are devoted for these tasks.
Vocal learning

Mammals:
- Humans
- Bats
- Dolphins/Whales
- Sea lions/Seals
- Elephants

Birds:
- Parrots
- Hummingbirds
- Songbirds

Over 5000 different species of songbirds
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.
Songbirds sing! Adult song is highly stereotyped

Zebra finch

There are two modes of singing:
1) Directed singing
2) Undirected singing
Songbirds learn to sing by imitating their tutor

if you deafen a bird, the song does not evolve properly→ the importance of auditory feedback
Reinforcement learning model for song acquisition

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

Konishi 1965; Marler 1970
Song system

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

*motor pathway*- if u lesion any of these song nuclei you disrupt the output of the song
• **HVC** (high vocal center)

• **RA** primary motor “cortex”

• brainstem motor areas
  – Muscles of the syrinx
  – Respiratory muscles

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 stimulation (electrical or optogentic)

  Hahnloser et al. (Nature, 2002)

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

→ the 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

Recording electrode

Stimulating electrode

Refectory period

HVC

Evoked spike

Spontaneous spike

Stimulus artefact
Collision test using optogenetic setup
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.
Picardo et al. use two-photon imaging and intracellular electrophysiology in the singing zebra finch to demonstrate that premotor cortical activity does not reflect ongoing song-related kinematics but, instead, appears to form an abstract population sequence during song performance.
HVC bursting produces continuous coverage of time in the song

- The population of HVC projection neurons is continuously active during singing
- Projection neurons and interneurons are rhythmically modulated by the song

These extremely sparse and precise patterns of activity suggest the entire ensemble of HVC
RA neurons could function to specify the timing of syllables, notes, and even the intervening silent “gaps.”
HVC bursting produces continuous coverage of time in the song.
The song

Recordings from RA neuron during the song
RA activity during singing

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
Sparse representation of time

Leonardo and Fee, 2005
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
Local manipulation of brain temperature

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

3% per 1 degree
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 generated within HVC? YES!!!
A chain model of song dynamics

A continuous-time model

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

Taken from Fee MCN2017 lecture
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).

Long & Fee (Nature, 2008)
Where are the (long timescale) dynamics? 

*Uva* may triggers short chains in HVC

Syllable modules in HVC
Multiunit activity in Uva shows syllable-onset-related bursts
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).
RA also gets input from LMAN!

What is the role of the learning pathway?
Subsong ("babbling") – i.e., the highly variable song in very young juveniles

- Subsong
- Plastic Song
- Crystallized
- Tutor Song

Song Variability

Similarity to Tutor

Age (days)

40d
60d
90d
• Question:
What are the mechanisms that produce subsong ("babbling") – i.e., the highly variable song in very young juveniles?
HVC-lesioned birds could still produce subsong!

Subsong does not require HVC

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

Babbling requires LMAN

![Diagram showing brain regions and vocalization frequencies](image)

- **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

**Bar Graphs**

- **Frequency of vocalizations (sec/day)**
  - **Control**: 1500
  - **no HVC**: 1000
  - **no RA**: 0
  - **no HVC no LMAN**: 0
  - **no LMAN (38-44 dph)**: 0
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 neurons exhibit activity also prior to subsong syllable offsets

Instantaneous firing rate (Hz)

Sound amplitude

200 ms

LMAN neurons exhibit activity also prior to subsong syllable offsets

Aronov et.al. (2008) Science
What drives plastic song production?

Aronov et al. (2008) Science

![Diagram showing brain regions and vocalization frequency](image.png)

- **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
- **no LMAN (45-67 dph)**: N=6/7
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
• 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.
Separate premotor pathways for stereotyped song and babbling

Sequence
Stereotypy
Precision

Uva

HVC

DLM

LMAN

RA

Adult song

Subsong

Randomness
Variability
Exploration

Motor Output
• **Question:**

What is the role of LMAN in older juveniles?

The AFP does more than generate variability!
Role of LMAN in older juveniles

Role of LMAN in older juveniles

LMAN is a generator of variability

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

Undirected singing rehearsal?

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.
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 → it doesn’t change the signal around an average but pushes the pitch away from the noise in more directional.

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.
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.

Andalman & Fee (PNAS, 2009)
Additional CAF manipulation

The Basal Ganglia Is Necessary for Learning Spectral, but Not Temporal features of Birdsong

Ali, Otchy, Pehlevan, Fantana, Burak and O"lveczky, Neuron 2013
LMAN is a generator of variability

- 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 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
- Motor Output

- Randomness
- Variability
- Exploration

- Babbling

- Uva
- HVC
- DLM
- LMAN
- RA

Motor Output
Summary

• Activity of the motor pathway is stereotyped.
• Activity of the learning pathway is variable.
• These two signals are combined at RA.
How does HVC take over?

• Adult song →

  HVC exhibits sparse bursts during singing.

How do the firing patterns in HVC change with development?
In subsong, HVC neurons bursts during singing, some locked to syllable onsets. However, more than 90% of projection neurons did not burst rhythmically. Bursting activity in HVC is sparse from the beginning.

Protosyllables are the earliest temporally structured song elements

42 dph

48 dph

52 dph

92 dph
Prototype syllable, or protosyllable—an identifiable rhythmic precursor to the fully formed syllable
Rhythmic sequences in HVC during the protosyllable stage

Most HVC premotor neurons show rhythmic bursts.

Gray boxes: Protosyllable

Early on during this stage the firing sequence spanned only the beginning of the protosyllable …
Example of rhythmic activity during protosyllables eventually grew to encompass the entire protosyllable.
How does the neural circuit manage to move from a single syllable into producing multiple distinct syllables in a complex motif?

Duplication! The original neural sequence used for the first protosyllable is repurposed (or duplicated) to produce a new protosyllable.
Shared neuron active during both β and γ

Neural splitting With development the number of shared neurons decreased, and the neurons produced syllable-specific activity.
Innate versus learned

• Is there any component in the vocal learning that is innate?
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?
Isolated birds are establishing a ‘song template’ very early, during the sensory period.

The lab of Ofer Tchernichovski

Konishi 1965; Marler 1970
Fehe´r et al. Nature 2009

The experiment: to determine whether normal wild-type song culture might emerge over multiple generations in an isolated colony founded by isolates.
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.
Cross fostered zebra finches has similar temporal structure to zebra finch song but the spectral was of Bengalese