How do songbirds sing birdsongs?

Michale Fee (MIT)

Liora Las

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).

Dr. Yarden Cohen

Next week

Thursday 16/5

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 into Adam and Eve by God.

Medieval monarch **Frederick II** (Holy Roman Emperor, 1194-1250 AD), tried a similar experiment, with disastrous results.

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 (marmosets)??
- 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
Neuron Perspective, 2019 https://doi.org/10.1016/j.neuron.2019.09.036

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.**

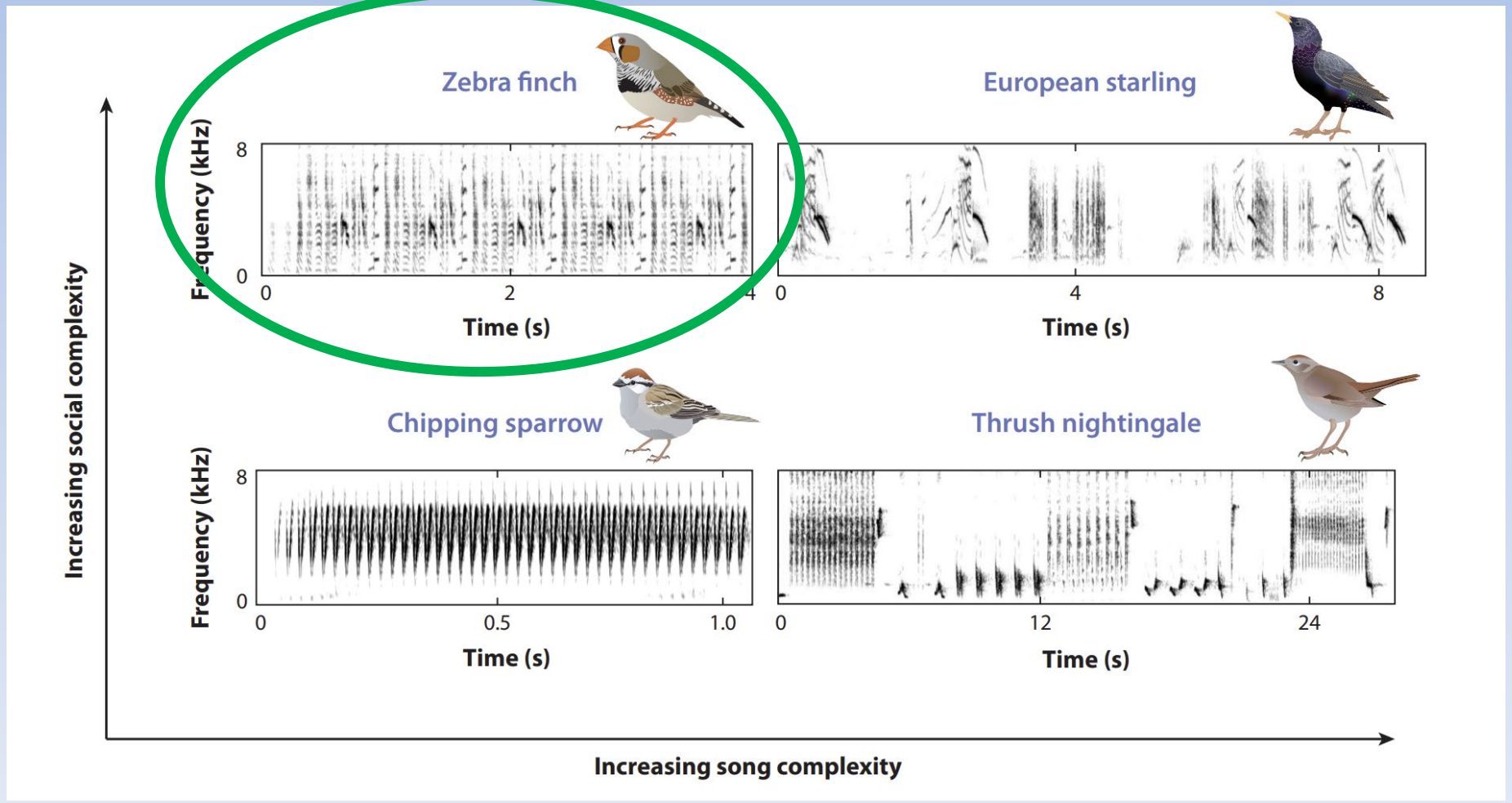
Singing is mostly a male activity.

Male's brains are specialized in ***singing***, ***female's*** brains are specialized in ***evaluating*** the song

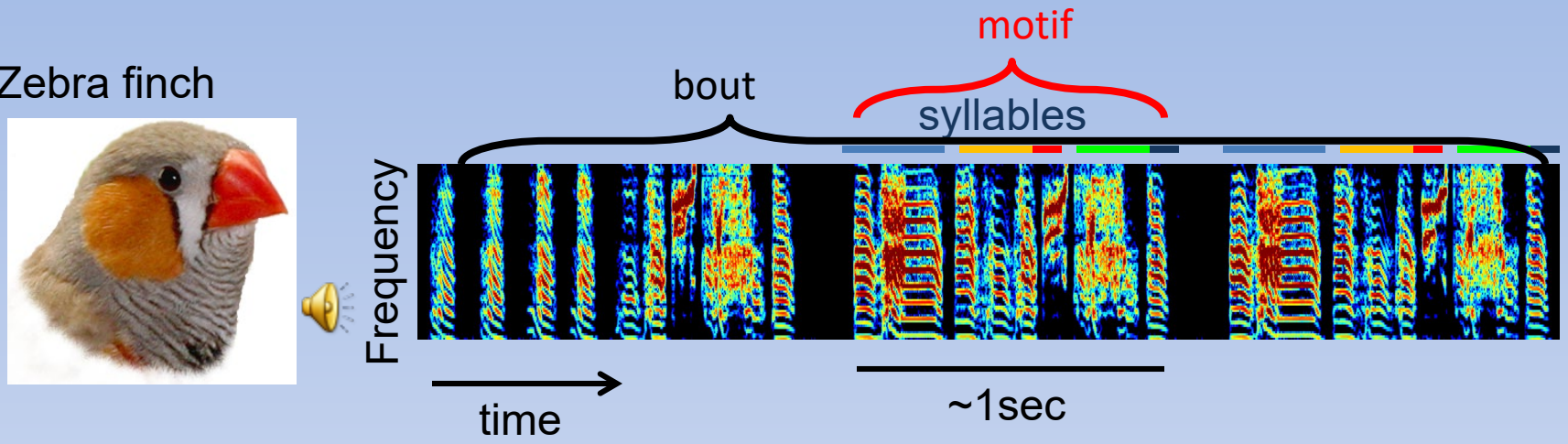
There are more than 5000 species of songbirds

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!

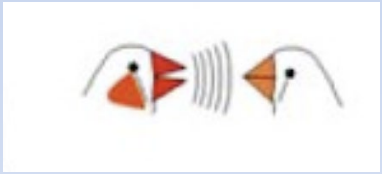


Songbirds sing! Adult song is highly stereotyped



There are two modes of singing:

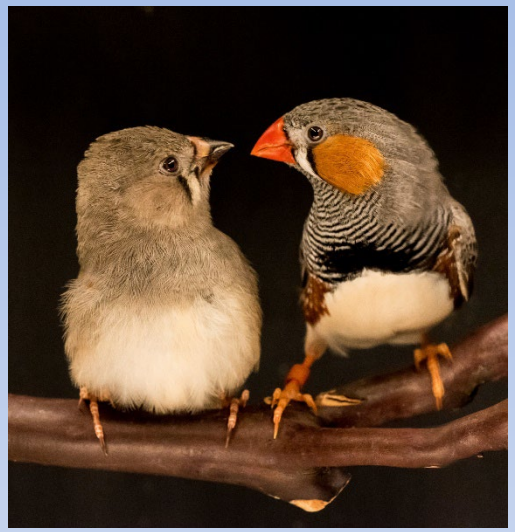
1) Directed singing

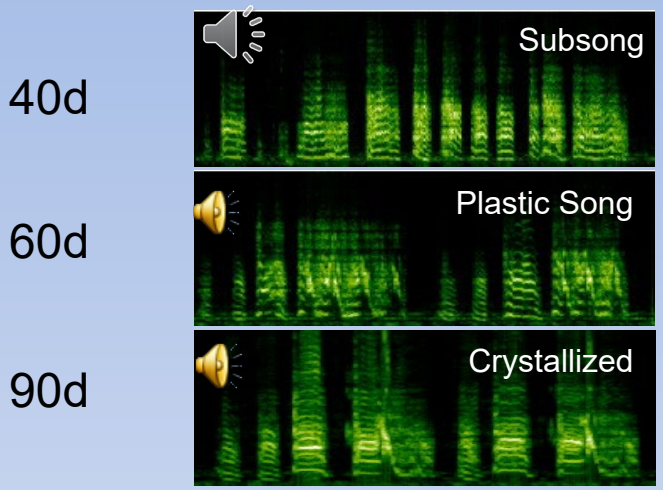


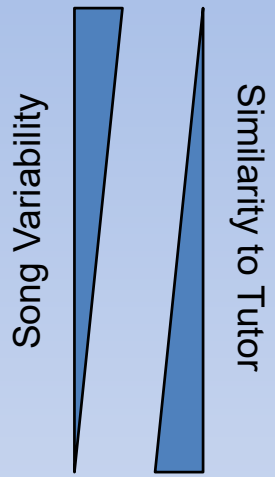
2) Undirected singing

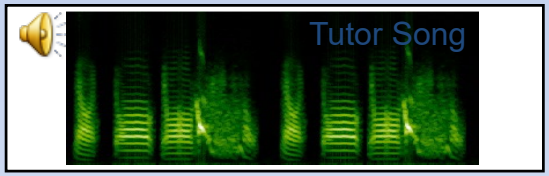
Ofer Tchernichovski's Lab (CUNY)

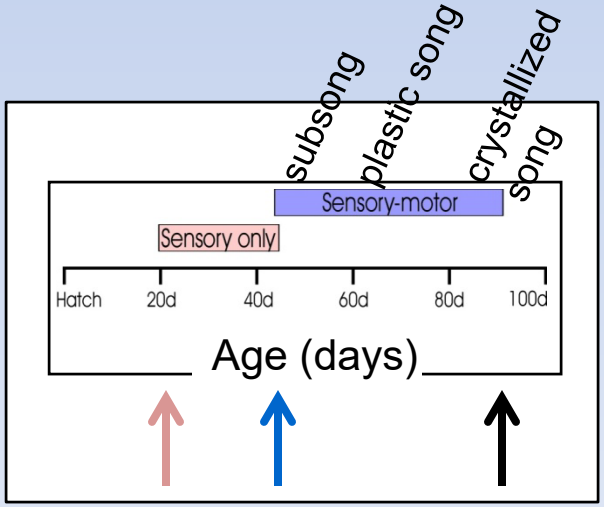
Songbirds learn to sing by imitating their tutor





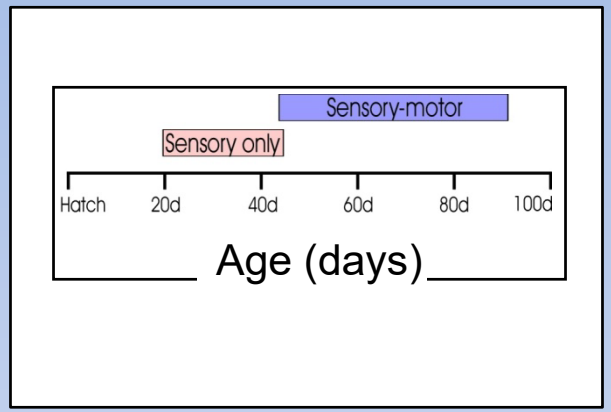






- The song is learned through ***trail-and-error process***
- If you deafen a bird, the song does not evolve properly→ the importance of auditory feedback

Reinforcement learning model for song acquisition

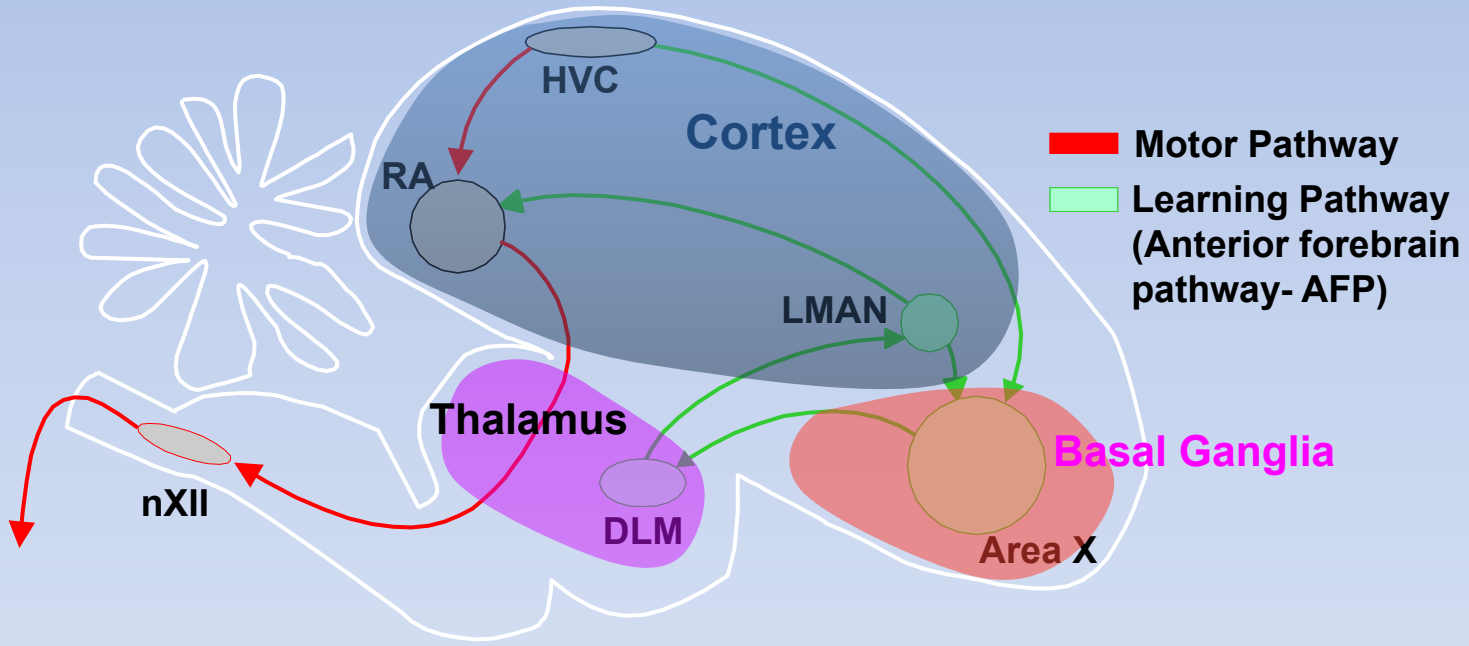


Konishi 1965; Marler 1970

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

The brain areas that are devoted to the Song system

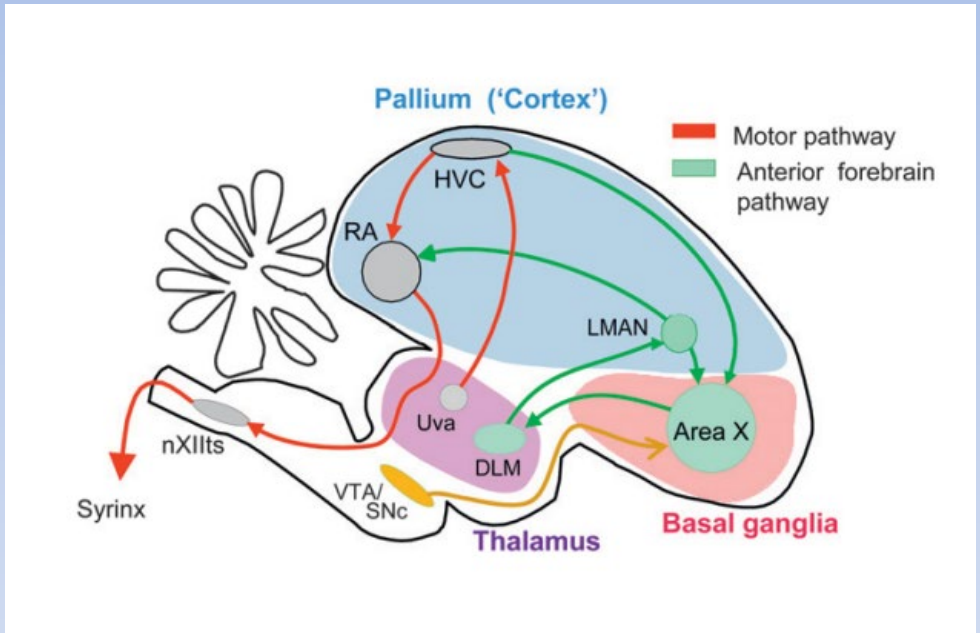
Many brain nuclei in avian telencephalon are derived from the pallial layer of embryos, which also gives rise to mammalian cortex.

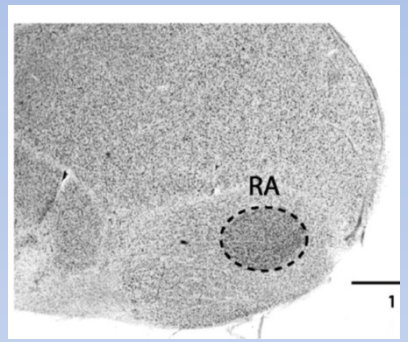


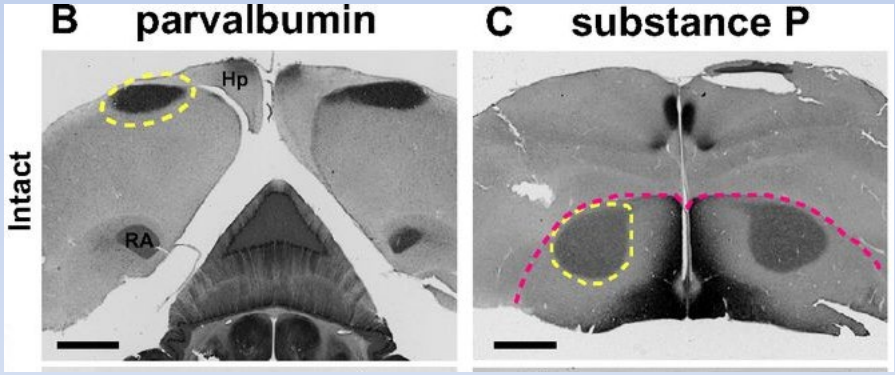
The motor pathway- if you lesion any of these song nuclei you disrupt the output of the song

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





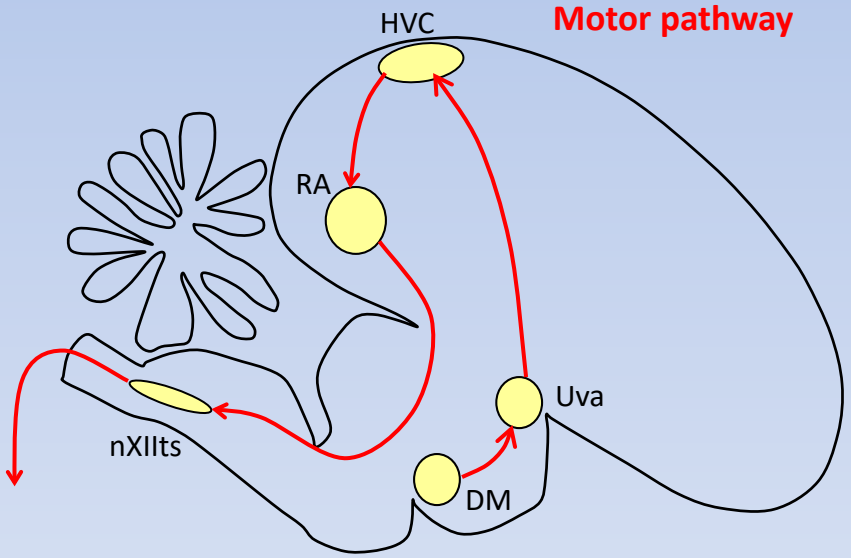


Birds lack a corpus callosum;
instead, connectivity between the two hemispheres is facilitated through the brainstem.

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



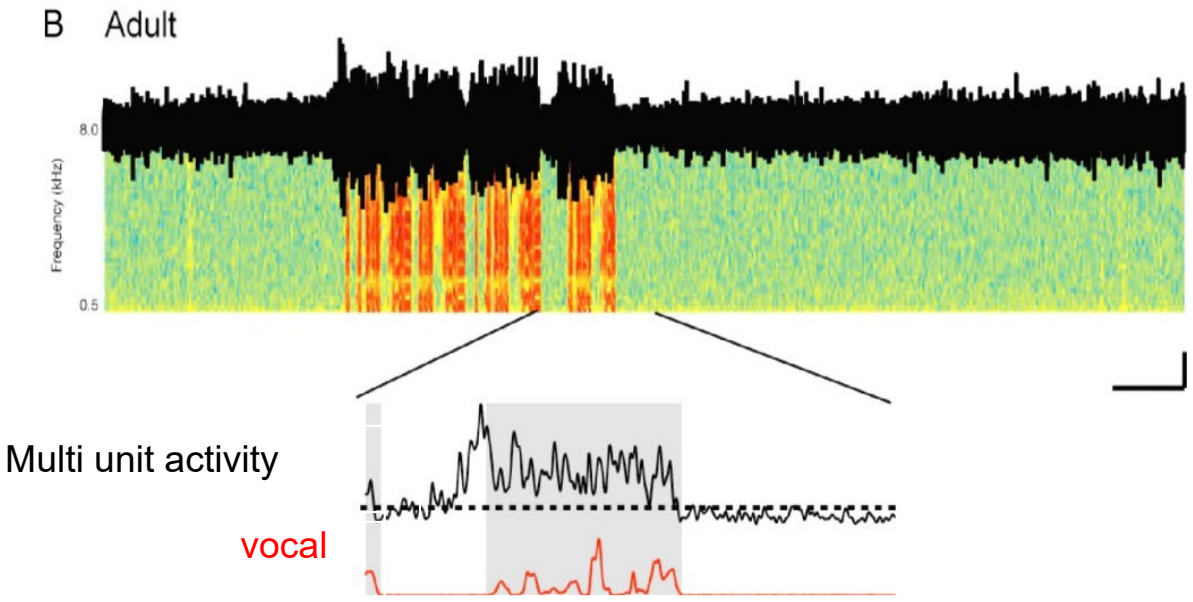
Nottebohm et al., (*J Comp Neurol*, 1976)

- **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

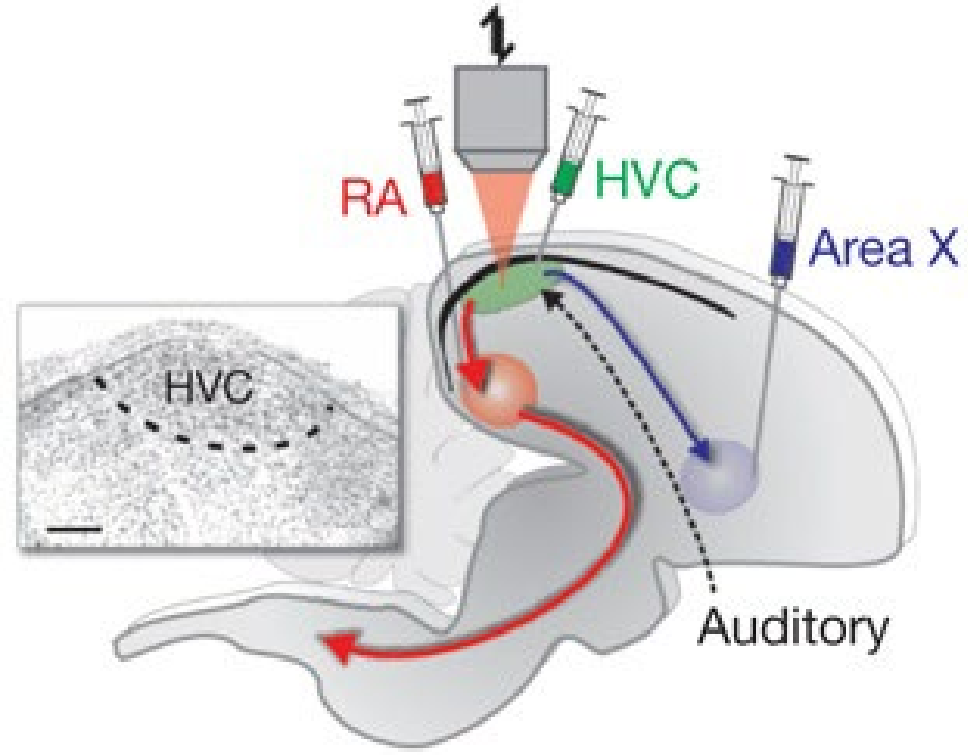


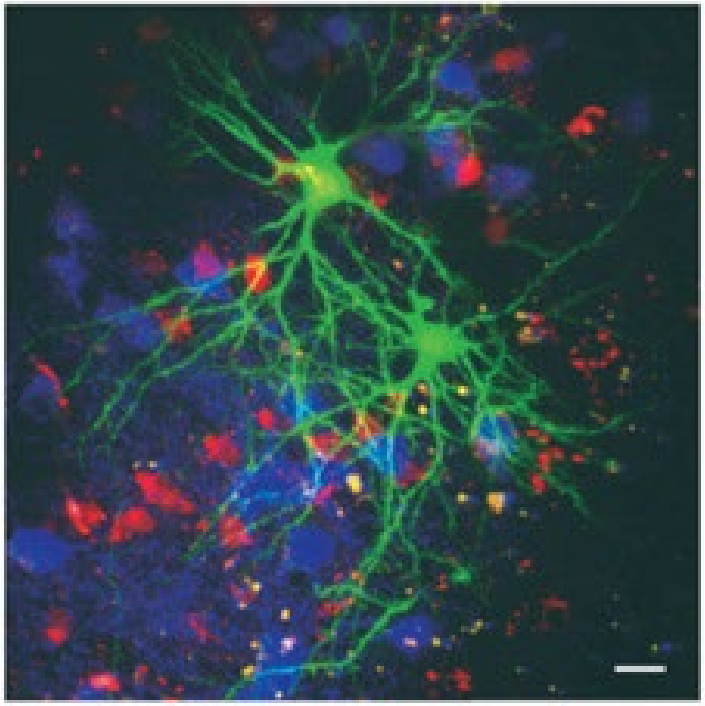
Recording from HVC showed a massive neuronal signal that was throughout the song- that was not well correlated with the song pattern.

Neuroanatomy suggested that there are three types of neurons
→ maybe there are also 3 distinct neuronal responses???

Three types of neurons in HVC:

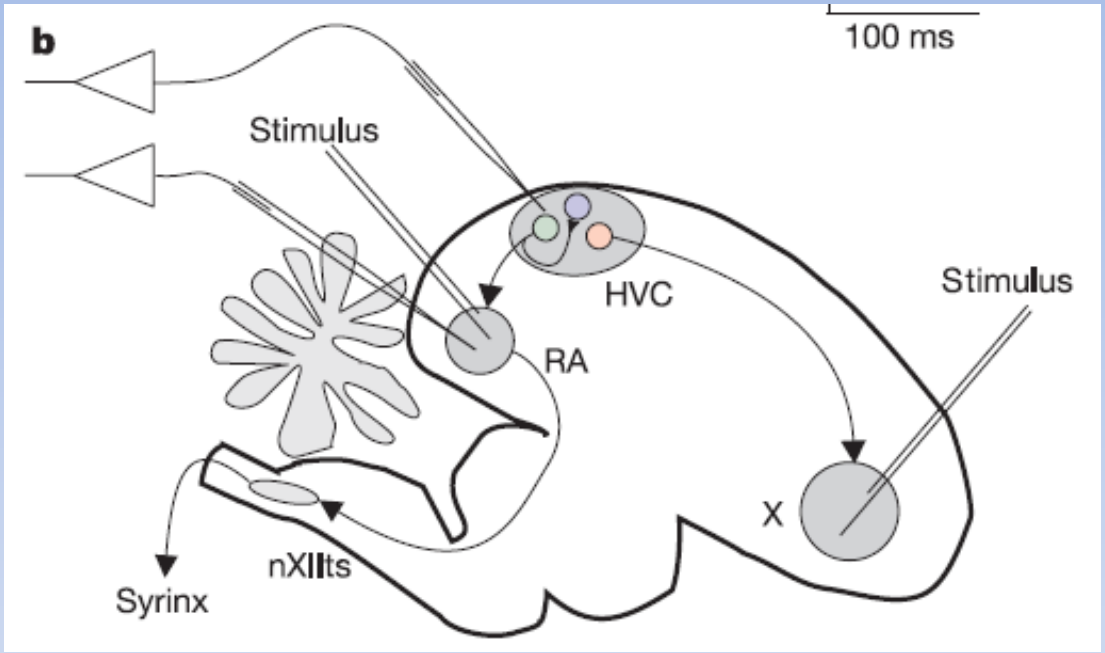
1) HVC-RA projecting neurons
2) HVC-X projecting neurons
3) interneurons





How to identify neurons you are recording from?

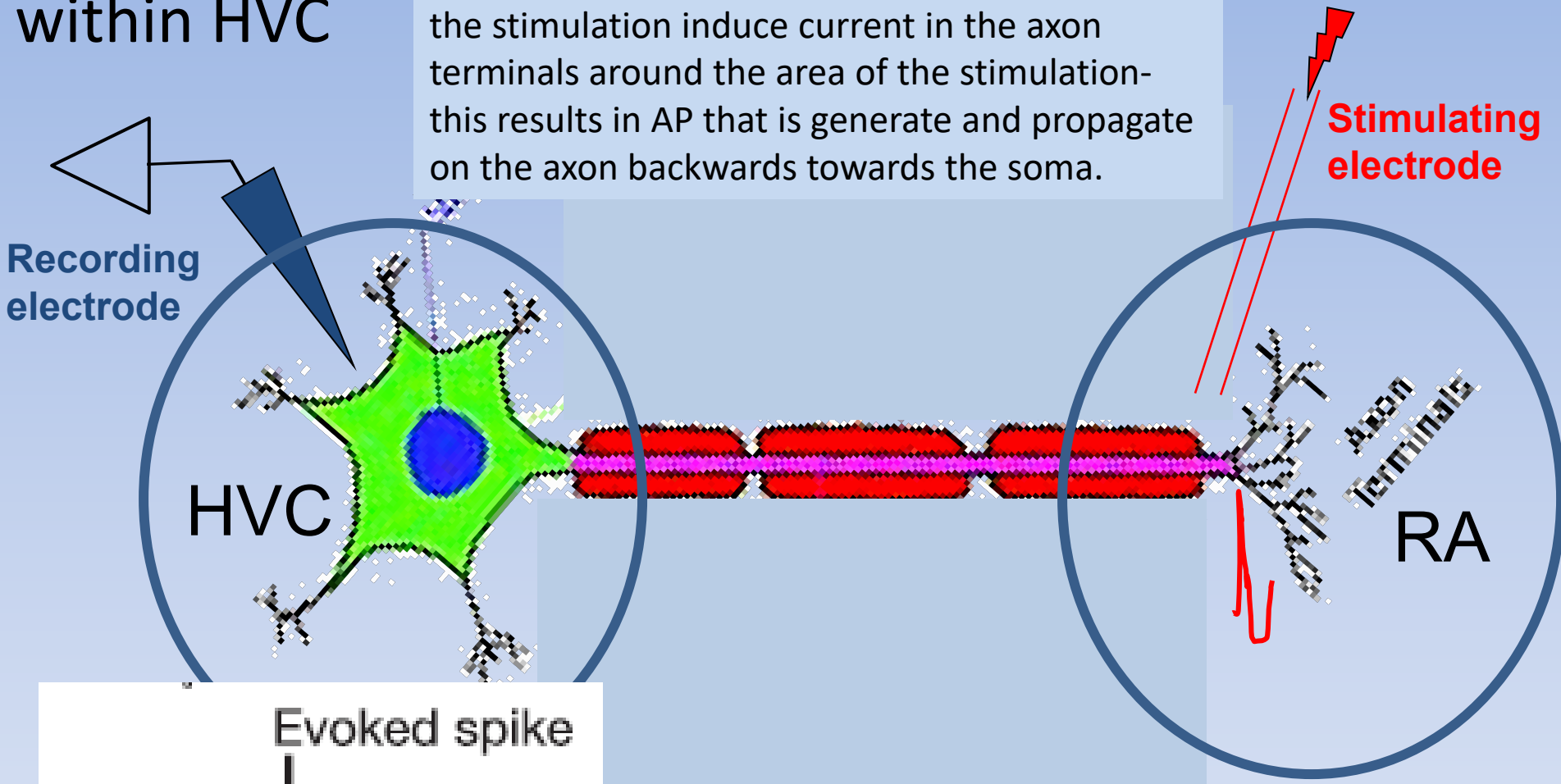
- Antidromic electrical stimulation

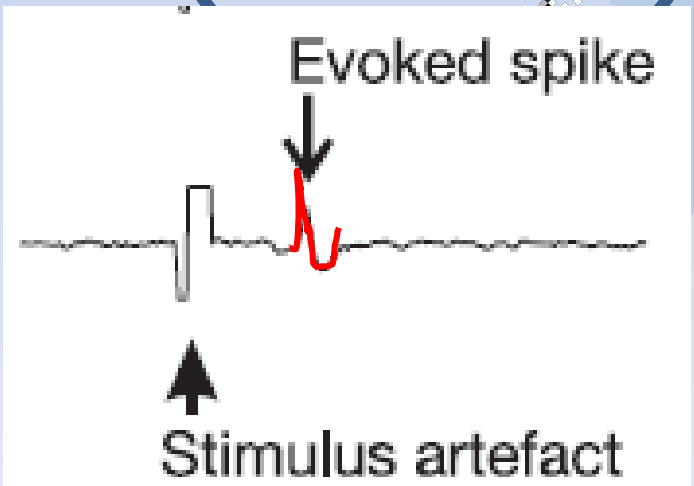


Hahnloser et al. (*Nature*, 2002)

Using antidromic stimulation to identify cell types within HVC

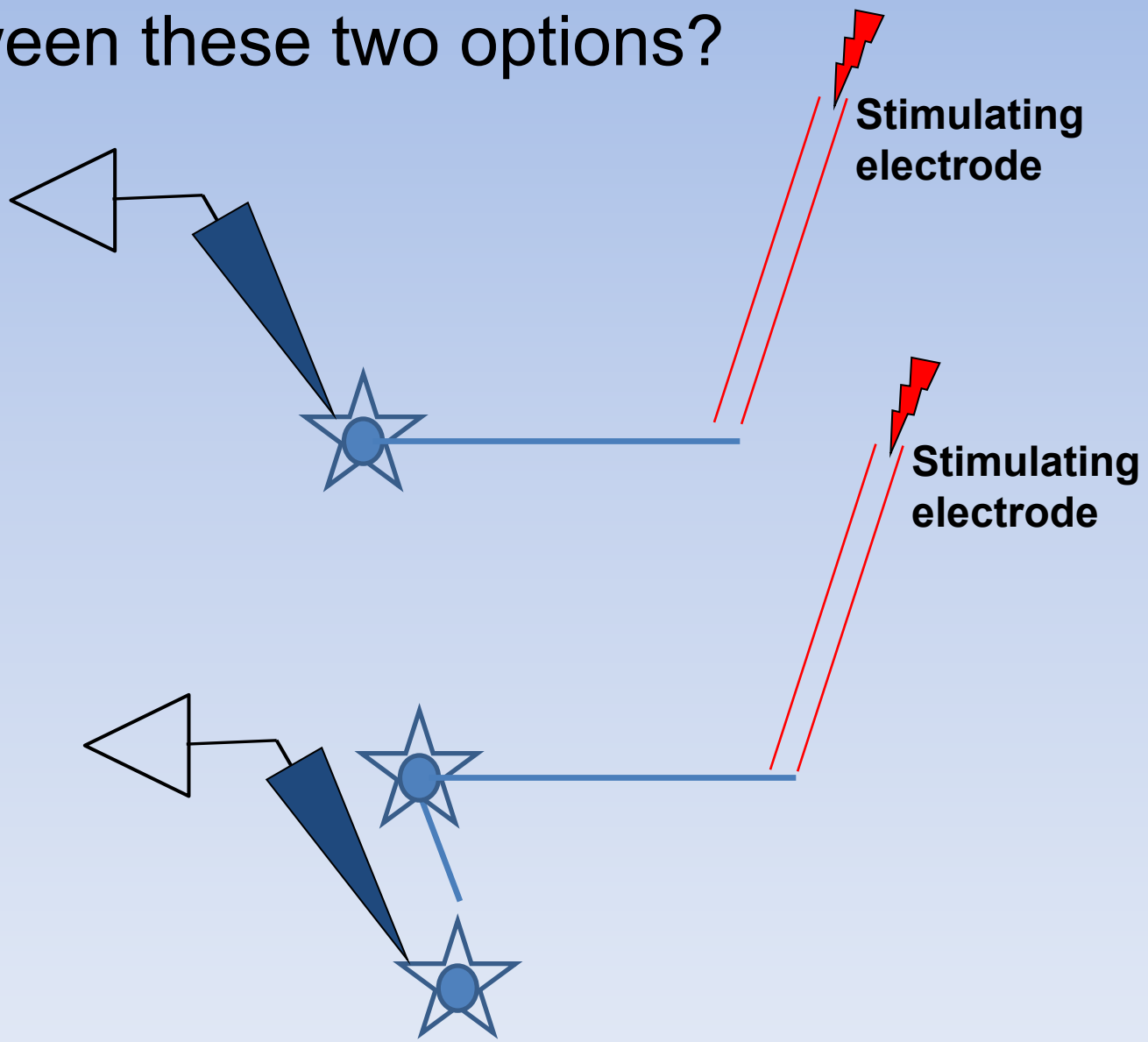
the stimulation induce current in the axon terminals around the area of the stimulation- this results in AP that is generate and propagate on the axon backwards towards the soma.





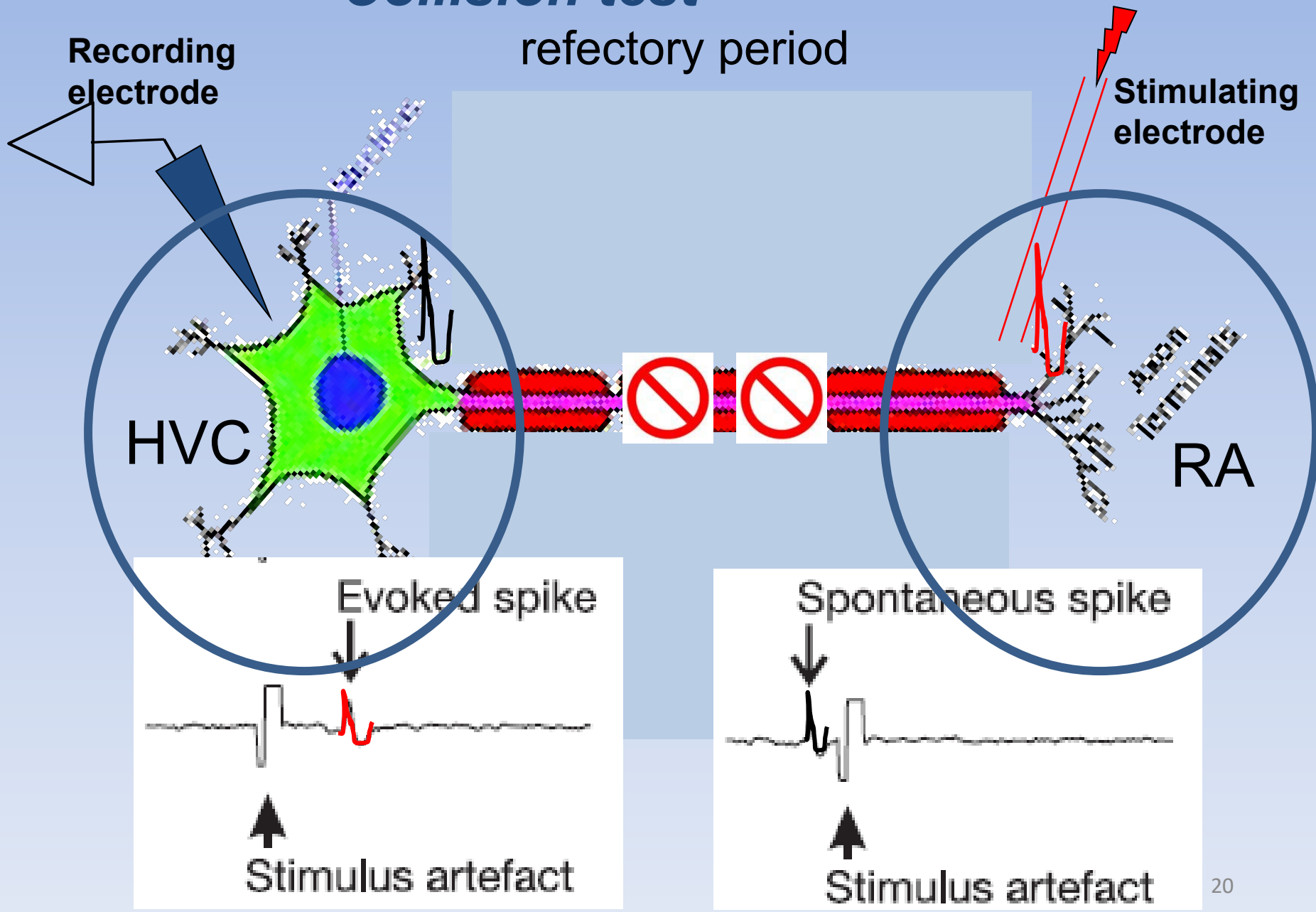
The recordings from the cell soma will first show artifact created by the stimulus- followed by an interval→ the spike traveling time on the axon and then the evoked spike

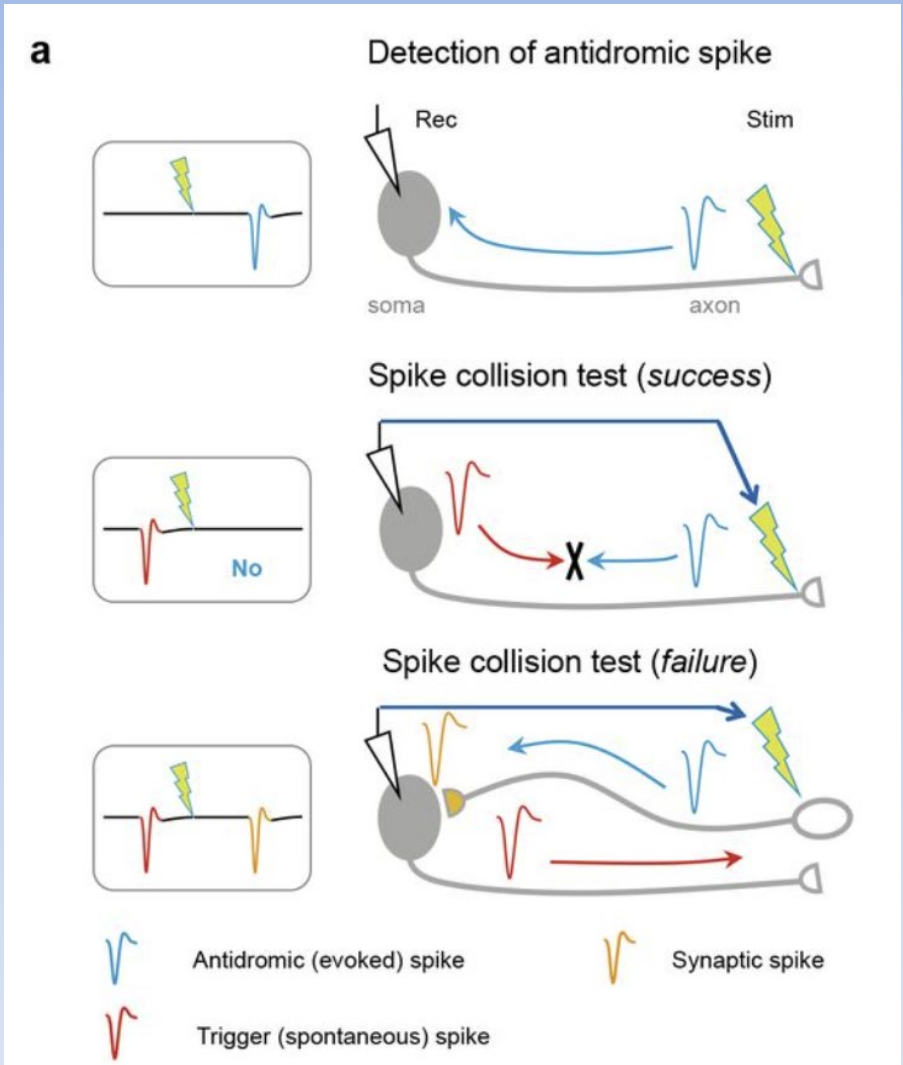
How do we differentiate between these two options?



Collision test

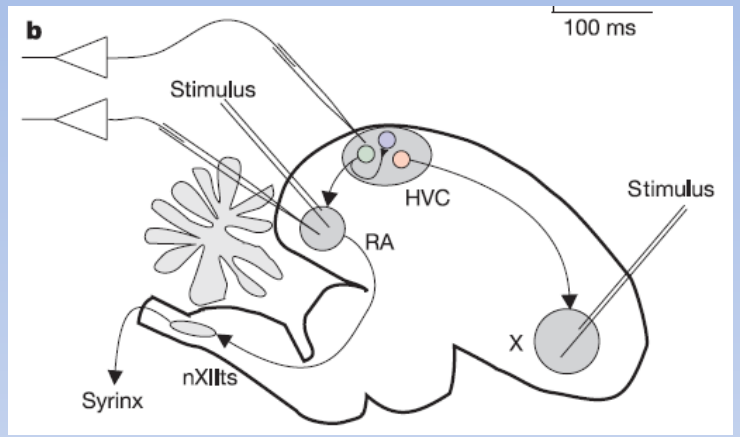
refectory period



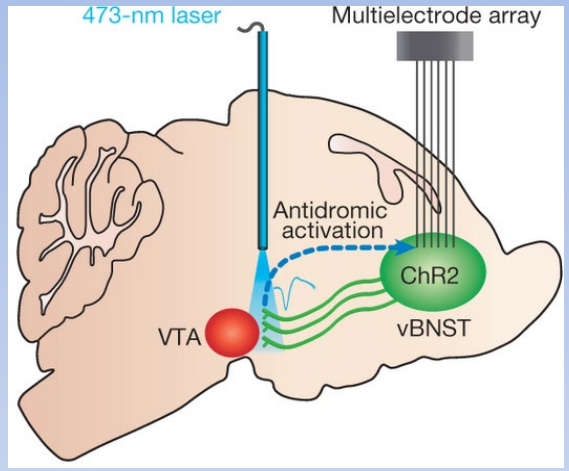

a
Detection of antidromic spike
Rec
Stim
soma
axon
Spike collision test (success)
No
Spike collision test (failure)
Antidromic (evoked) spike
Synaptic spike
Trigger (spontaneous) spike

How to identify neurons you are recording from?

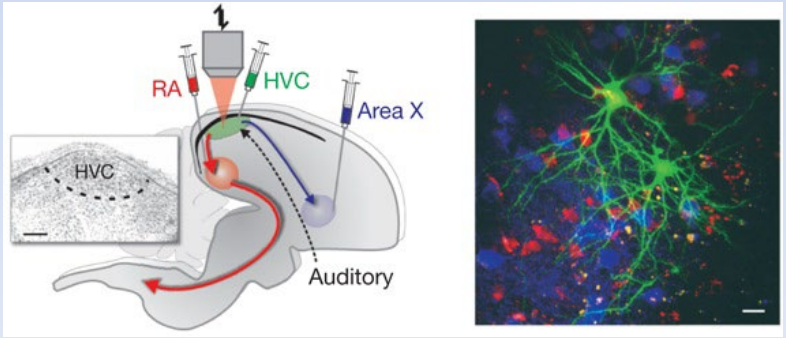
- Antidromic stimulation (electrical or optogentic)



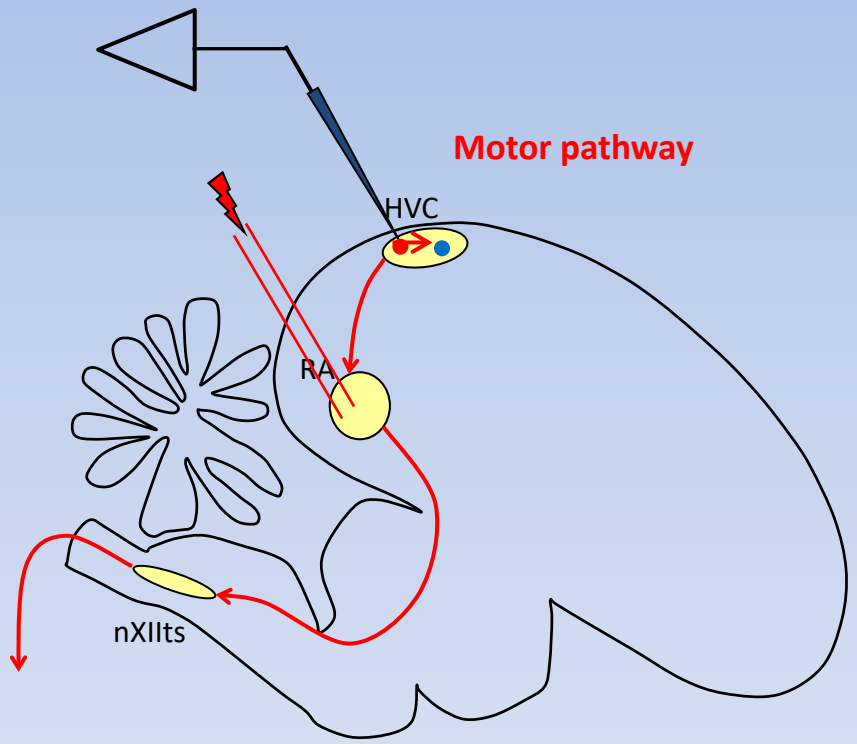
Hahnloser et al. (*Nature*, 2002)

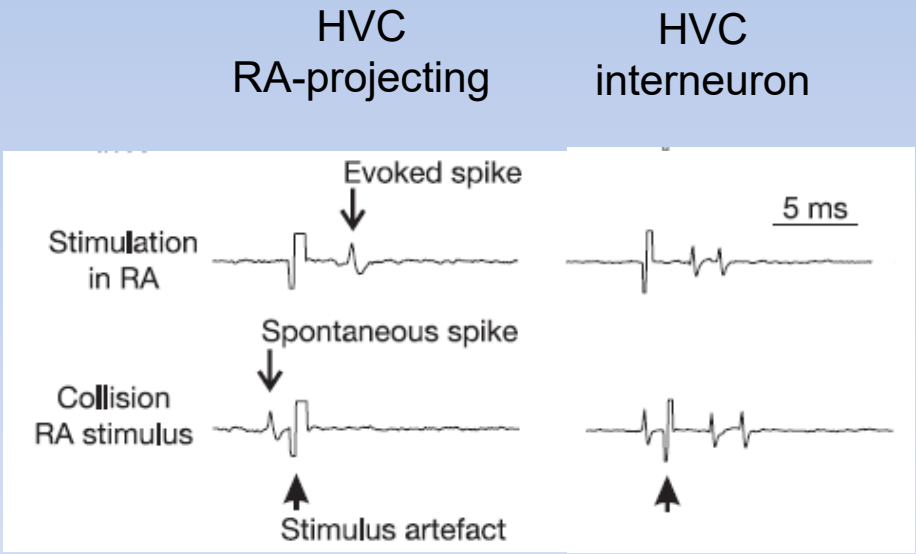


- Labeling (genetically or injections)

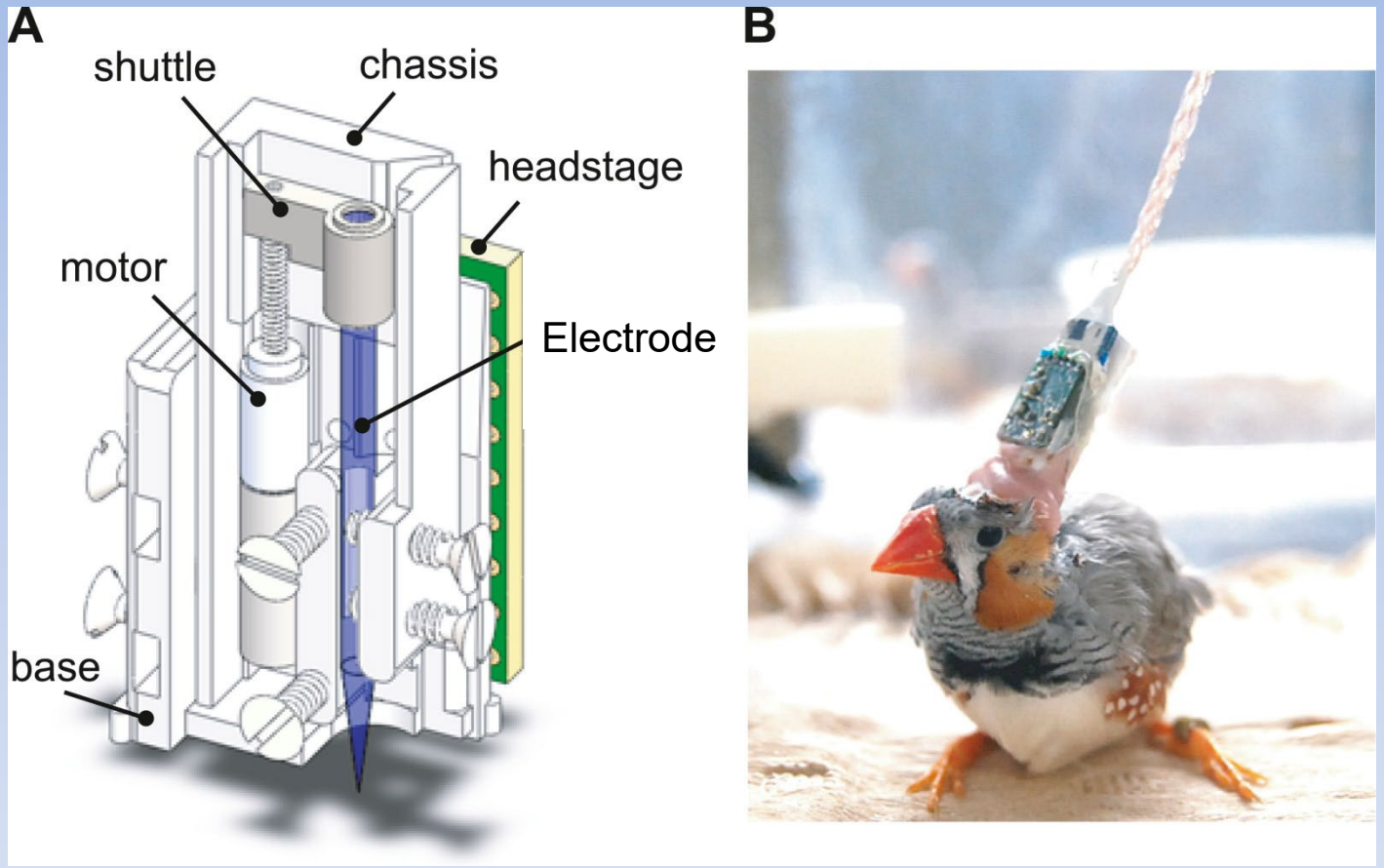


Using antidromic stimulation to identify cell types within HVC

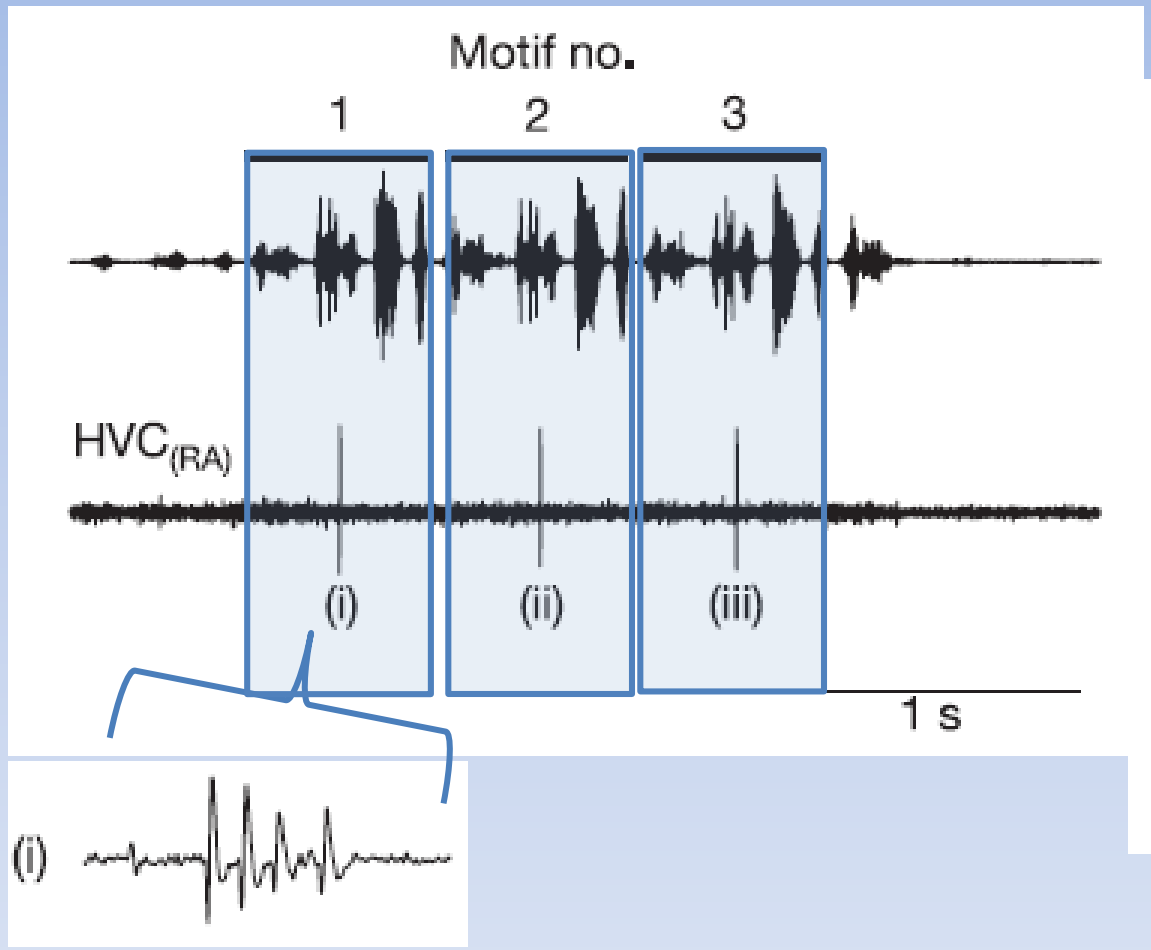


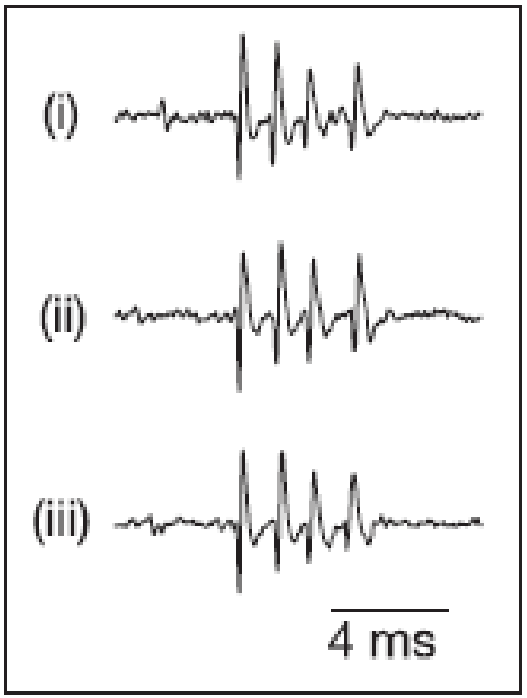


Hahnloser et al. (*Nature*, 2002)


A
shuttle
chassis
headstage
motor
Electrode
base
B

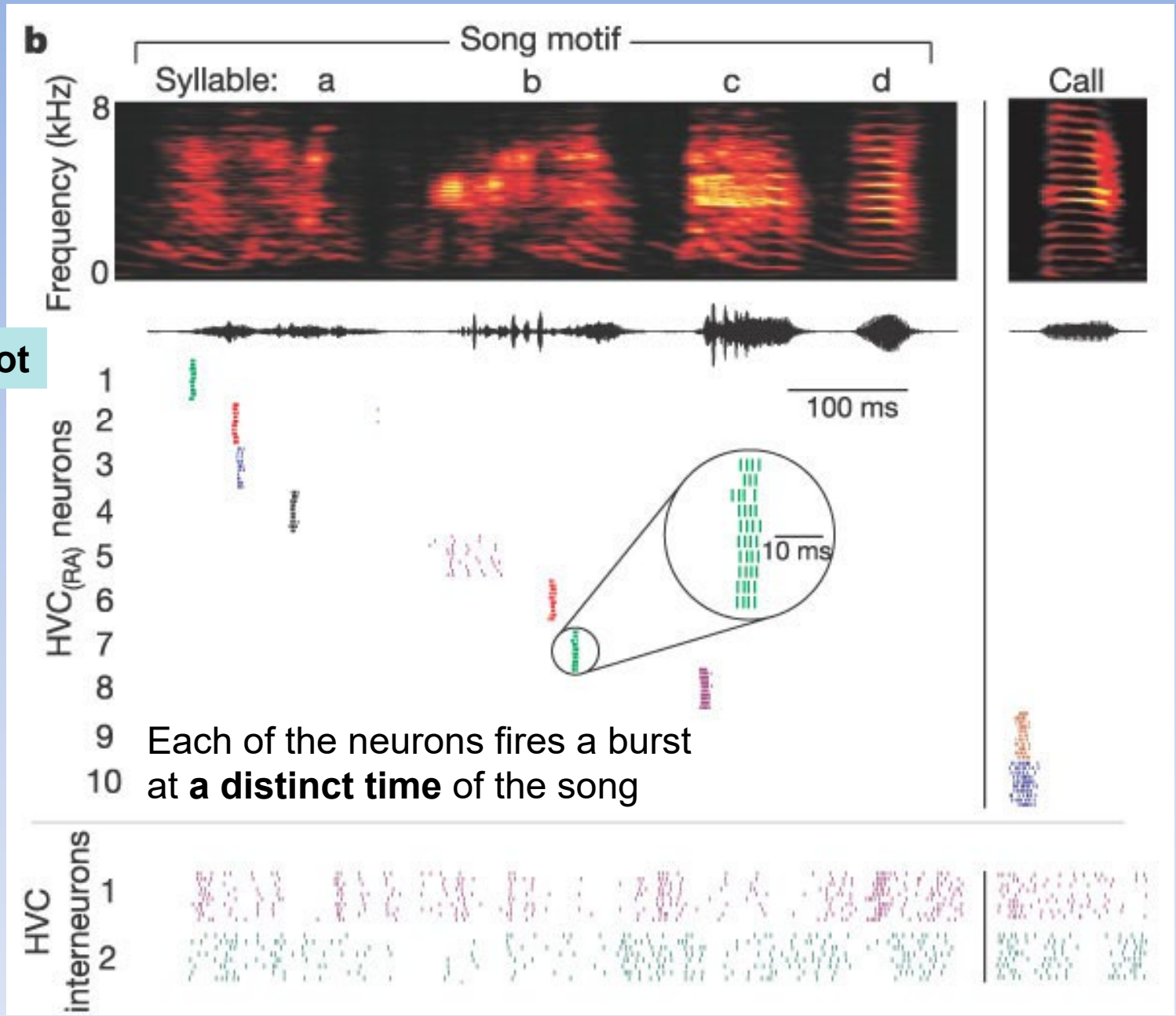
Activity of HVC-RA neurons during singing





Hahnloser et al. (*Nature*, 2002)

raster plot



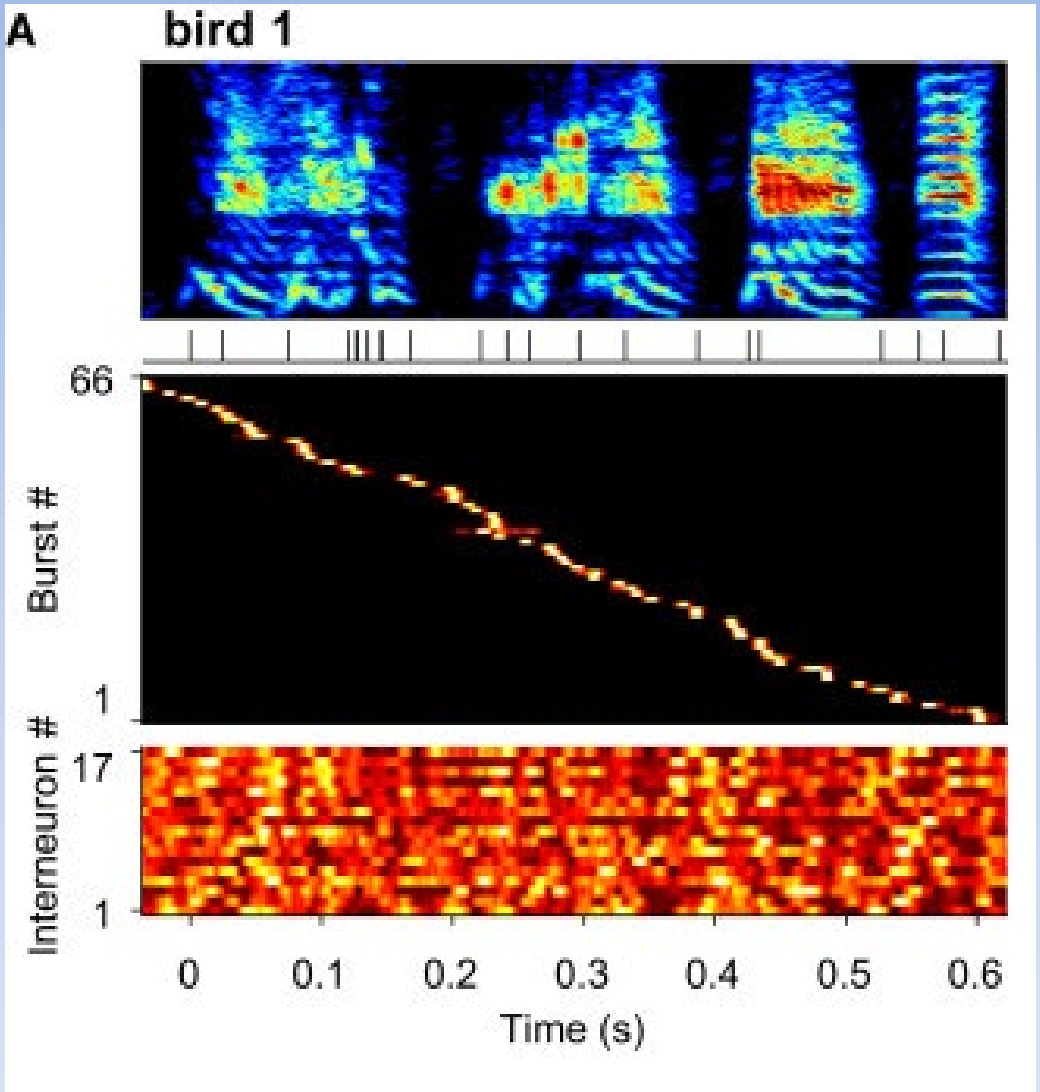
This suggest a *sparse* sequence of activity that propagates through the song.

Hahnloser et al. (*Nature*, 2002)

HVC bursting produces continuous coverage of time in the song

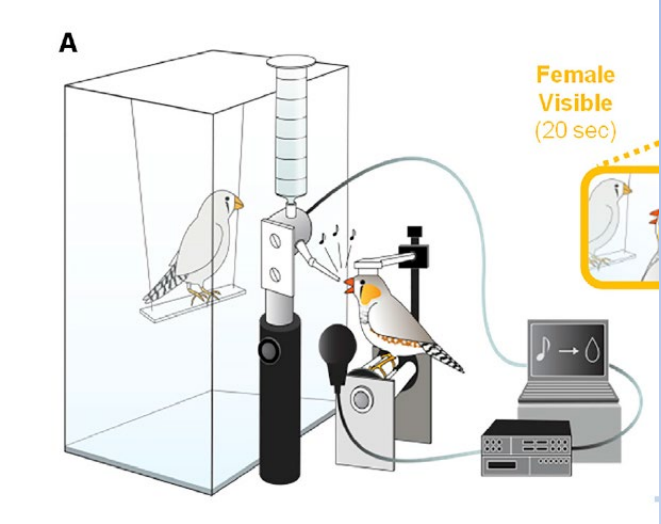
The population of HVC projection neurons is ***continuously active*** during singing

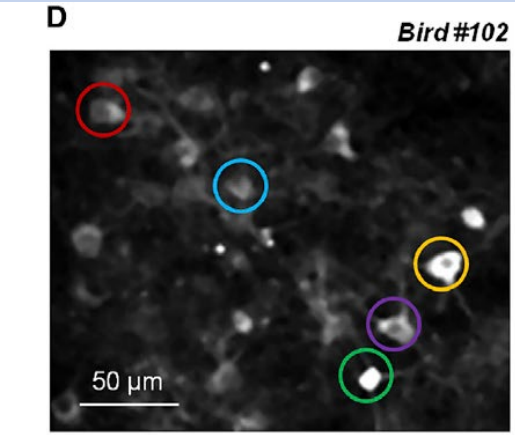
These extremely sparse and precise patterns of activity suggest the entire ensemble of HVC_{RA} neurons could function to specify the timing of syllables, notes, and even the intervening silent "gaps."

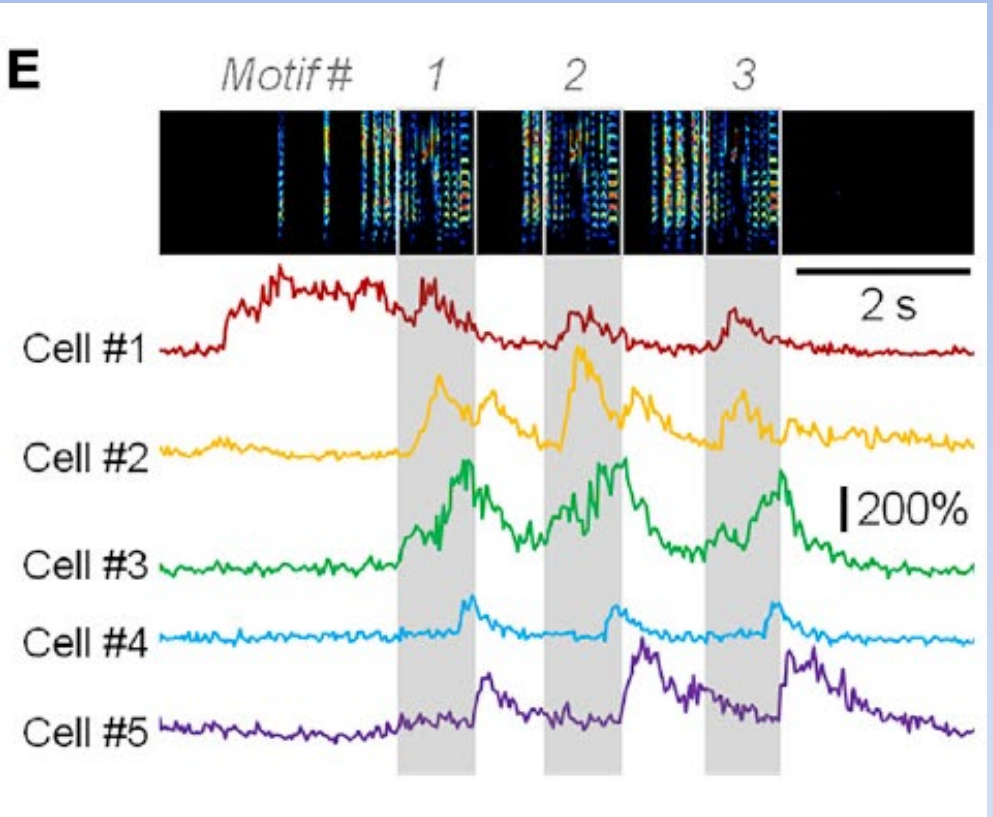


Rhythmic Continuous-Time Coding in the Songbird Analog of Vocal Motor Cortex
Galen F.Lynch[14]Tatsuo S.Okubo[14]AlexanderHanuschkin[23]Richard H.R.Hahnloser[23]Michale S.Fee[1]

The sparse activity of HVC neurons and its location makes it perfect for two-photon calcium imaging



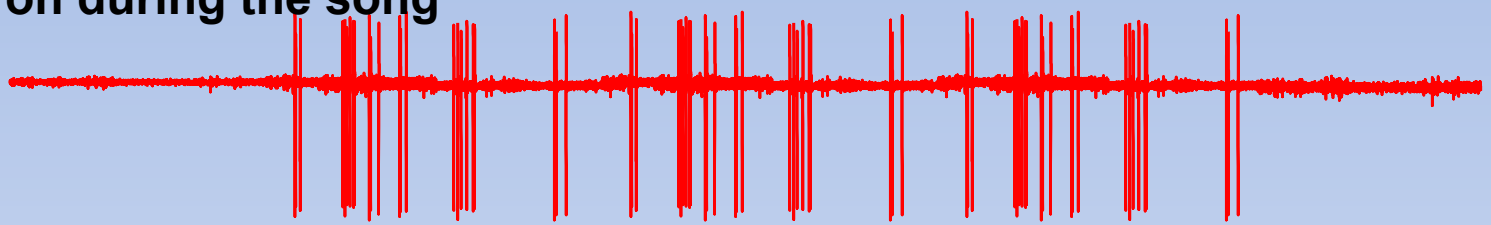


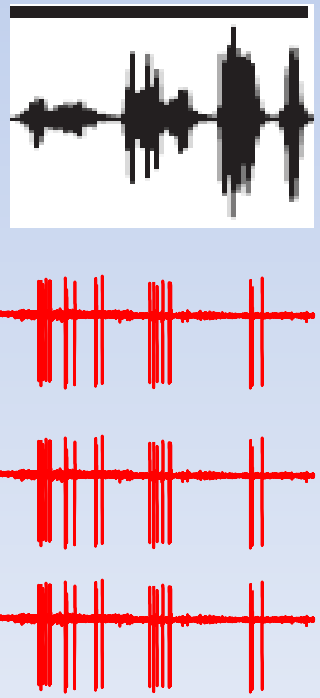


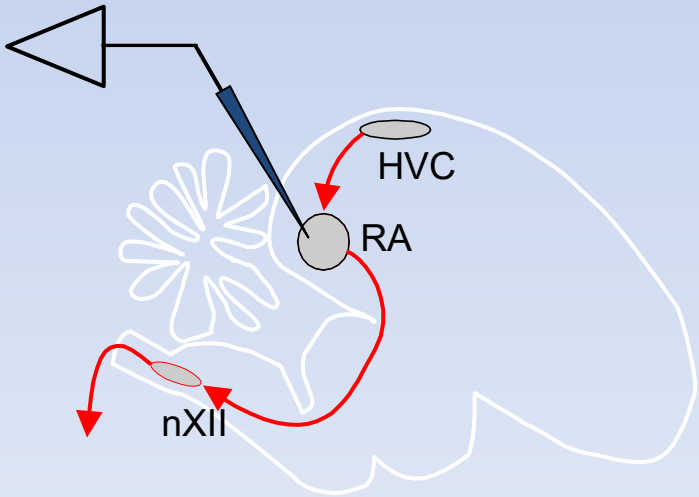
Population-Level Representation of a Temporal Sequence Underlying Song production in the Zebra Finch. 2016, Neuron

Michel A. Picardo, Josh Merel, ..., Eftychios A. Pnevmatikakis,Liam Paninski, Michael A. Long

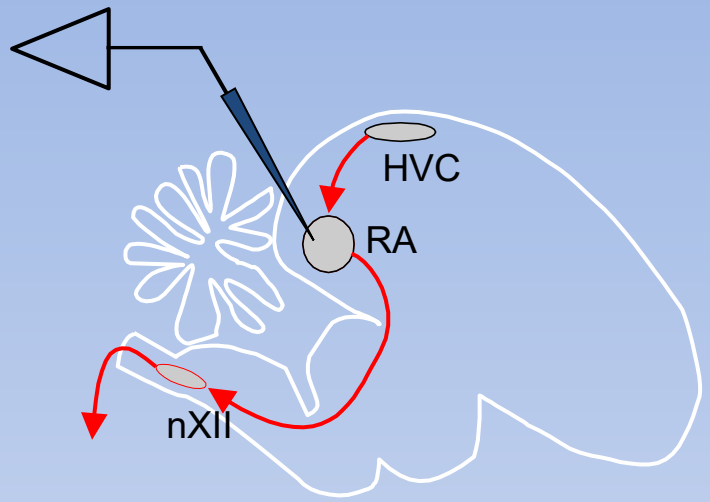
Recordings from RA neuron during the song



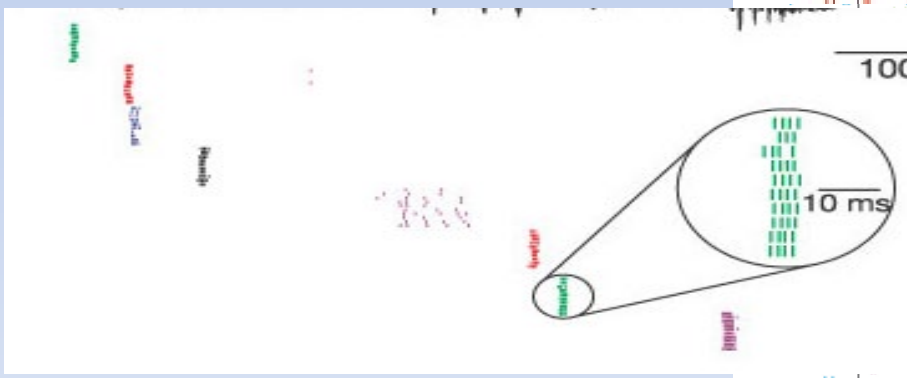


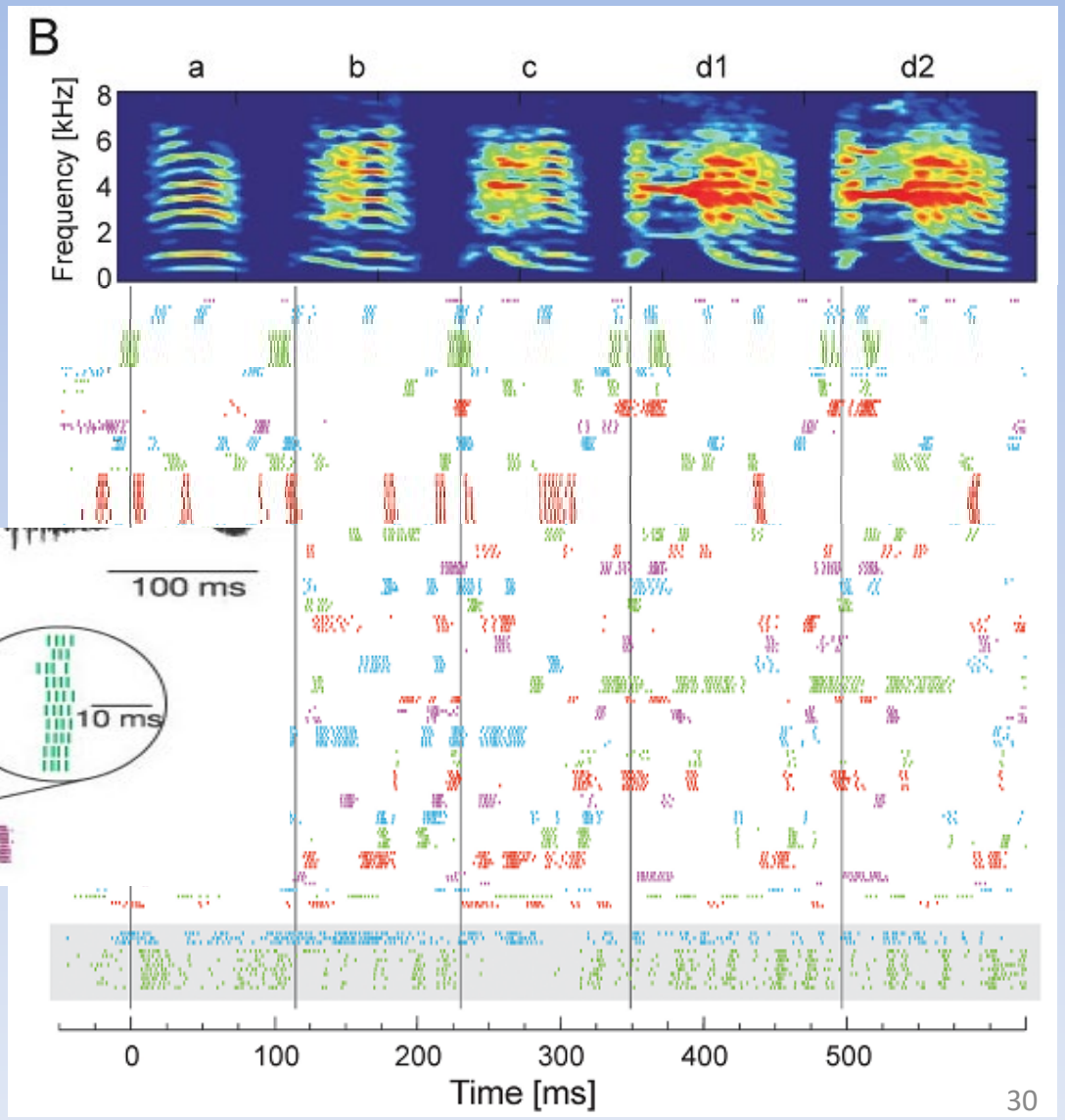


RA activity during singing



HVC-RA neurons

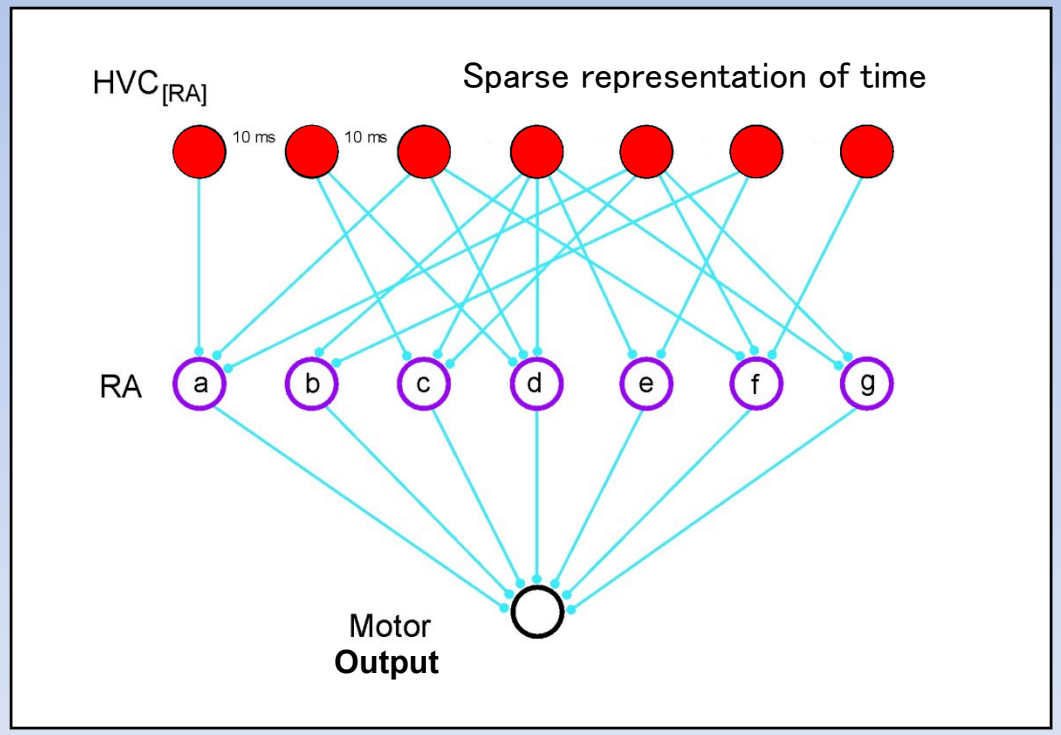


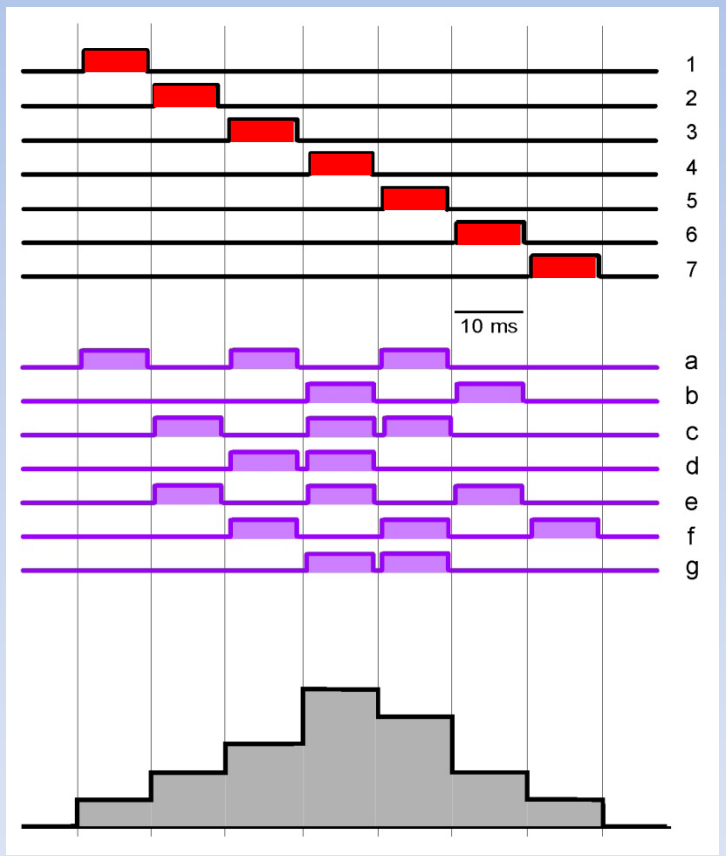


Leonardo & Fee (*J Neurosci*, 2005)

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.

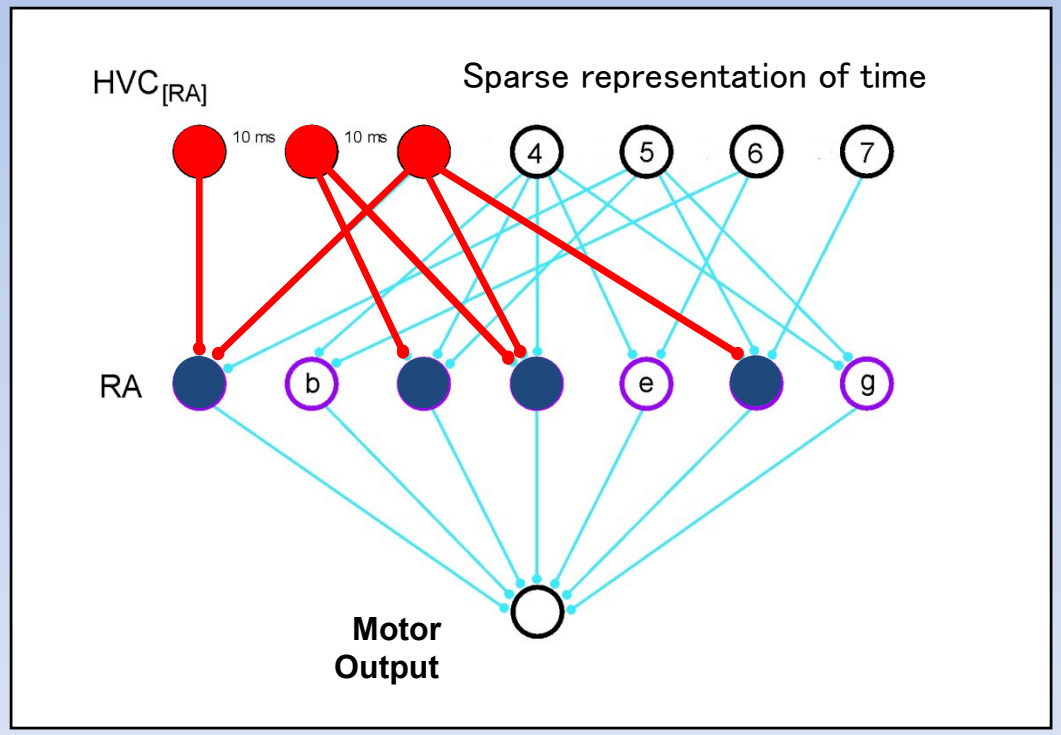


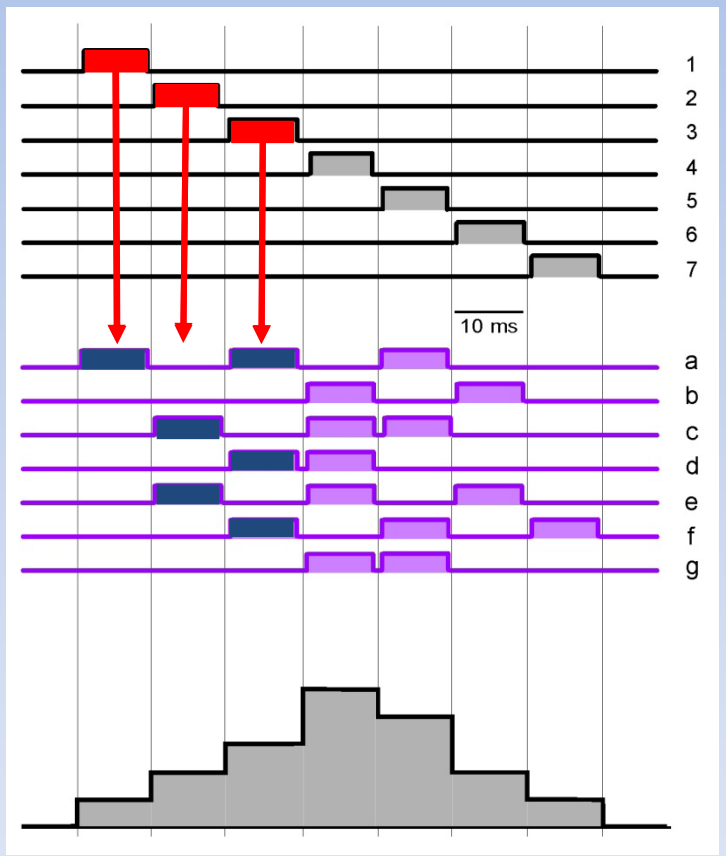


Leonardo and Fee, 2005

Simple sequence generation circuit

The output of HVC cell goes to more then one RA neuron → **each RA neuro will be activated more than just once in a motif.** The summation of RA neurons converge onto to the mn in the brainstem.

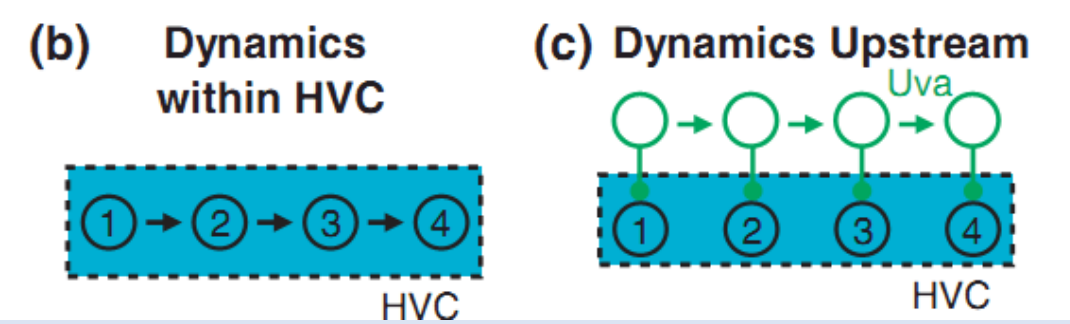


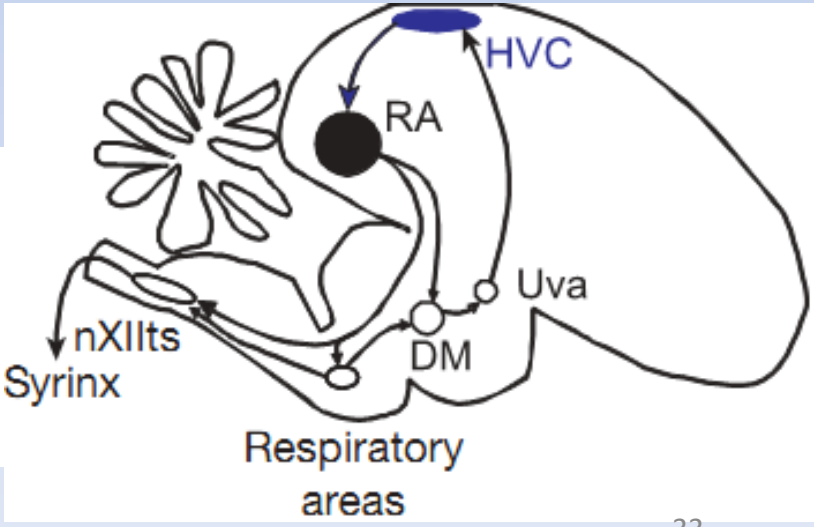


Leonardo and Fee, 2005

Part 1: Summary

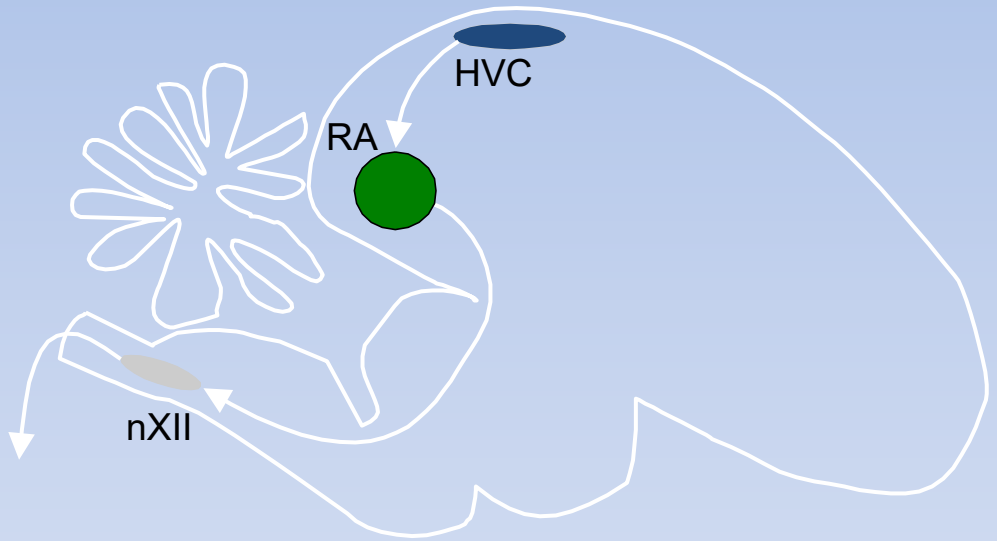
- HVC exhibits sparse bursts during singing.
- RA transforms the sparse code into multiple bursts which then drive motoneurons.
- BUT: Where are these patterns of activity coming from? What is driving HVC to fire at a particular moment?

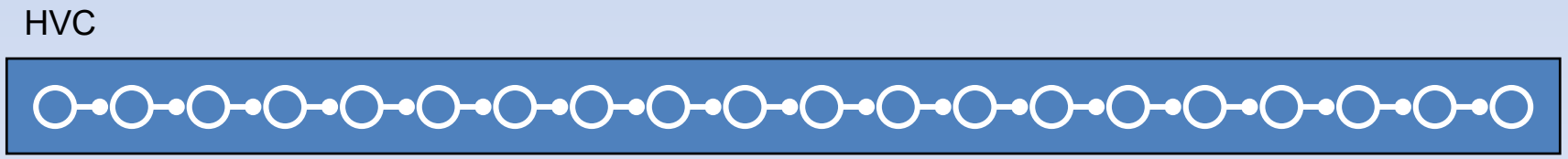




Maybe its within HVC?
one chain model for all song dynamics

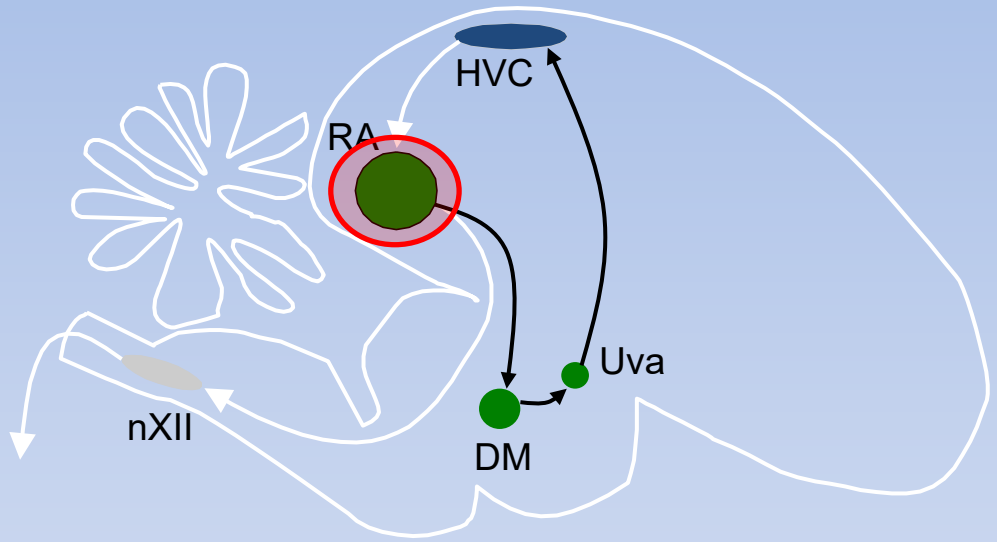
A continuous-time model



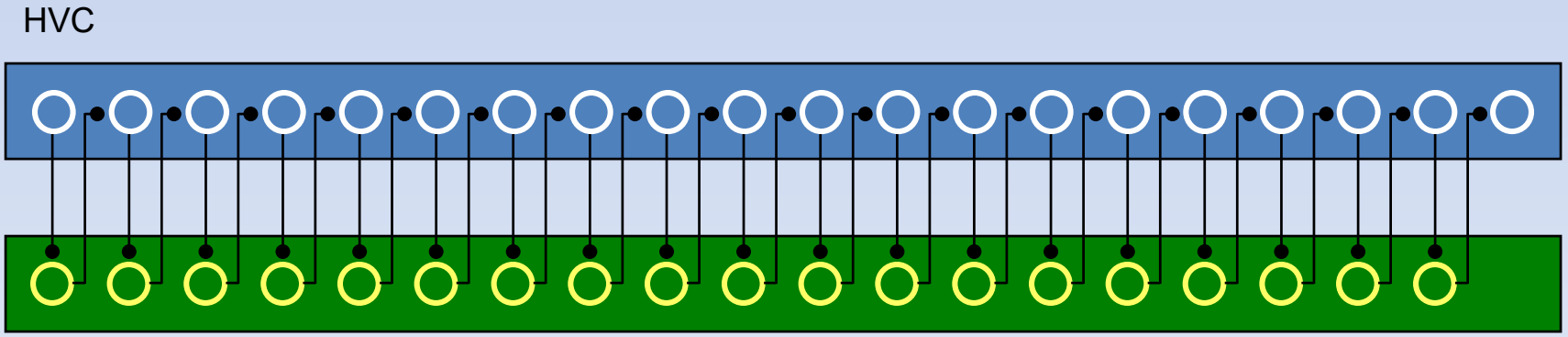


Li and Greenside, 2006
Jin et al, 2007
Long, Jin, Fee, 2010

OR: The chain might have links outside of HVC



?

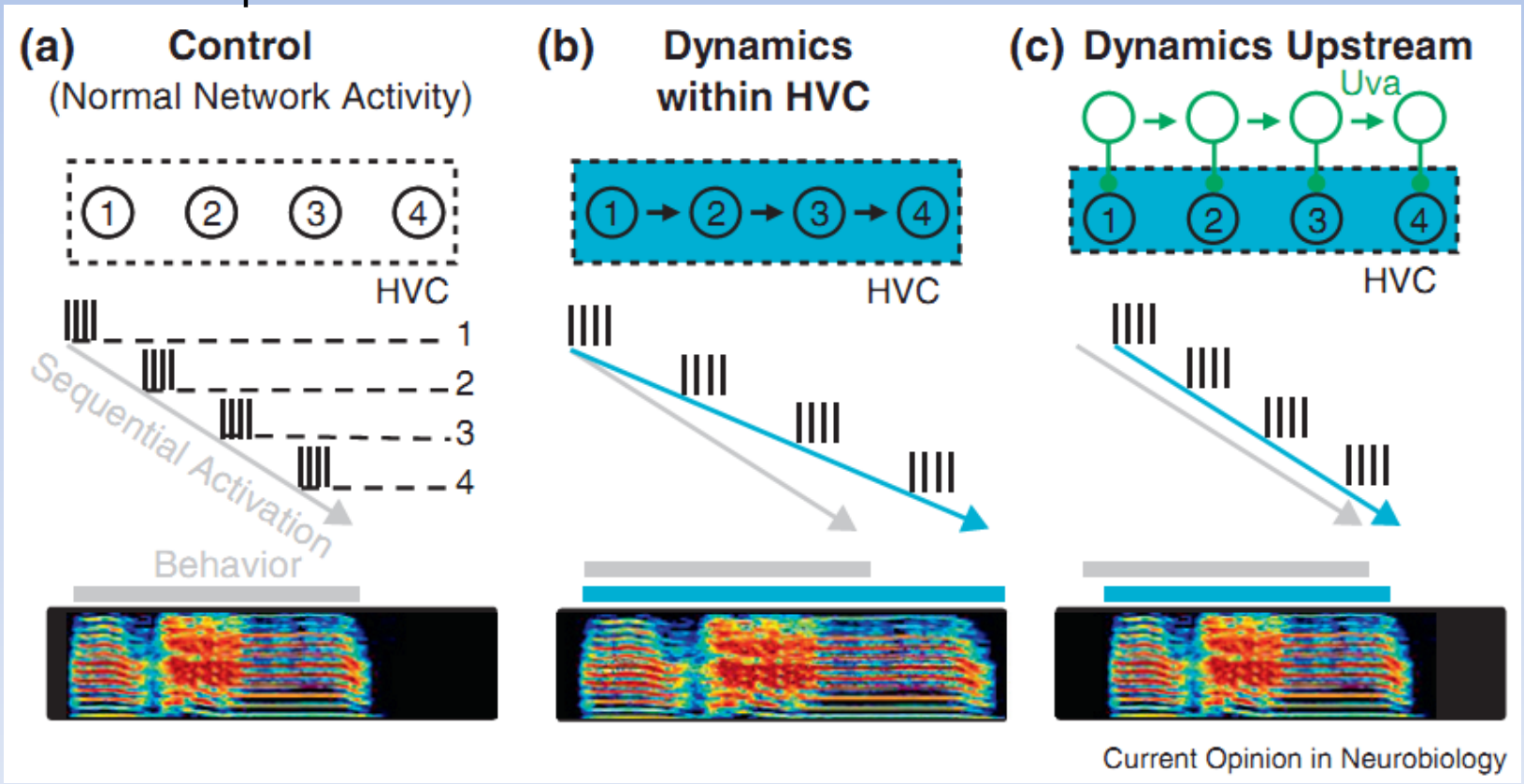


Hamaguchi, Tanaka, Mooney, 2016

What and where is the mechanism that determines tempo? Are the slow dynamics generated within HVC?

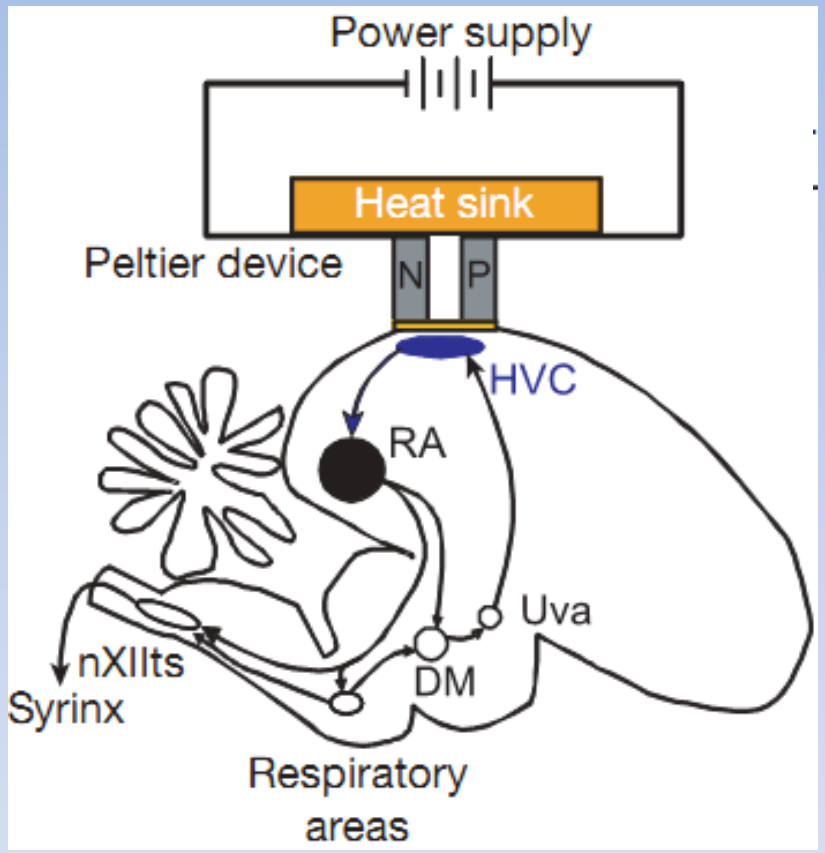
If song tempo is determined by the activity of the HVC local network, then song should slow as HVC is cooled.

Theoretical predictions



Fee & Long (*Curr Opin Neurobiol*, 2011)

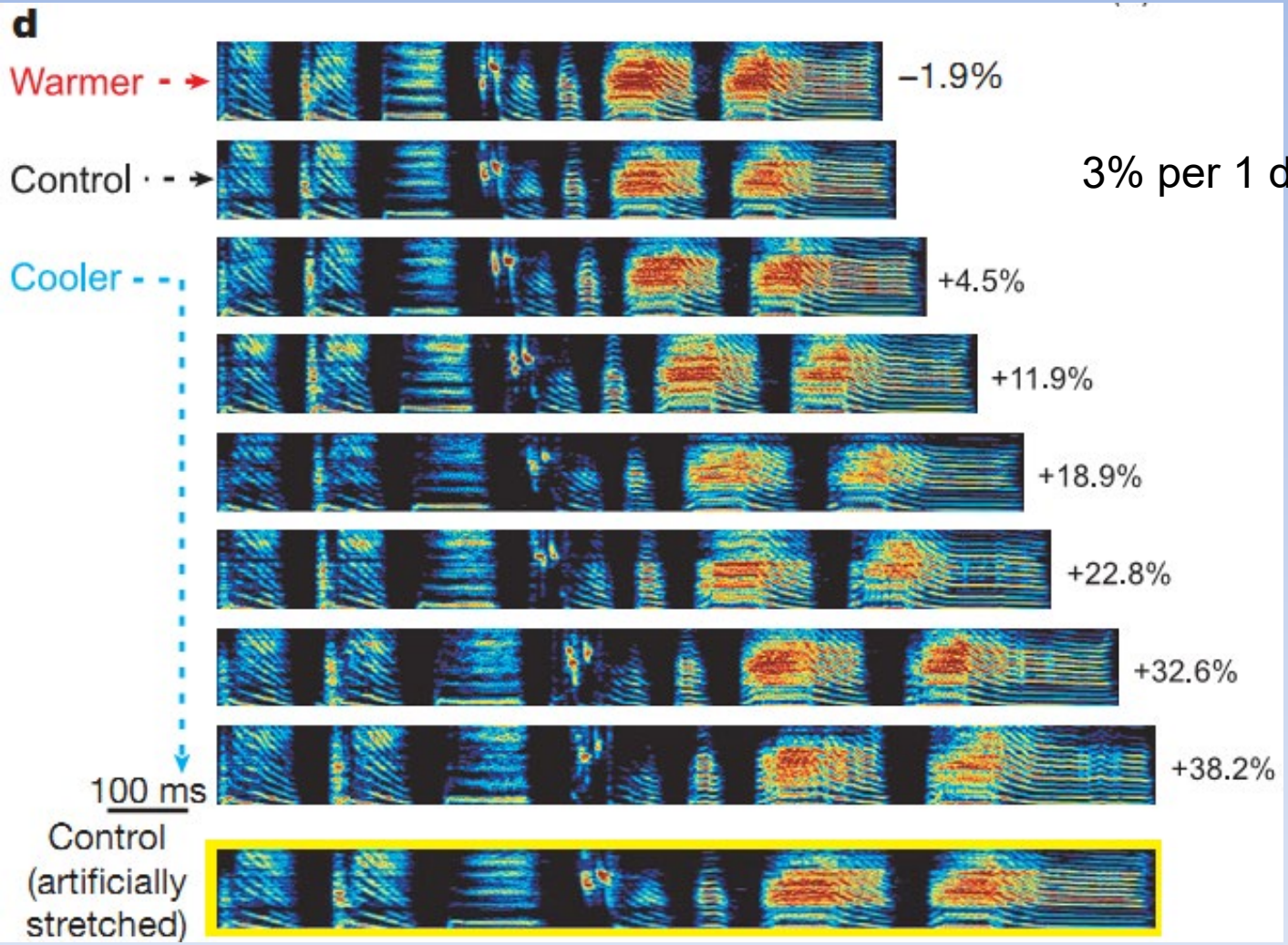
Local manipulation of brain temperature



Long & Fee (*Nature*, 2008)

The temperature changes are very local, and rapid (within 10 seconds)

Cooling of HVC causes slowing of song...

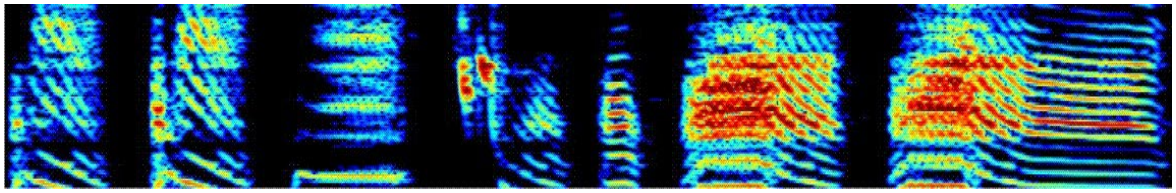


3% per 1 degree

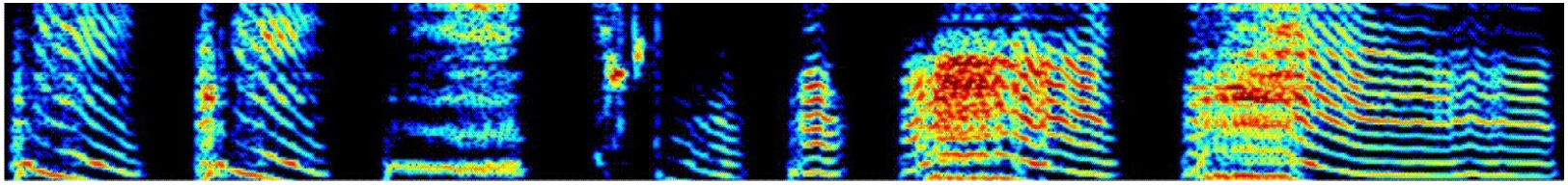
Long & Fee (*Nature*, 2008)

Stretching of song is highly uniform

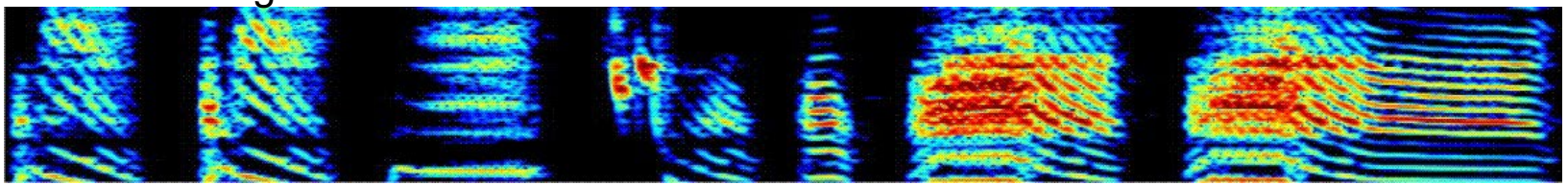
Control: *T=40 ºC*



Cooled: *T=30 ºC*



Control: image stretched

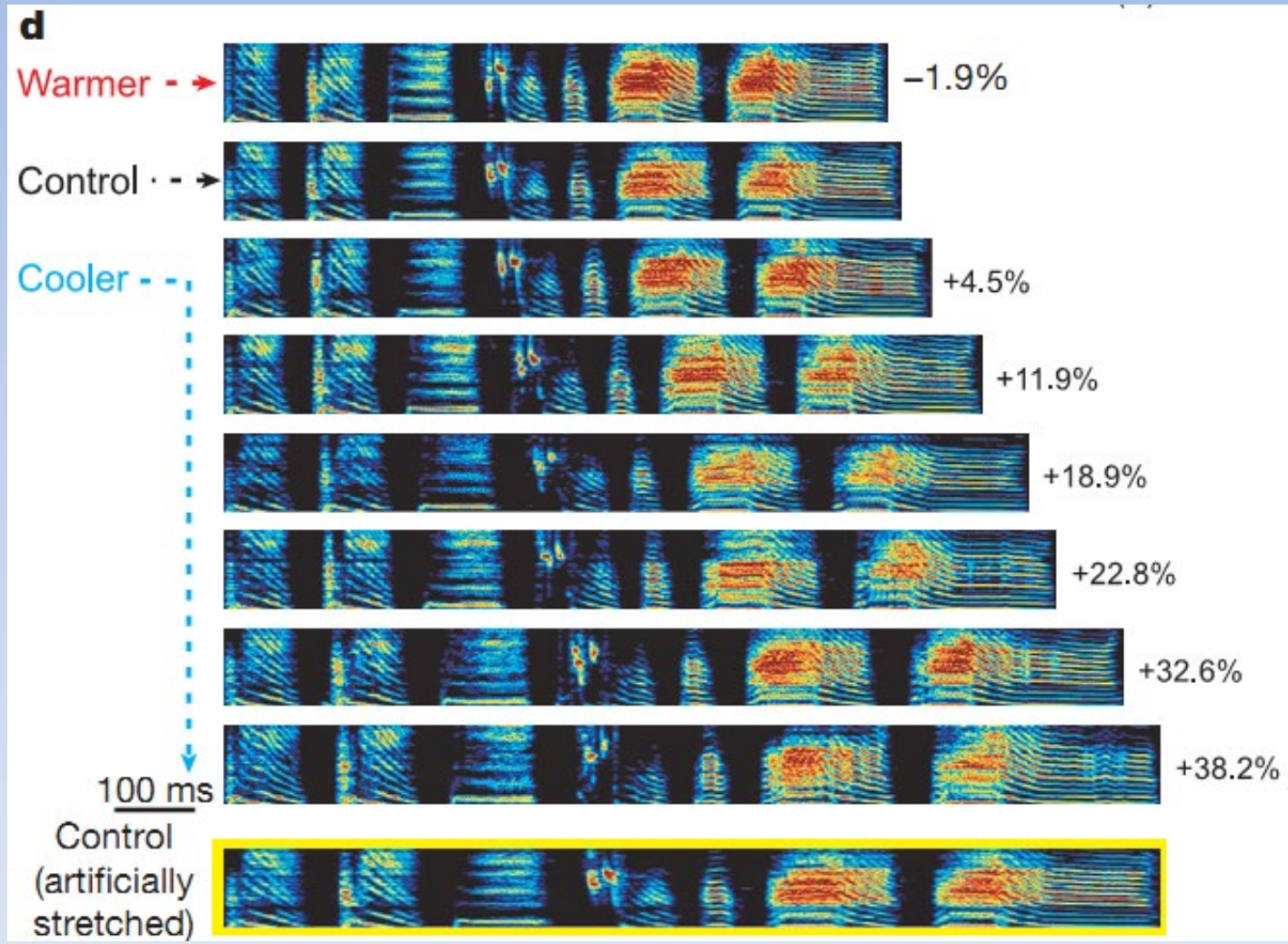


Cooling of HVC: Stretching of song is highly uniform!

song tempo slowed similarly across all timescales:

1) individual notes (~10 ms),
2) entire motif (~1s)
3) the silent gaps

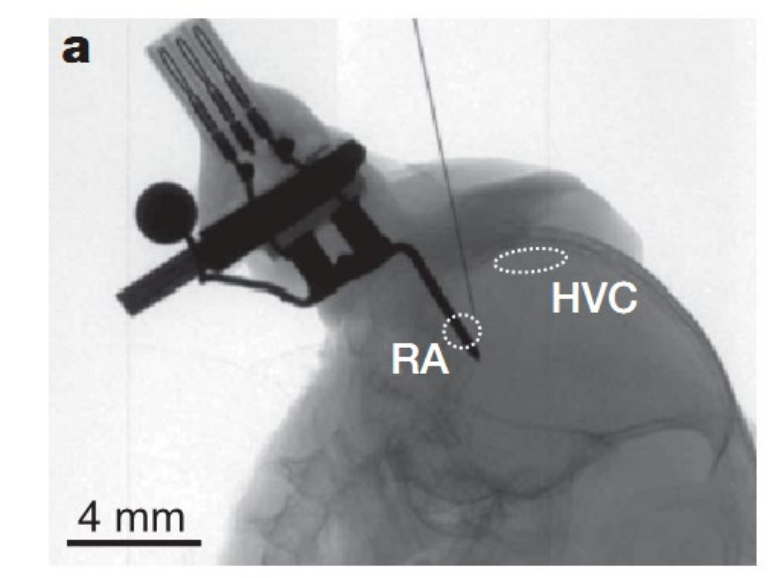
3% per 1 degree

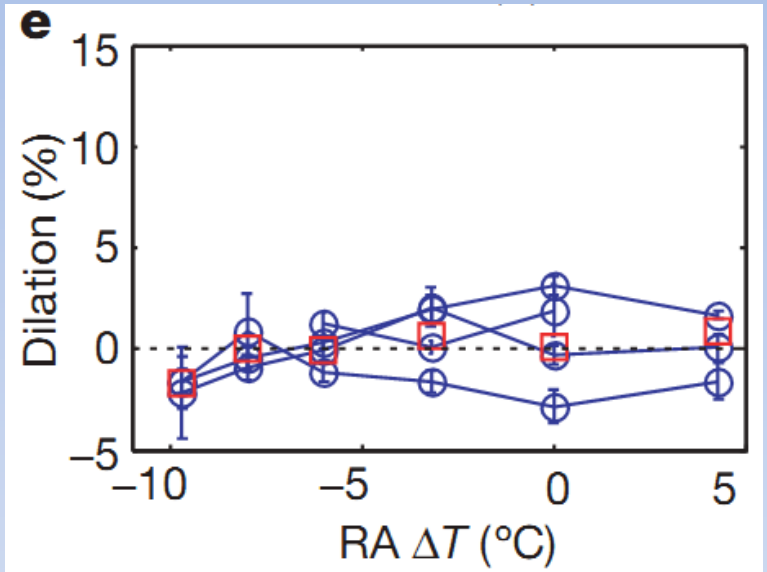


Long & Fee (*Nature*, 2008)

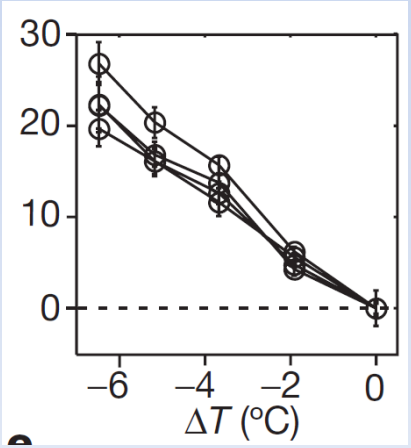
Cooling RA has no effect on song timing at any timescale, including song speed or the structure of the notes

Song timing is not controlled by dynamics within RA (from bilateral cooling in RA)



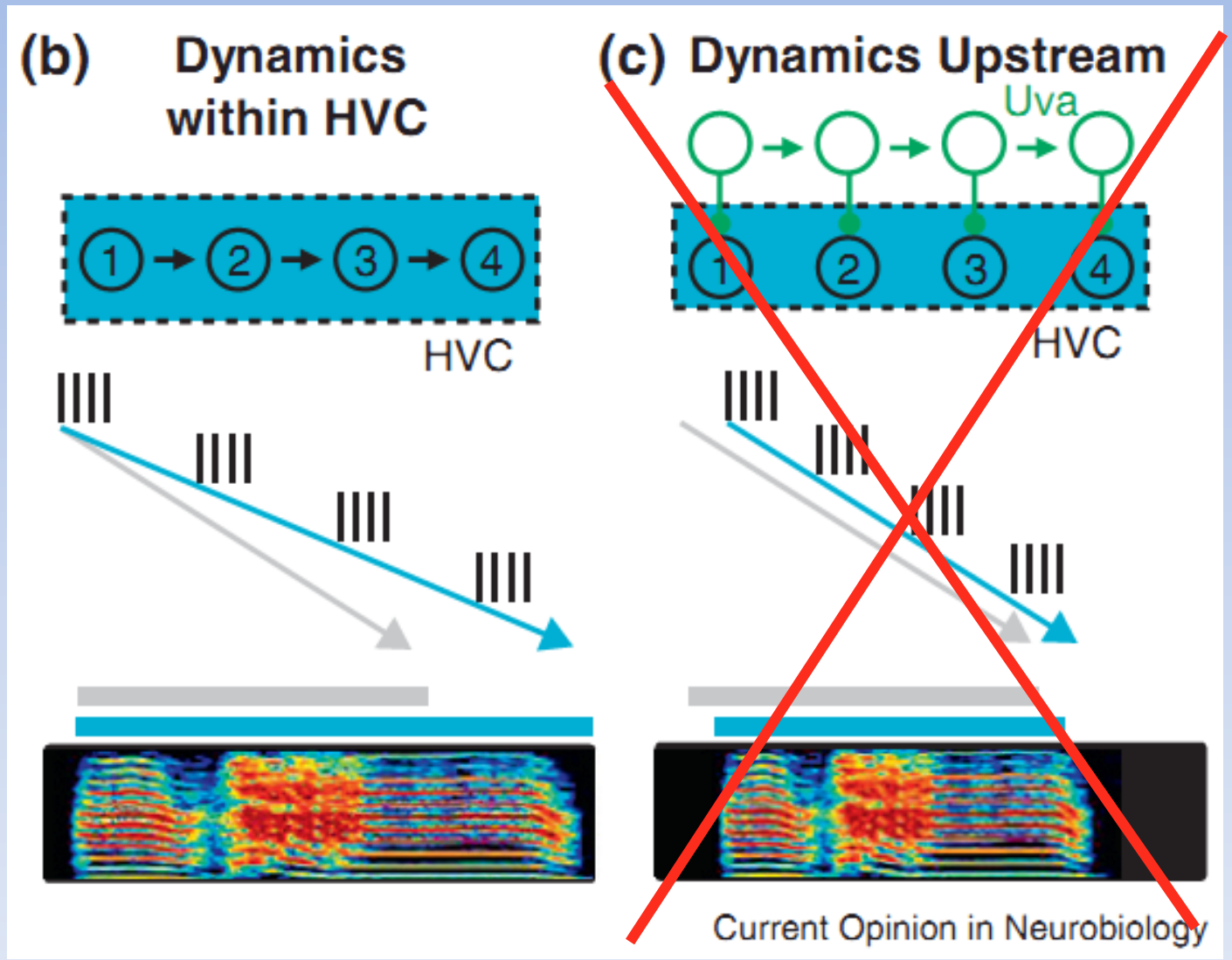


In contrast to the huge effect cooling in HVC



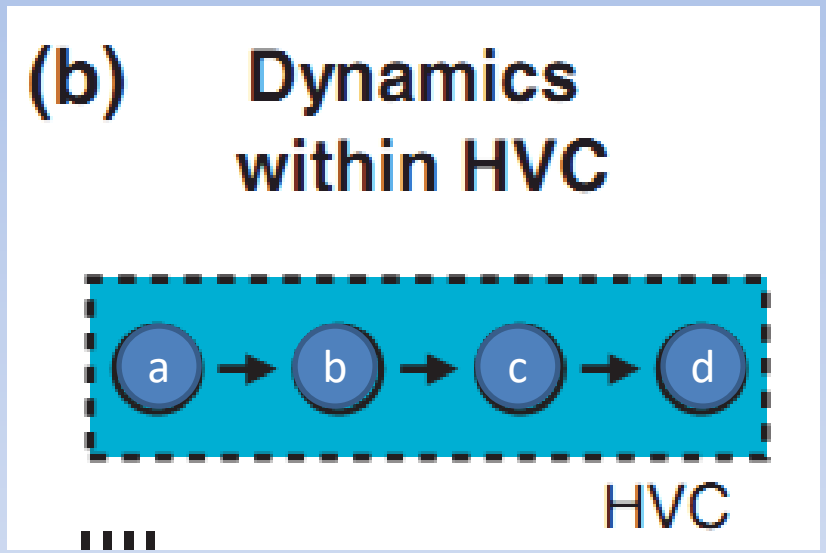
Long & Fee (*Nature*, 2008)

Are the ***short dynamics (within one syllable)*** generated within HVC? YES!!!



Fee & Long (*Curr Opin Neurobiol*, 2011)

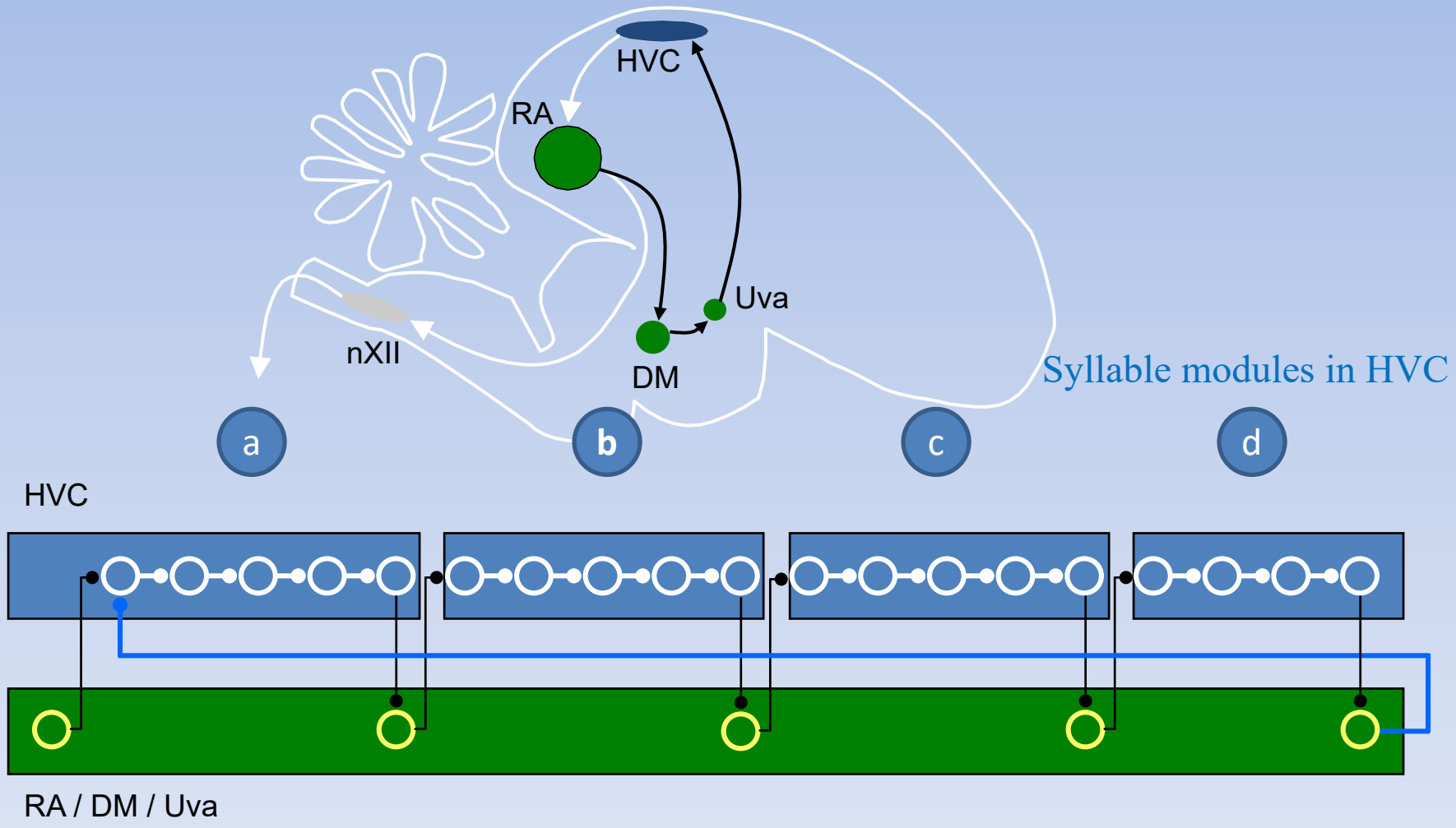
Where are the **long timescale** (switching between *syllables*) dynamics?



Syllable modules in HVC

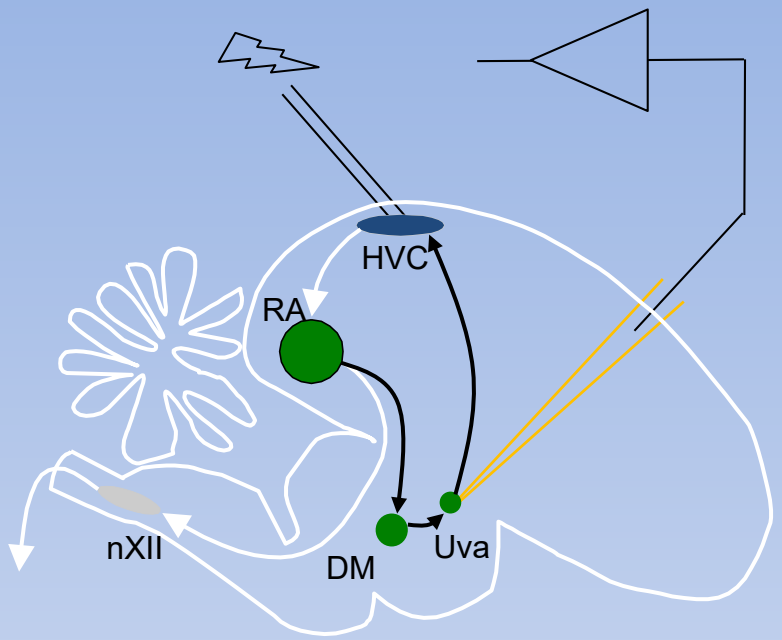
Fee & Long (*Curr Opin Neurobiol*, 2011)

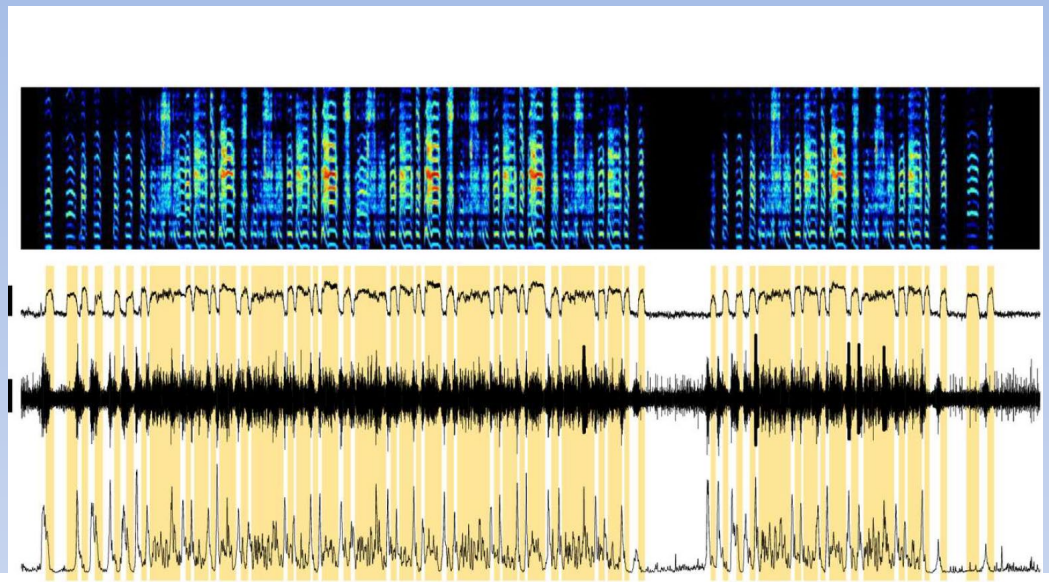
Uva likely triggers short chains in HVC

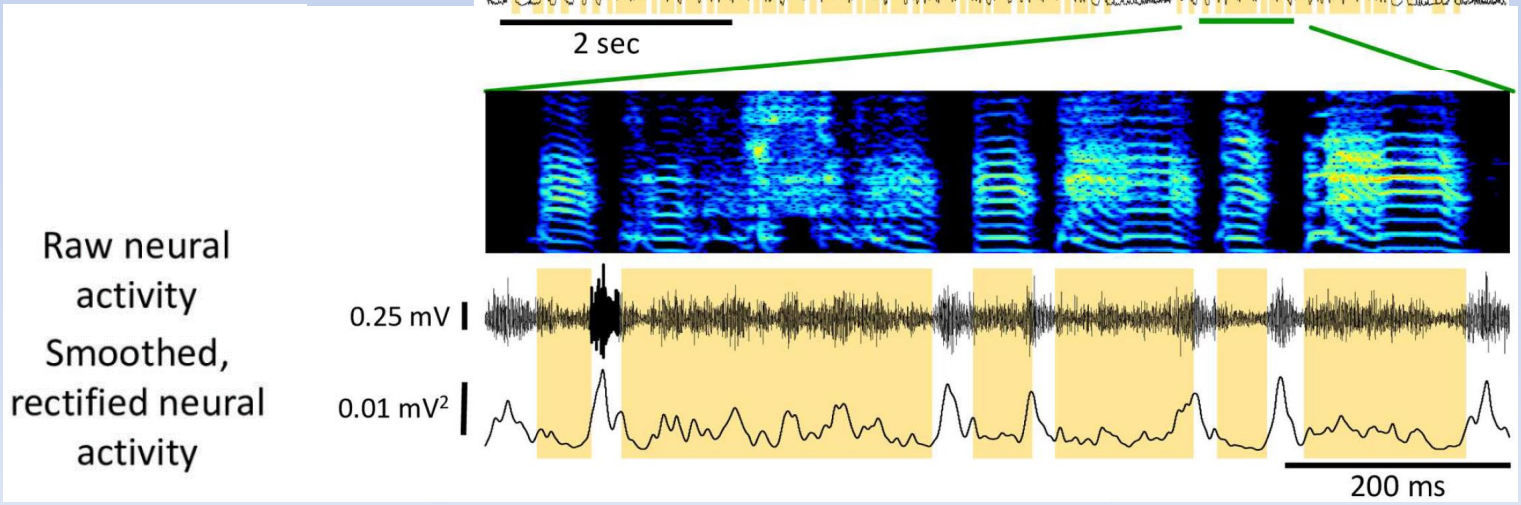


Modified from Fee MCN2017 lecture

Multiunit activity in Uva shows syllable-onset-related bursts







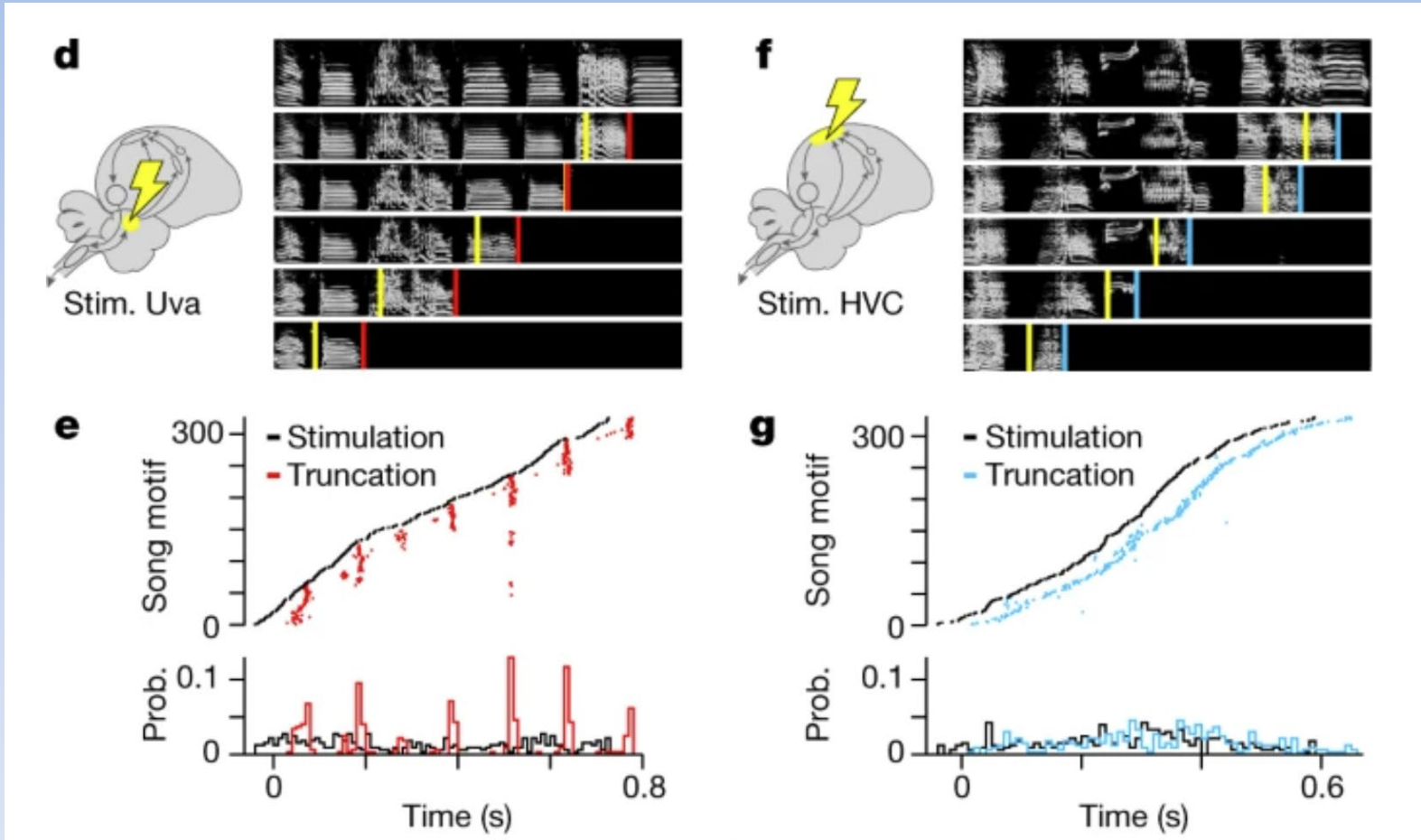
Danish, Aronov, Fee, 2017

Uva - the initiator for syllable

Using stimulation to induce song truncation

Uva perturbation led to truncations at the end of syllables, indicating that thalamic activity is critical for starting the following syllable but not for completing the ongoing syllable.

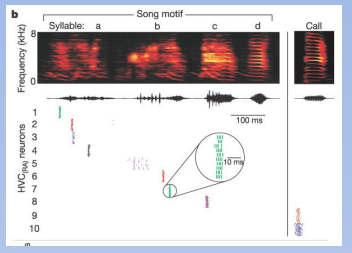
(in contrast to stimulation in HVC that induce truncation in a fix time delay)

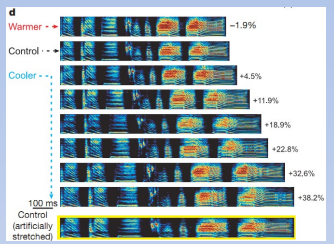


Thalamus drives vocal onsets in the zebra finch courtship song

Nature 2023 Felix W. Moll … & Michael A. Long

Part 1: Summary – A simple model of vocal sequence generation in adult birds



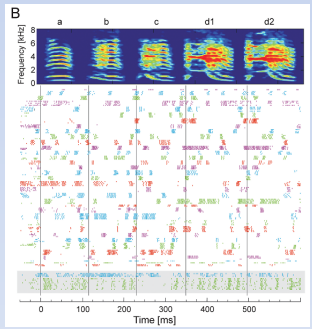


HVC:

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

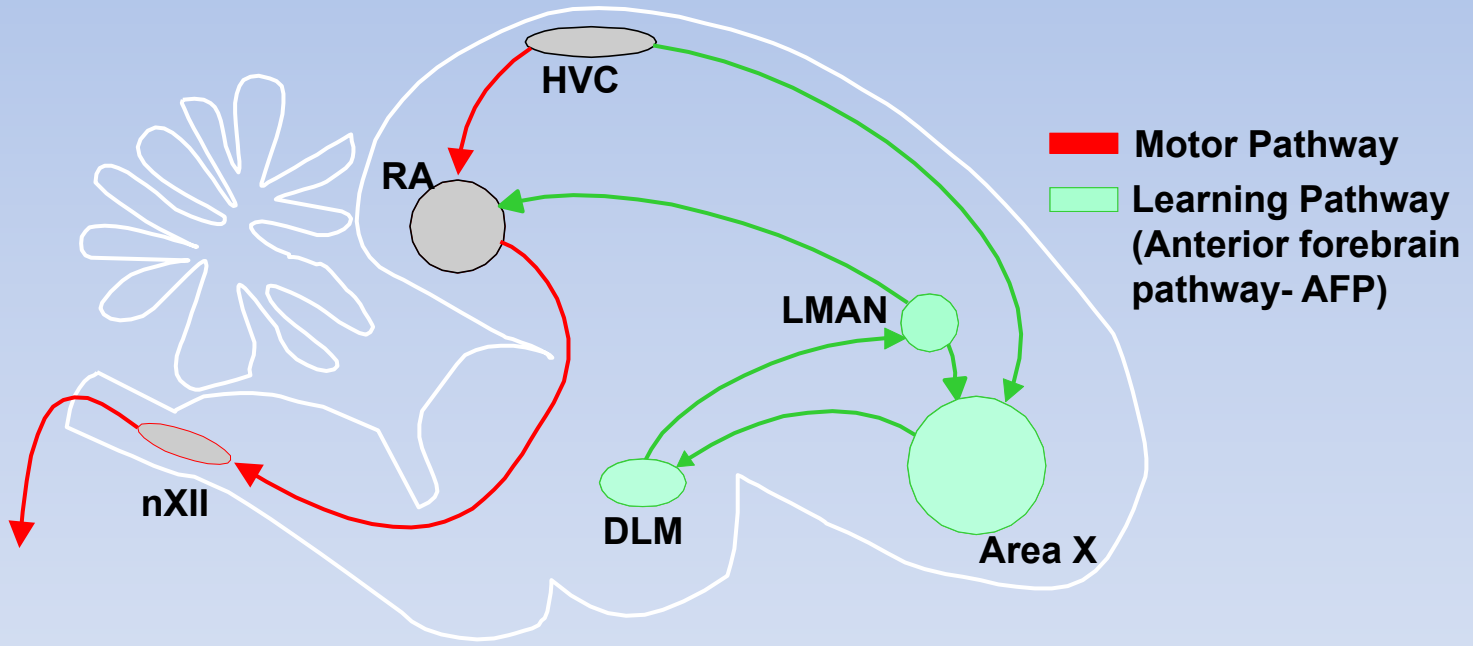
RA:

- 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 - 20 ms).



15 min break

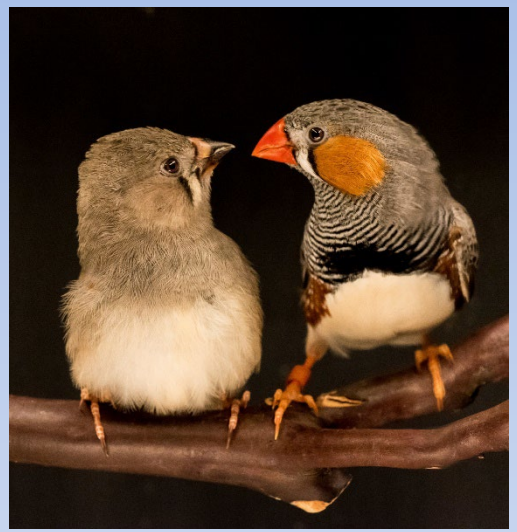
RA also gets input from LMAN!
What is the role of the learning pathway?

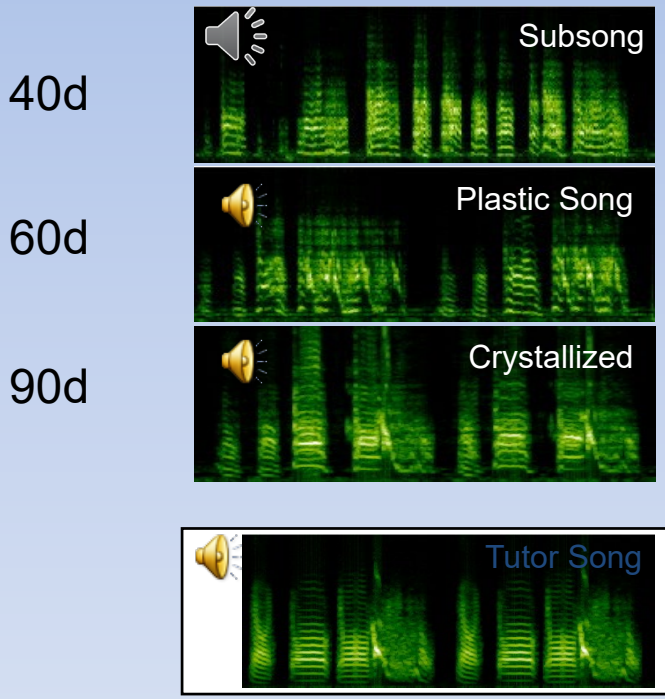


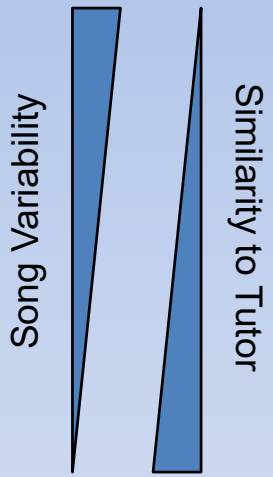
The motor pathway- if you lesion any of these song nuclei you disrupt the output of the song

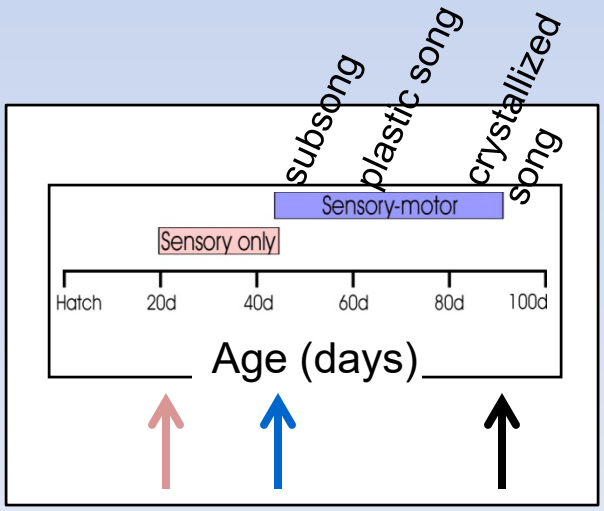
The learning pathway is not necessary for adult song production, but is required for learning (Bottjer, 1984, Scharff and Nottebohm, 1991, Goldberg @ Fee , 2010)

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?

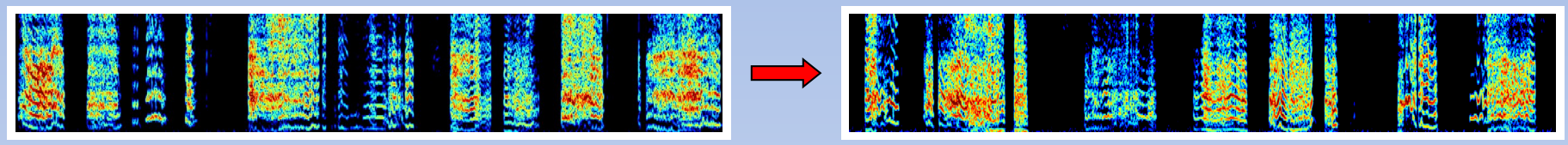
Do we need HVC for producing subsong?

What would you do to test this?

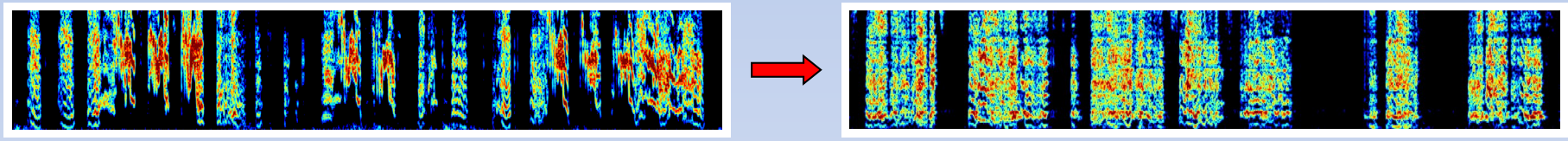
HVC-lesioned birds could still produce subsong!

Control **no HVC**

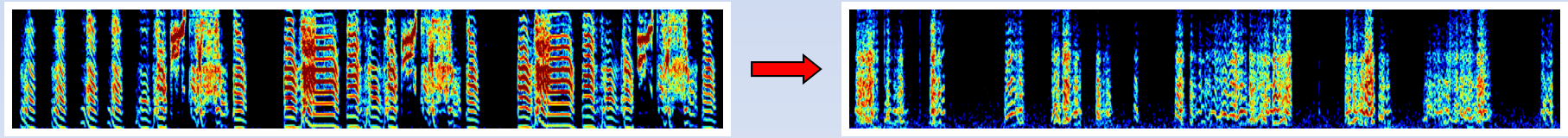
Subsong stage (37 dph)



Plastic song stage (50 dph)



Adult

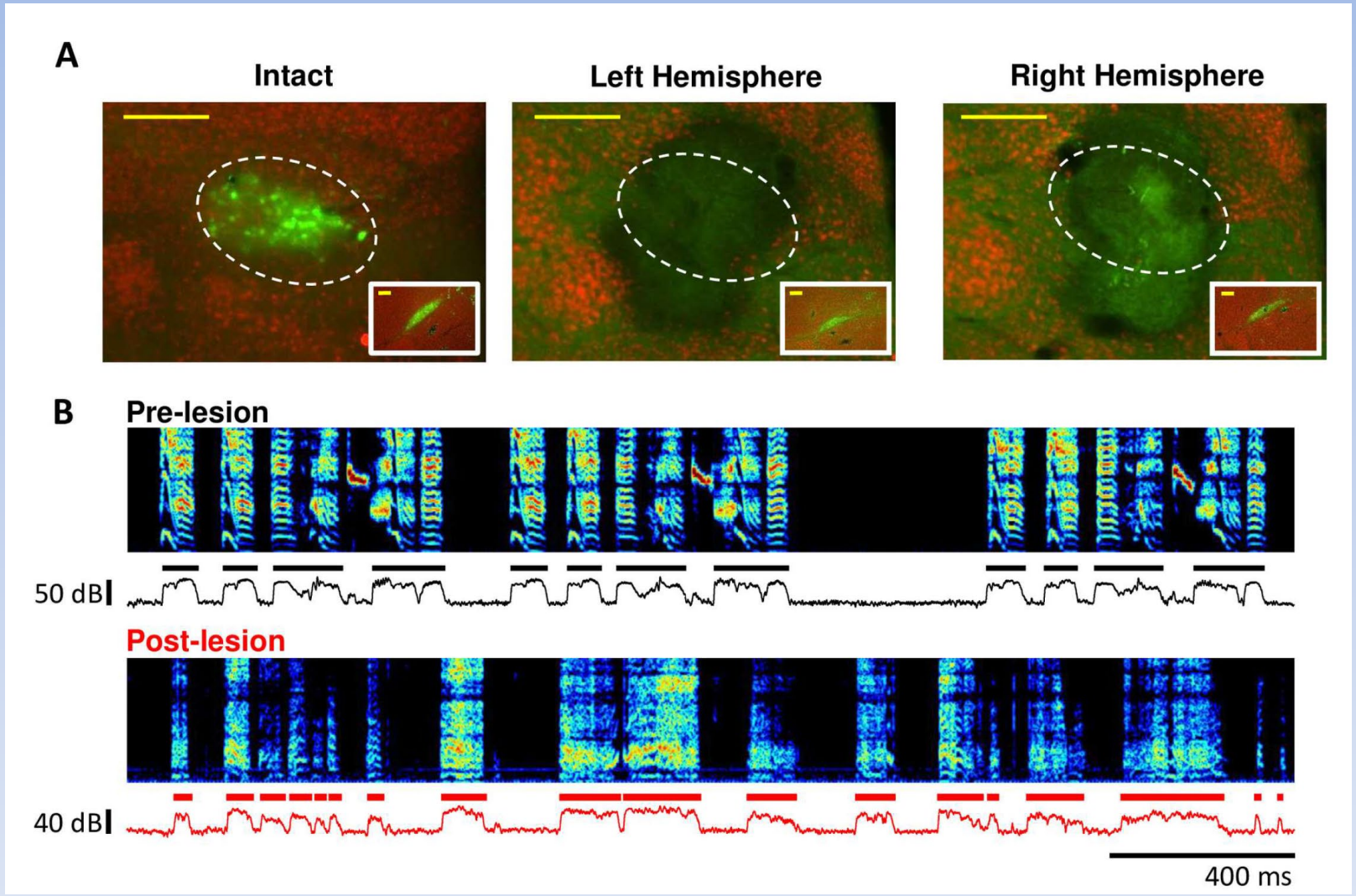


250 ms

Subsong does not require HVC

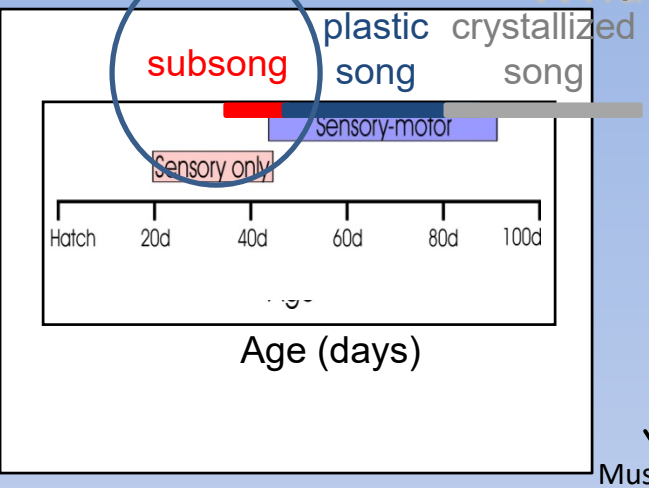
Aronov et.al. (2008) *Science*

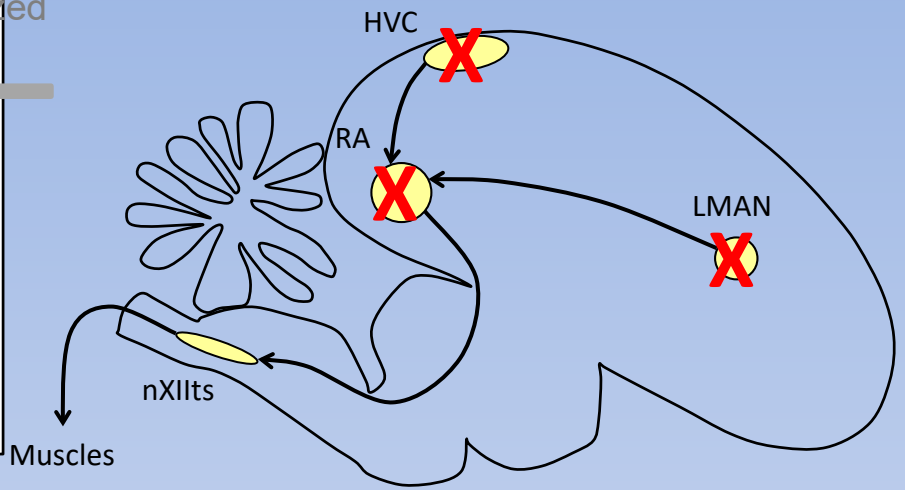
Uva lesions abolish song stereotypy

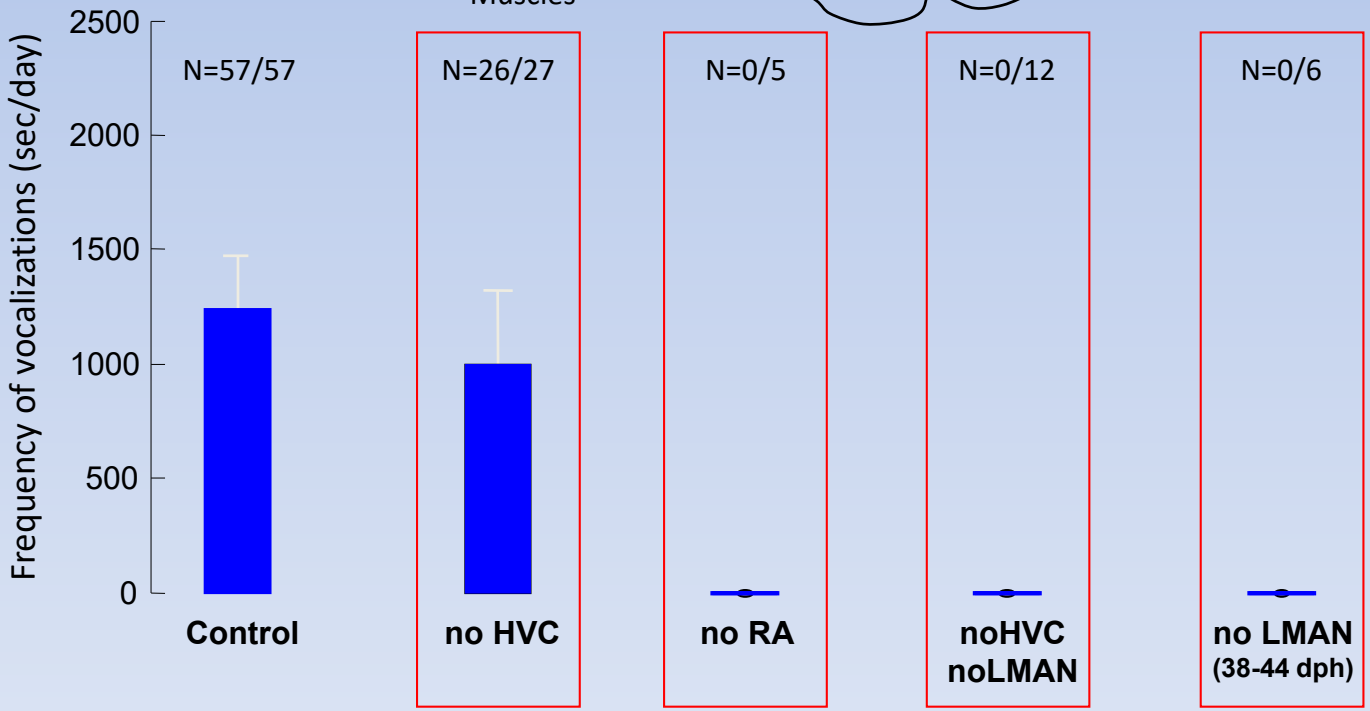


Danish, Aronov, Fee, 2017

What drives ***subsong*** production?



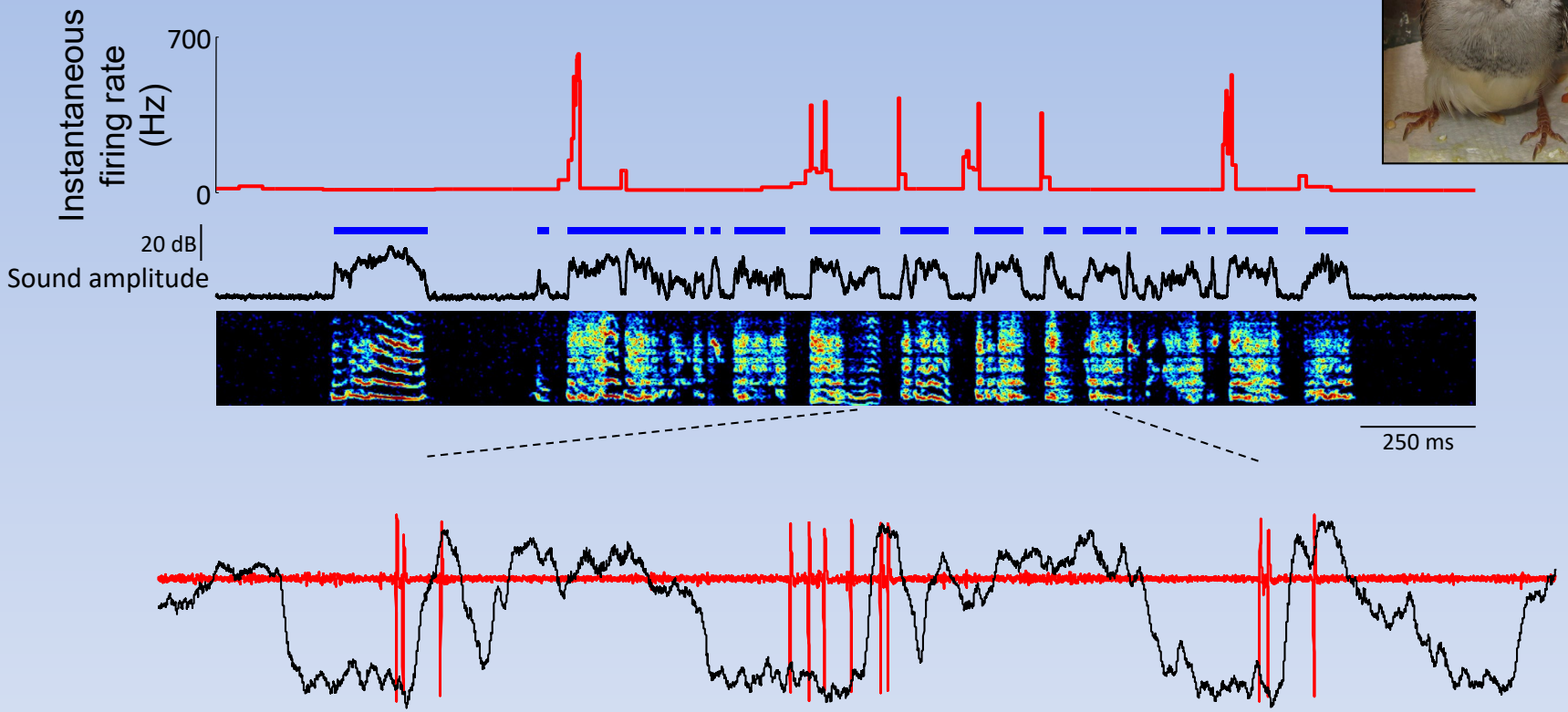




Babbling requires LMAN

LMAN drives subsong

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

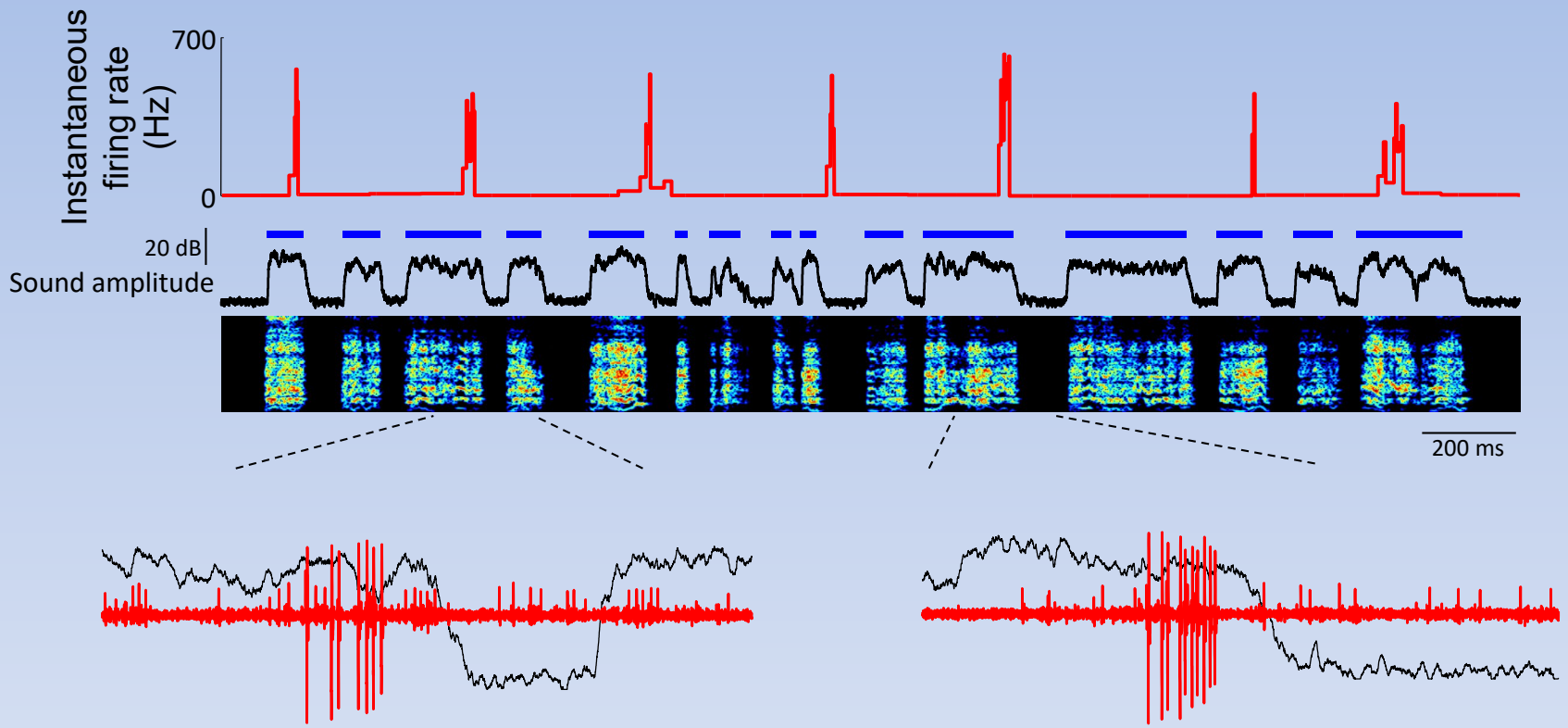


this suggest that babbling is not a result of immature motor pathway but it is actually deriving by this learning circuit

Aronov et.al. (2008) *Science*

LMAN drives subsong

LMAN neurons exhibit activity also prior to subsong syllable offsets

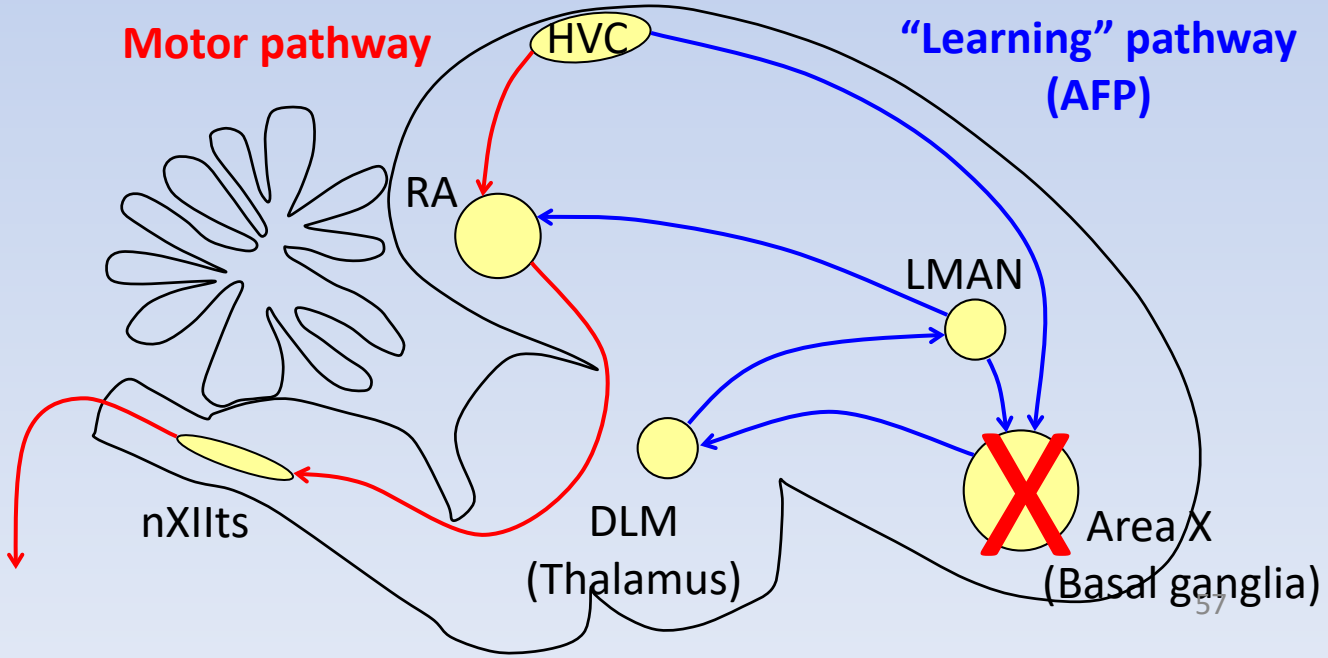


Aronov et.al. (2008) *Science*

- 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.

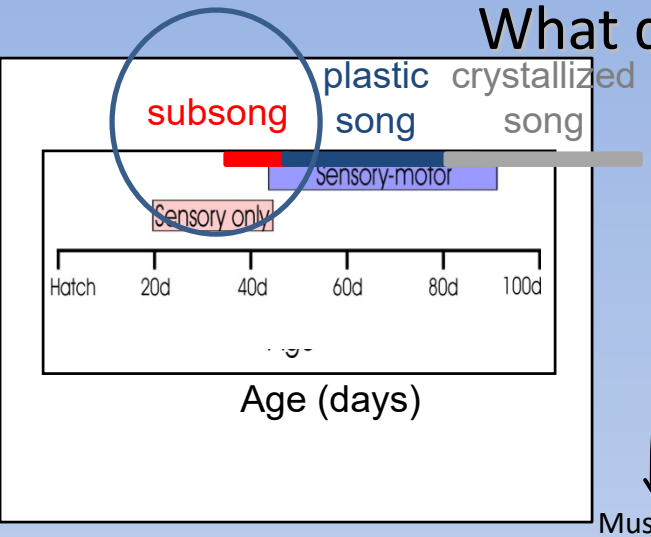
motor thalamus plays a key role in the expression of exploratory juvenile behaviors during learning

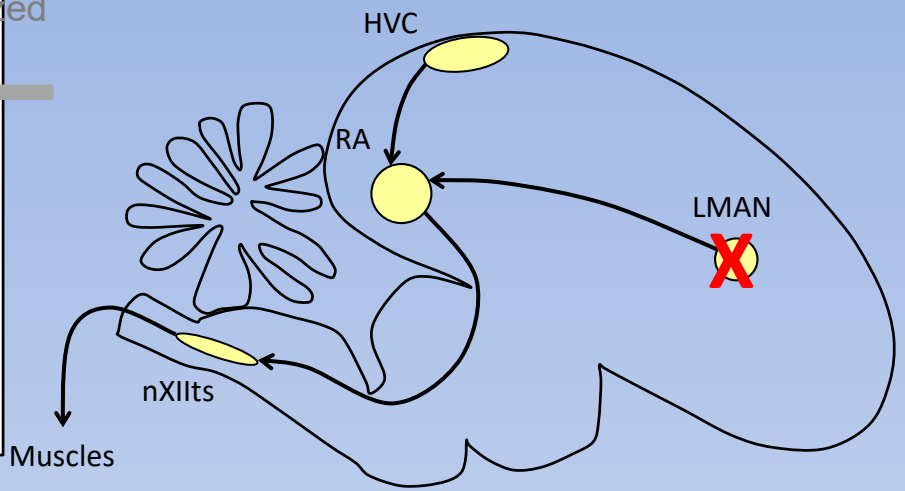


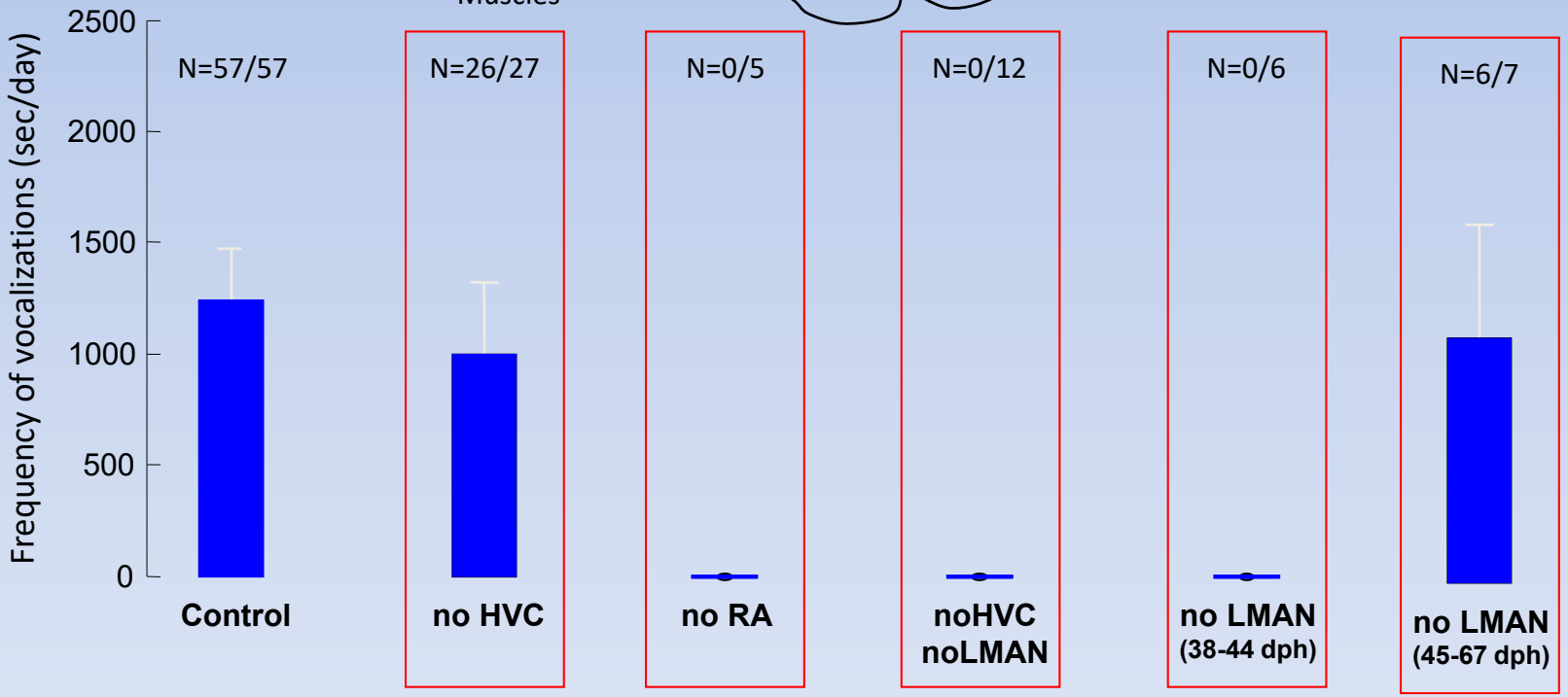
- **Question**:

  What is the role of LMAN in older juveniles?

What drives ***plastic*** song production?

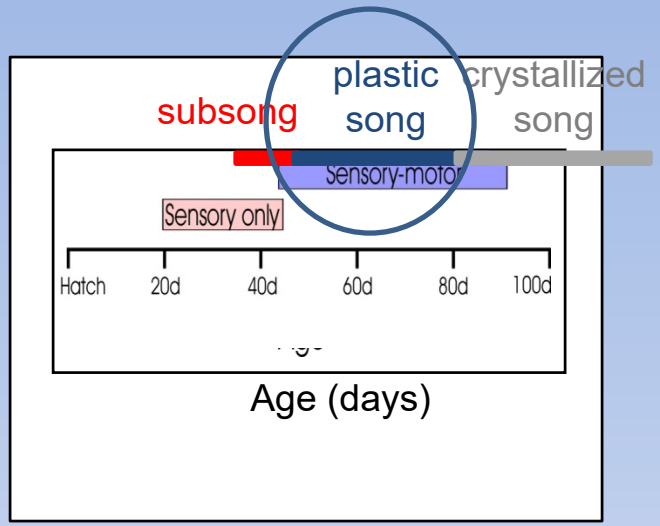


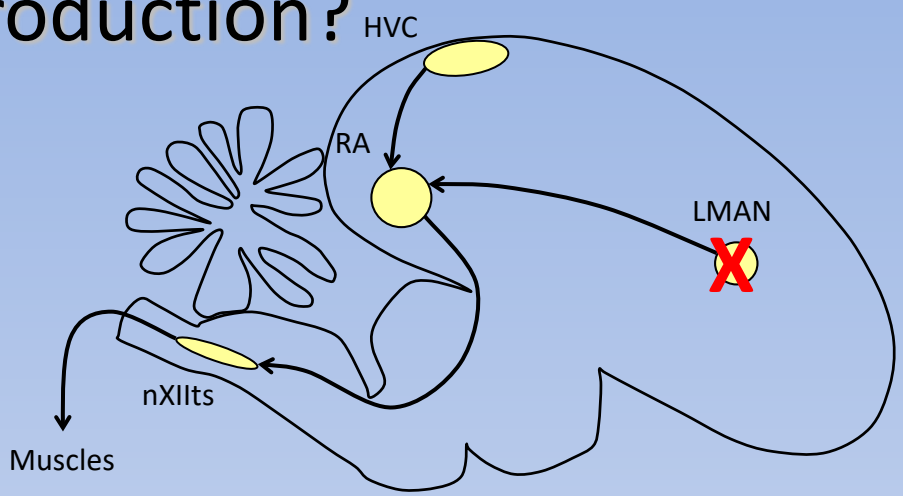




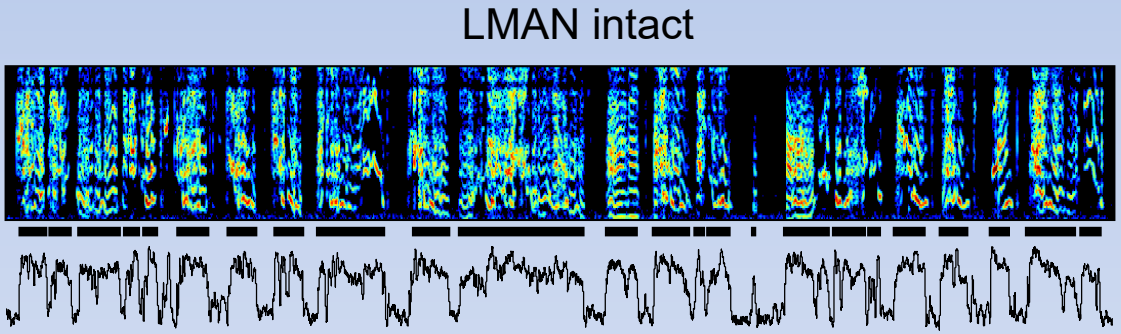
Aronov et.al. (2008) *Science*

What drives plastic song production?

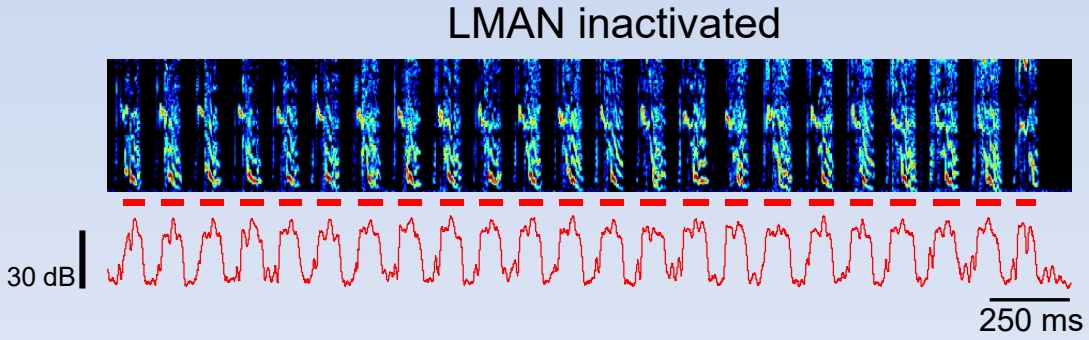




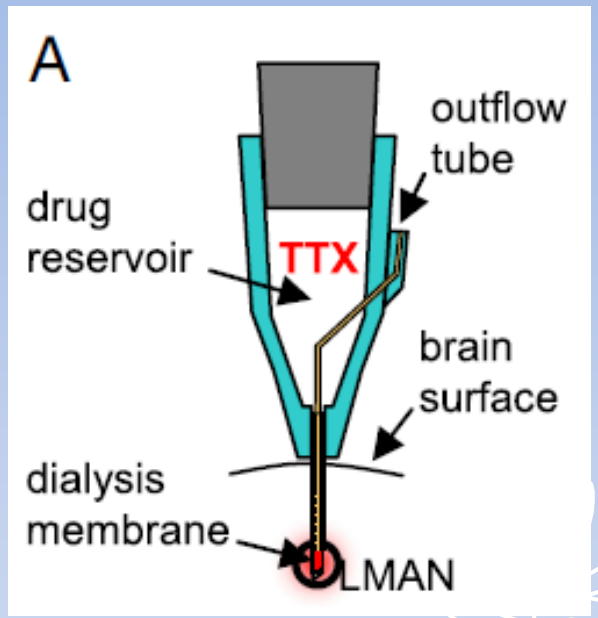
Subsong/plastic song requires LMAN

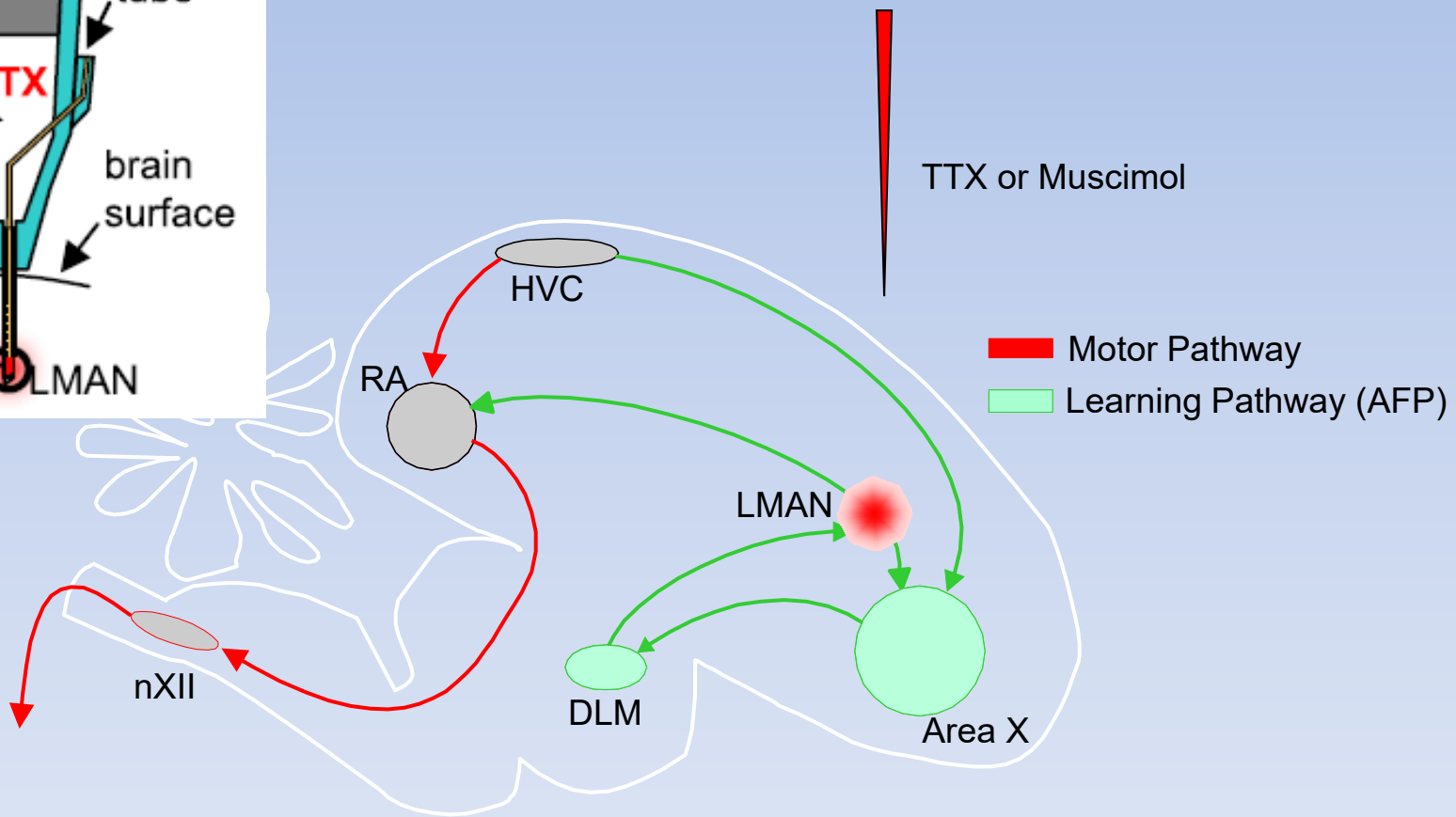


Babbling requires LMAN –> LMAN give rise to a high variability sounds, whereas HVC is slowly taking over to give structure into the song



Role of LMAN in older juveniles

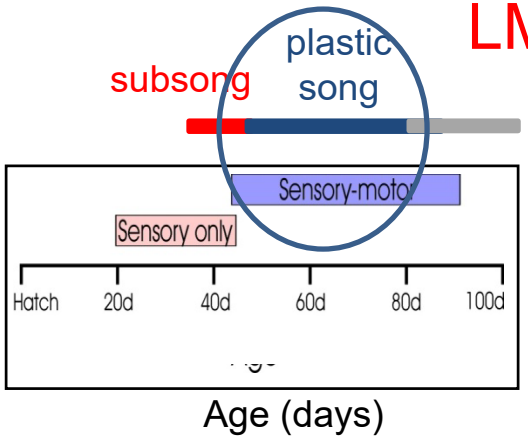


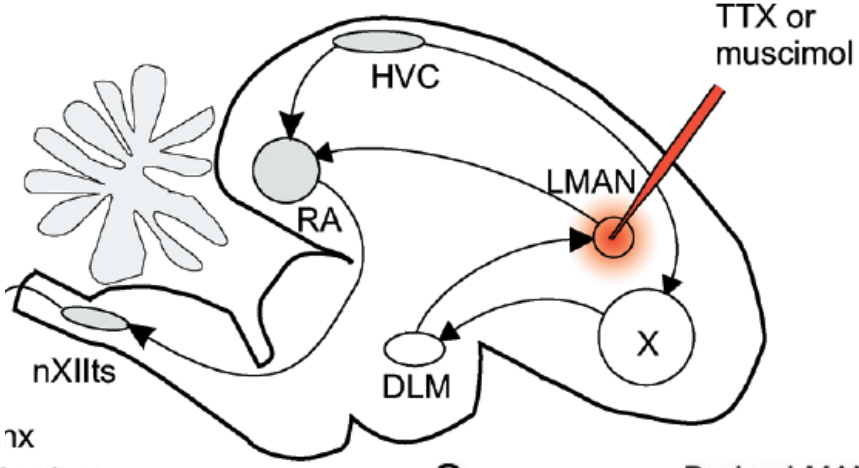


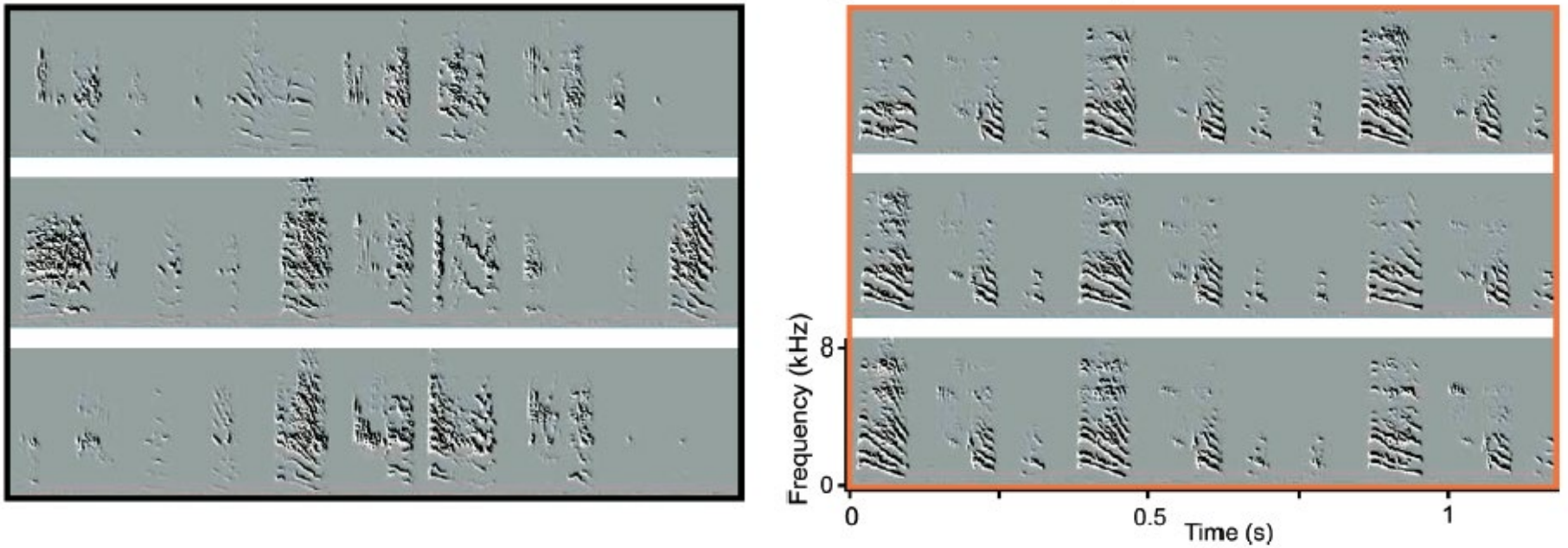
Ölveczky BP, Andalman AS, Fee MS (2005) Vocal Experimentation in the Juvenile Songbird Requires a Basal Ganglia Circuit.

Role of LMAN in older juveniles

LMAN is a generator of variability

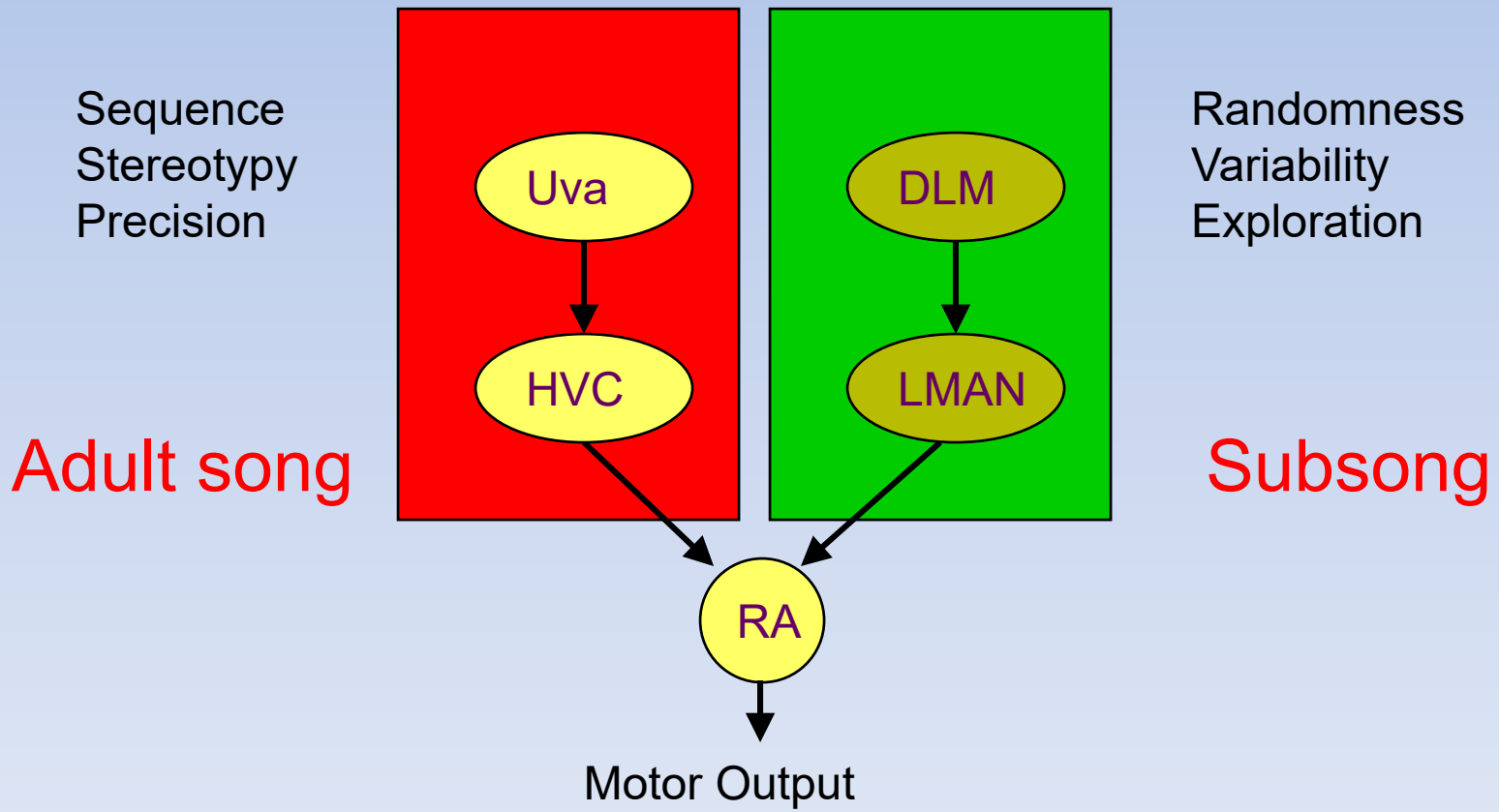




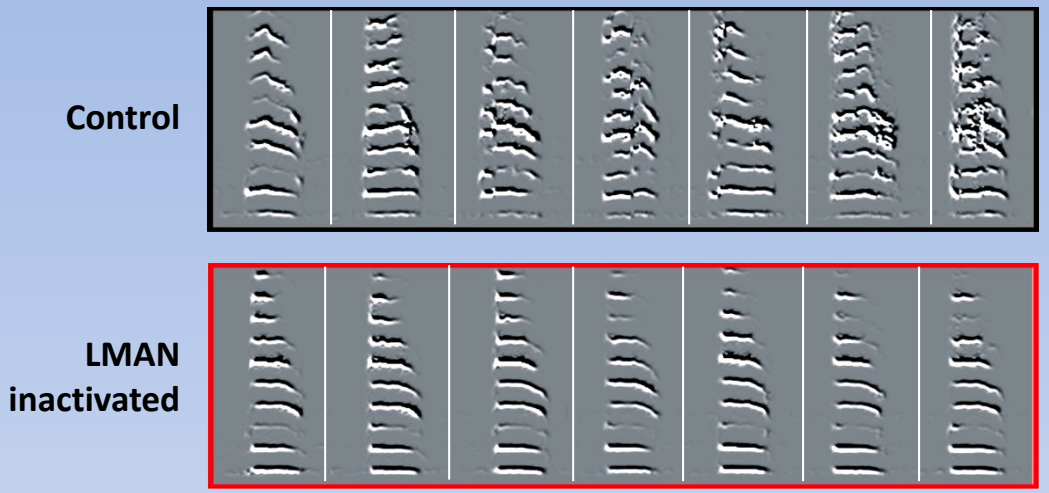


Ölveczky BP, Andalman AS, Fee MS (2005) Vocal Experimentation in the Juvenile Songbird Requires a Basal Ganglia Circuit. PLoS Biol 3(5): e153. doi:10.1371/journal.pbio.0030153

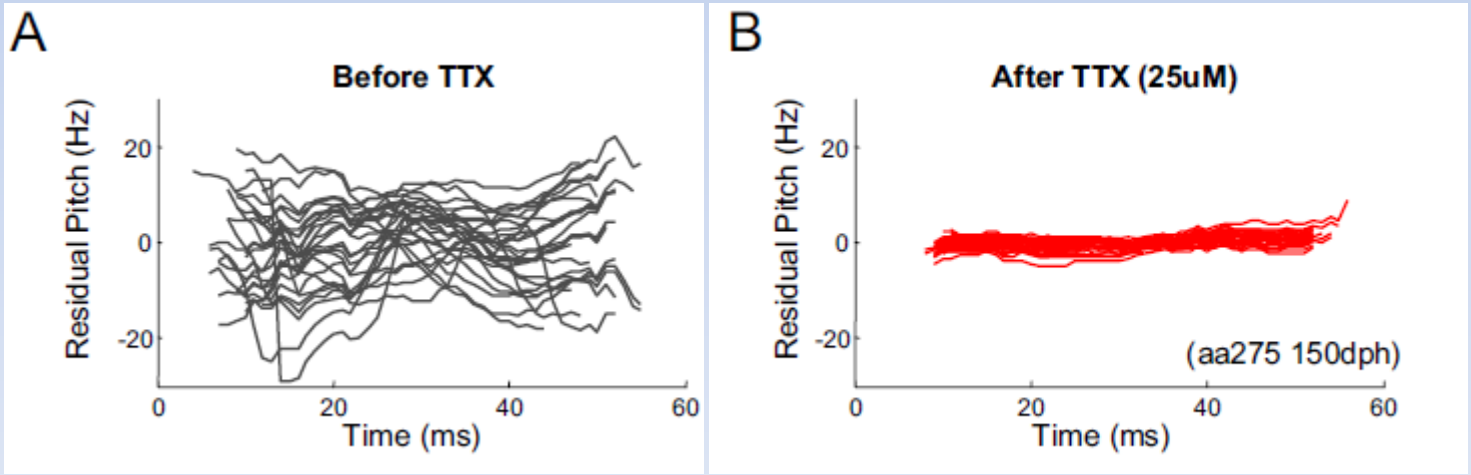
Separate premotor pathways for stereotyped song and babbling



Reduced variability in pitch after LMAN inactivation during crystalized song

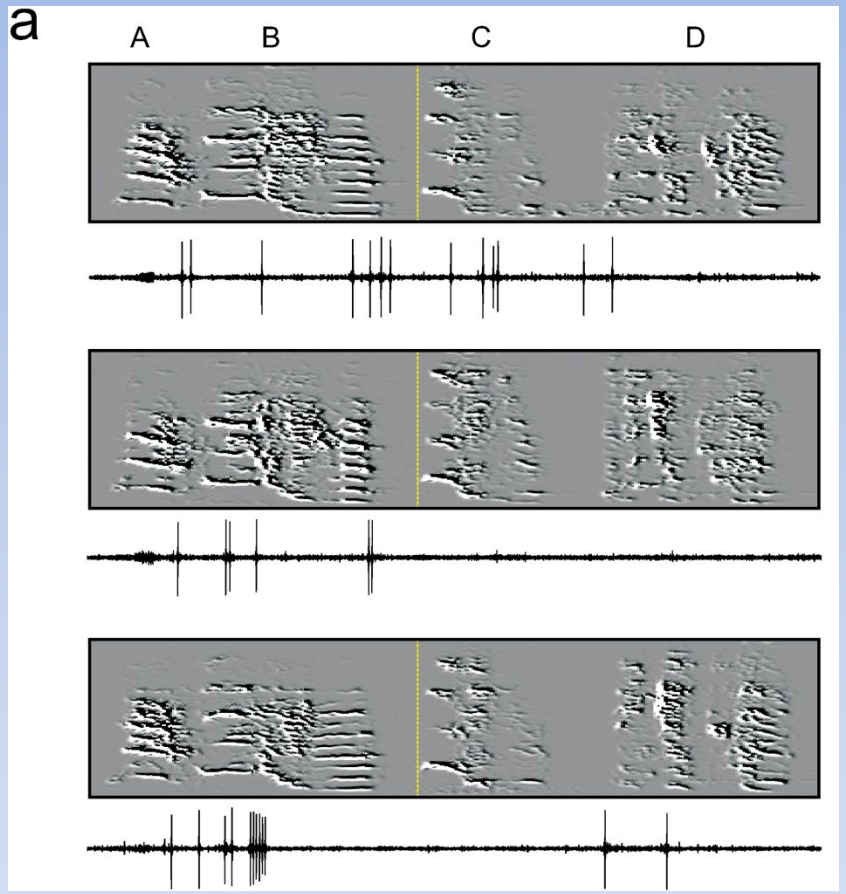


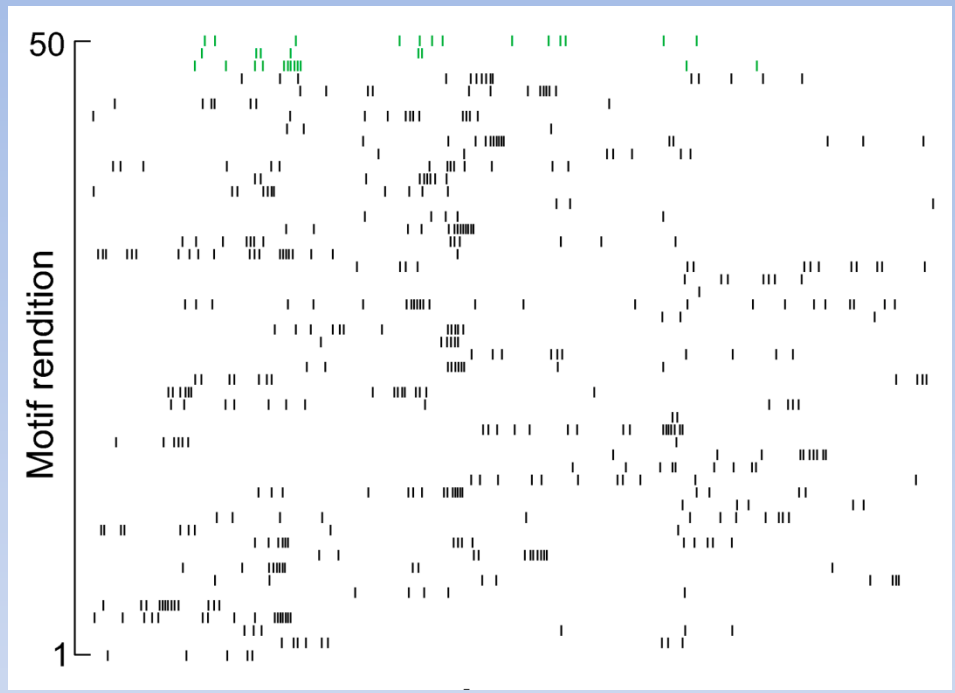
LMAN involves in injecting stochastic noise into the naive behavior so to have more variation on which to select the better performance.



Ölveczky BP, Andalman AS, Fee MS (2005) Vocal Experimentation in the Juvenile Songbird Requires a Basal Ganglia Circuit. PLoS Biol 3(5): e153. doi:10.1371/journal.pbio.0030153

LMAN activity in older juveniles





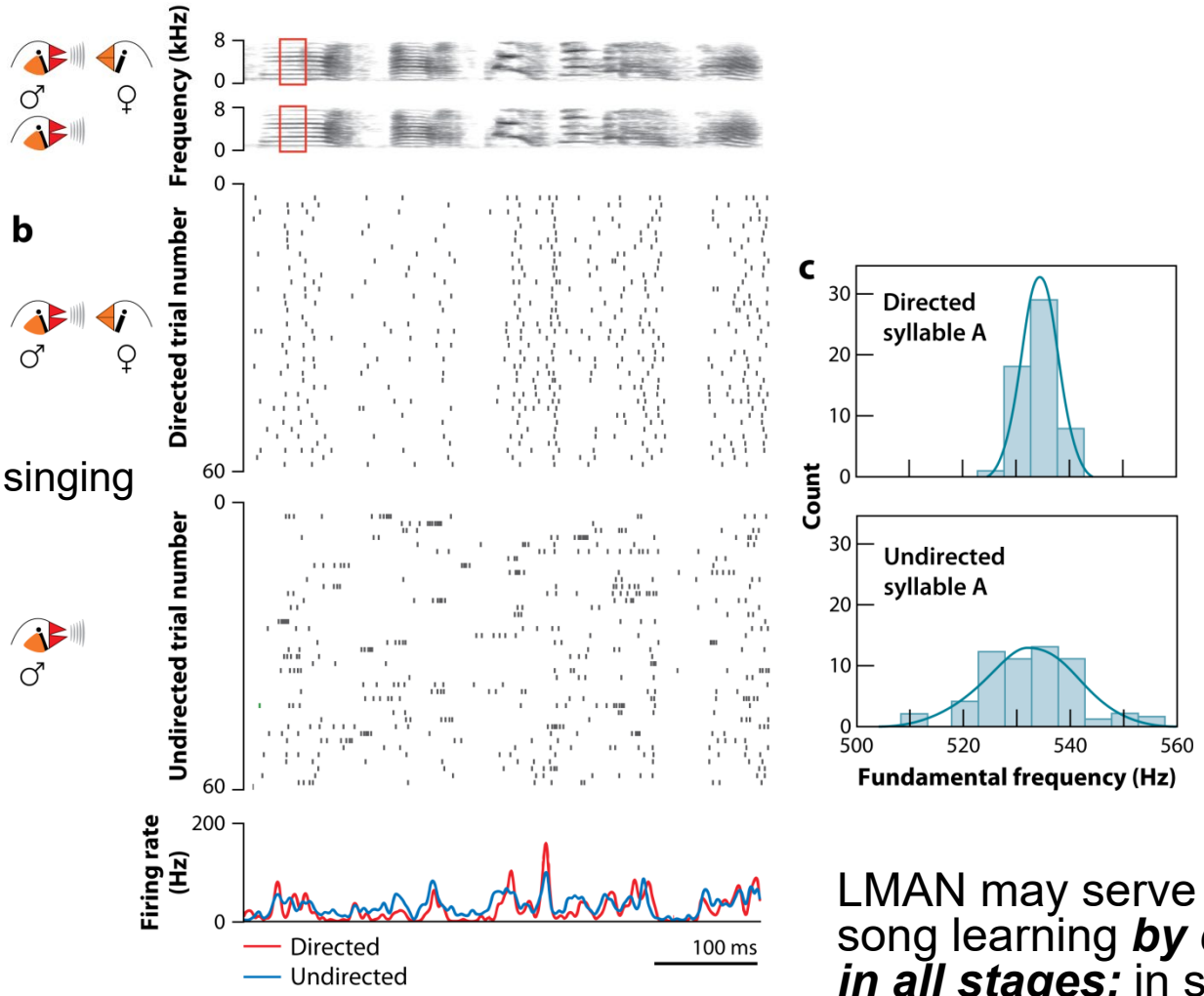
Ölveczky BP, Andalman AS, Fee MS (2005) Vocal Experimentation in the Juvenile Songbird Requires a Basal Ganglia Circuit. PLoS Biol 3(5): e153. doi:10.1371/journal.pbio.0030153

The crystallized songs of male zebra finches display different amounts of acoustic variability depending on social context

Directed singing

Undirected singing

rehearsal?



Brainard MS, Doupe AJ. 2013.
Annu. Rev. Neurosci. 36:489–517
Kao et al. (Journal of Neuroscience, 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.

Role of LMAN in learning

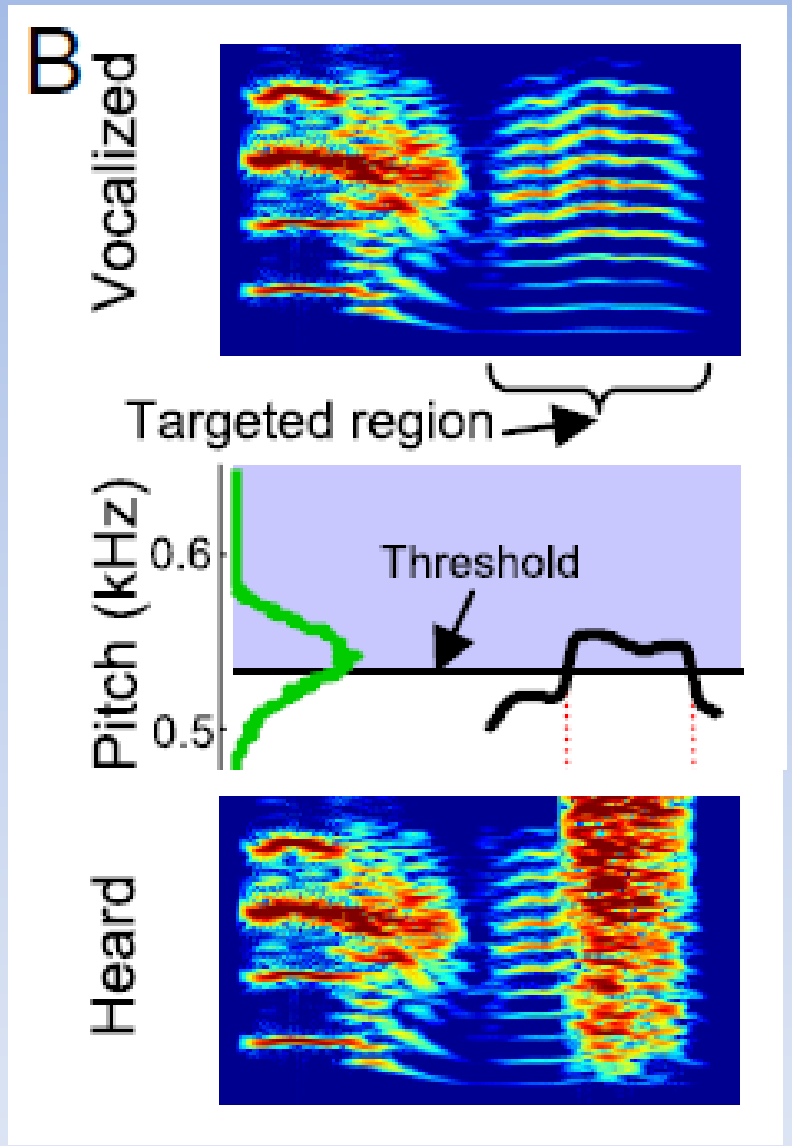
- Question
  - **Is variability purely random or is it biased?**

- Difficulty
  - Song learning is a slow process.

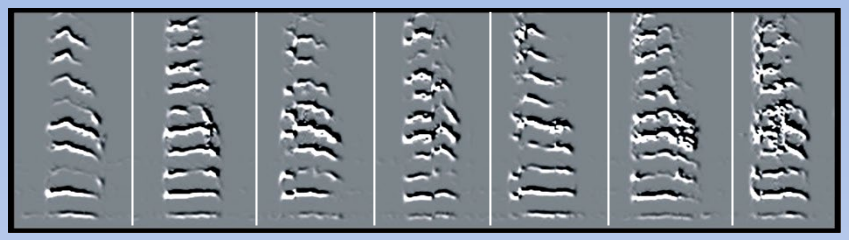
- Strategy
  - Use real-time feedback to induce error in the song artificially (using young adult- (1) sing a lot, (2) still show high variablilty)

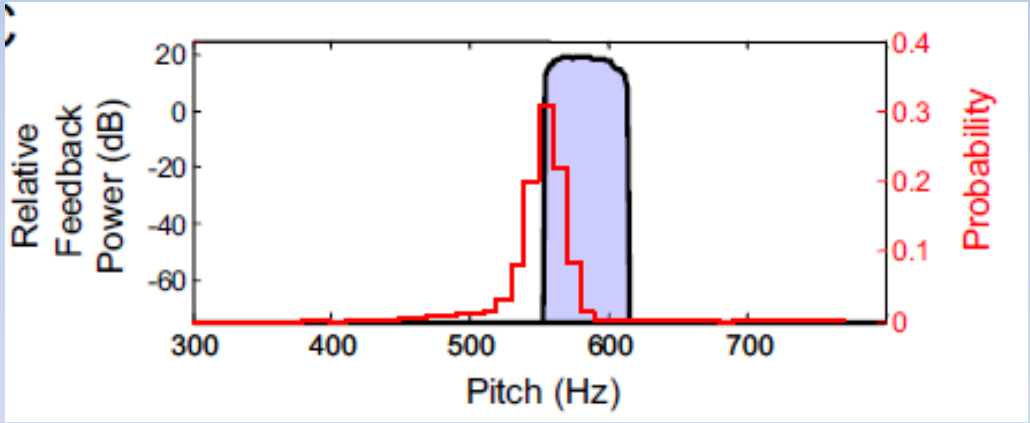
The AFP does more than generate variability!

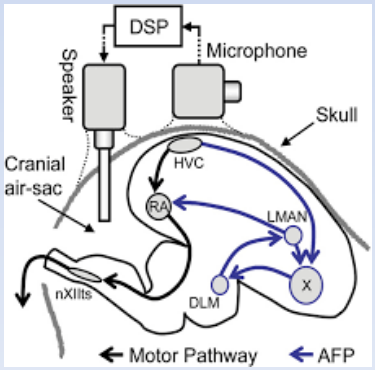
Conditional auditory feedback



Control

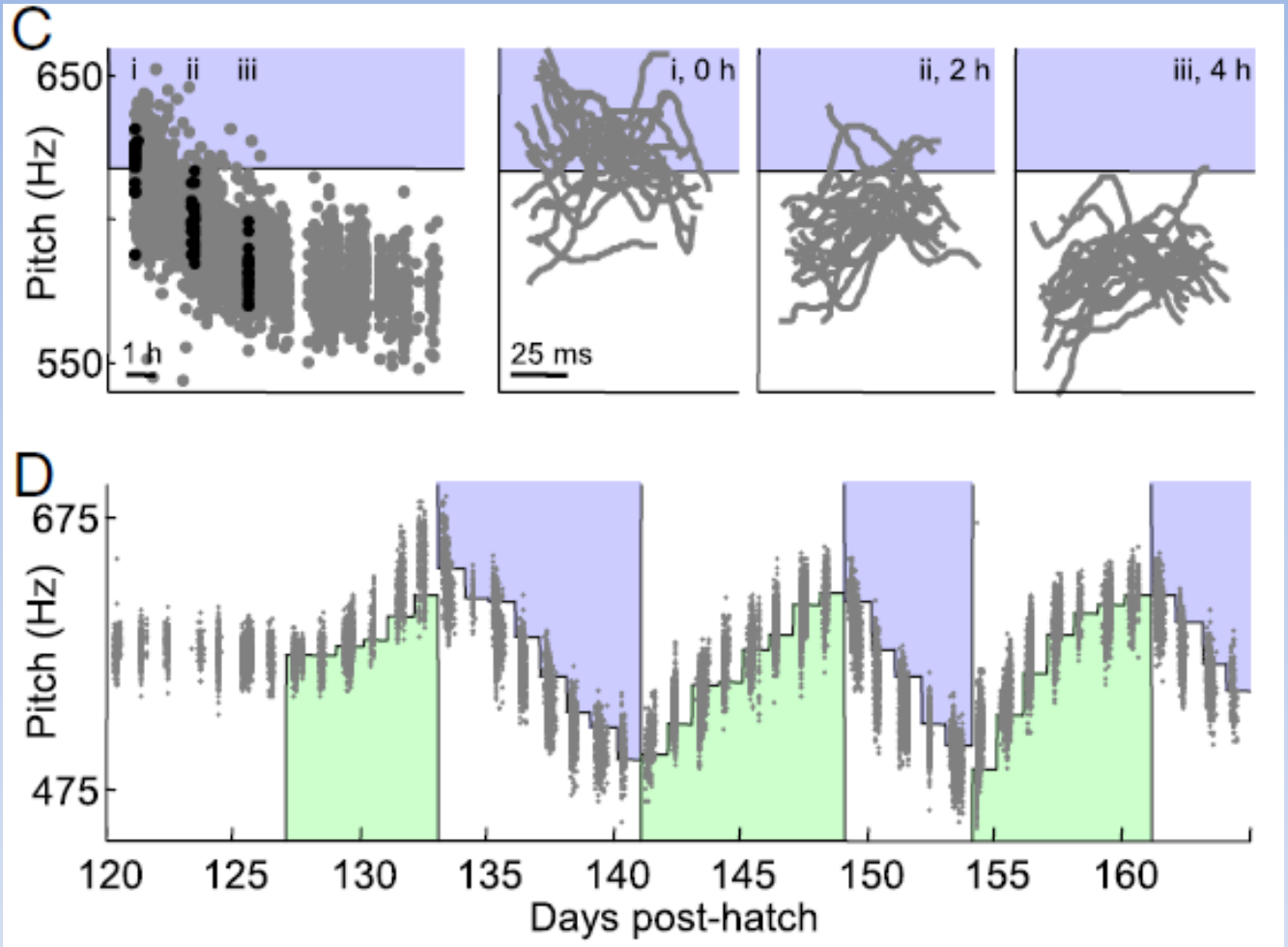






Tumer & Brainard (Nature, 2007)
Andalman & Fee (PNAS, 2009)

Behavioral results – young adult sings more undirected song



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 a more directional way.

Andalman & Fee (PNAS, 2009)

Two possibilities

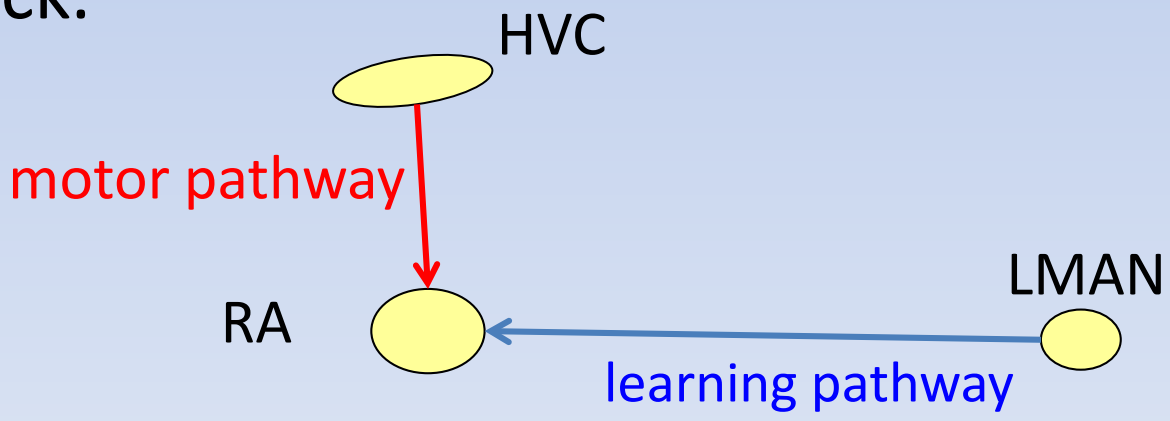
Where does the learning take place?

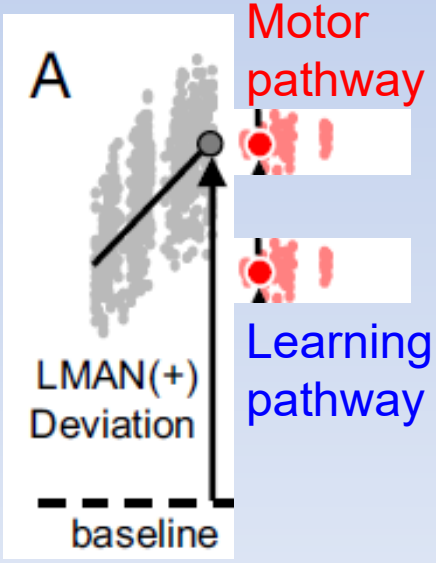
- Hypothesis 1: motor pathway

  → LMAN inactivation will not change the pitch.

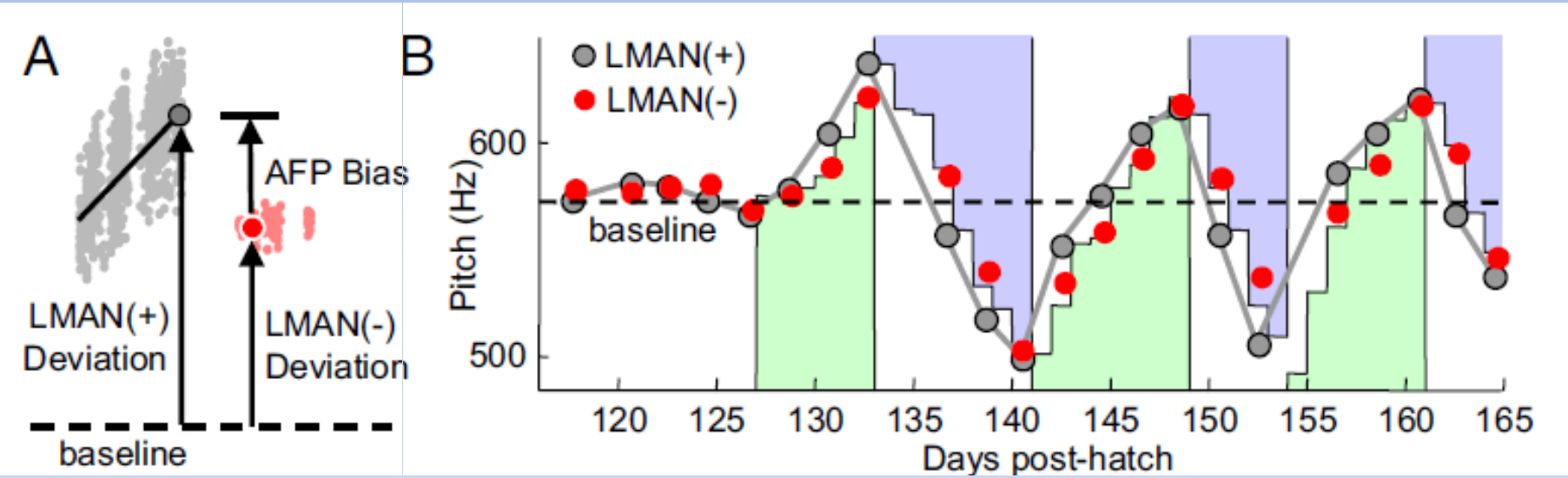
- Hypothesis 2 : learning pathway

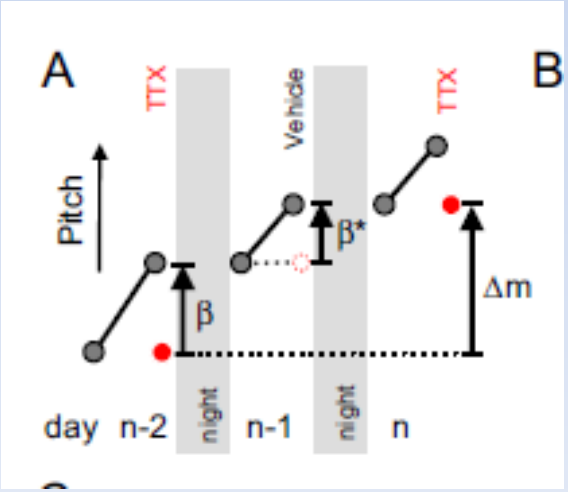
  → LMAN inactivation will change the pitch to go back.





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)

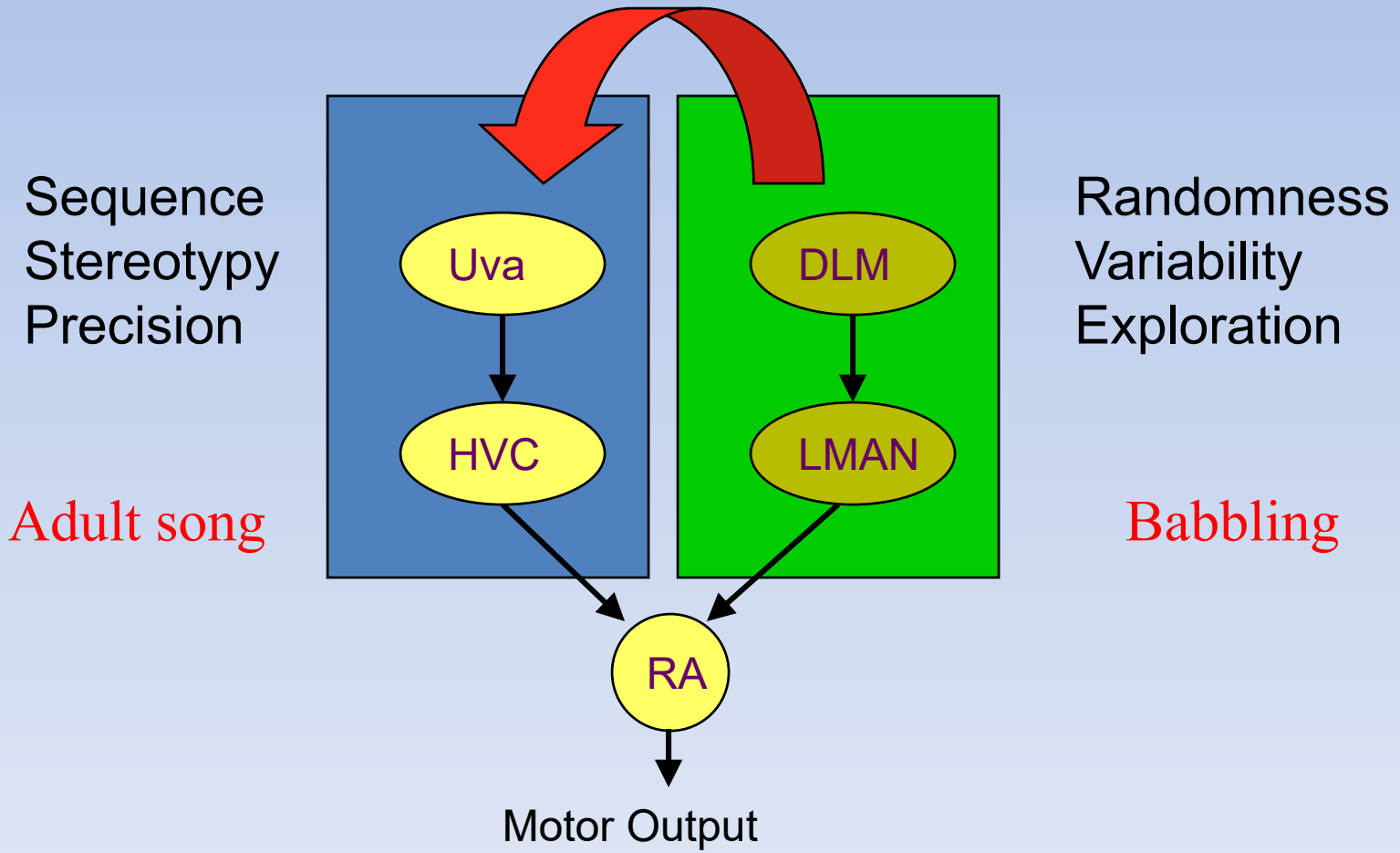
LMAN is a generator of variability

- 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 all stages:*** in subsong, plastic song, and even in adult song (Kao et al, 2005).

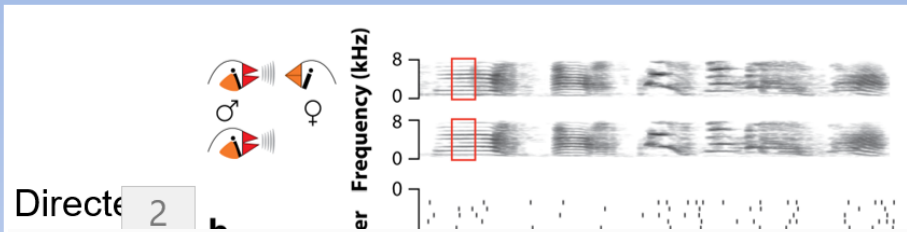
- ***LMAN adds variability to enable exploration***. This variability produced by the learning pathway is ***not purely random***, but instead ***biased***.
- This bias is ***consolidated*** in the motor pathway after one day delay.

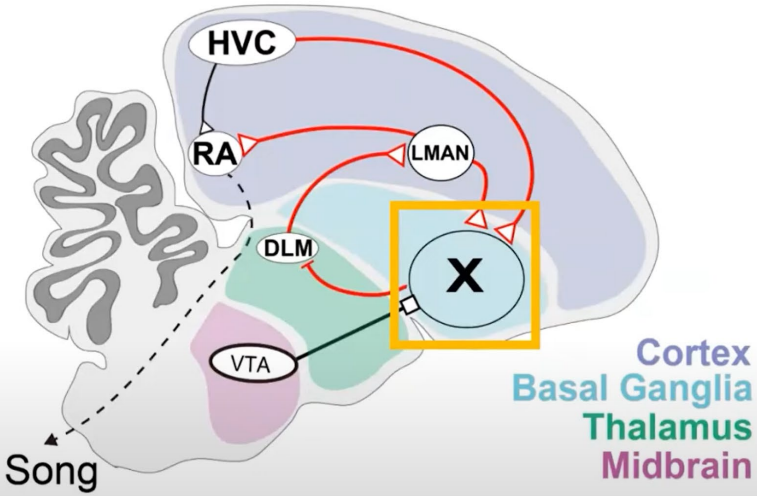
Separate premotor pathways for stereotyped song and babbling

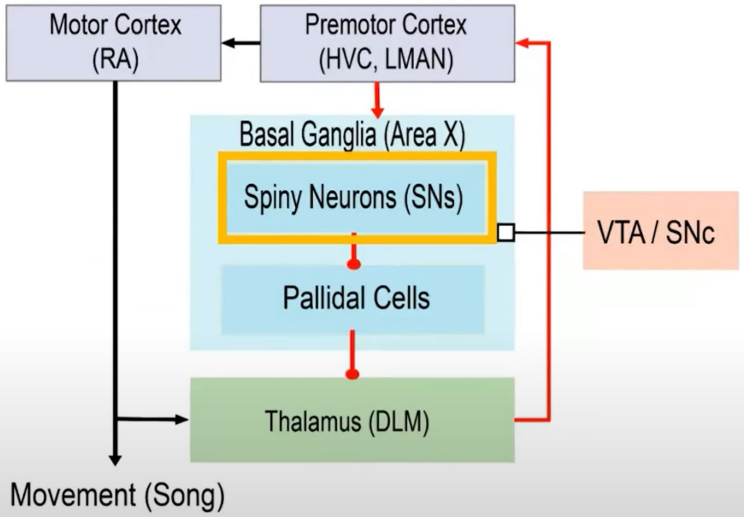


Going back to adult: what is behind the stereotype directed singing vs the more variable undirected song

The crystallized songs of male zebra finches display different amounts of acoustic variability depending on social context





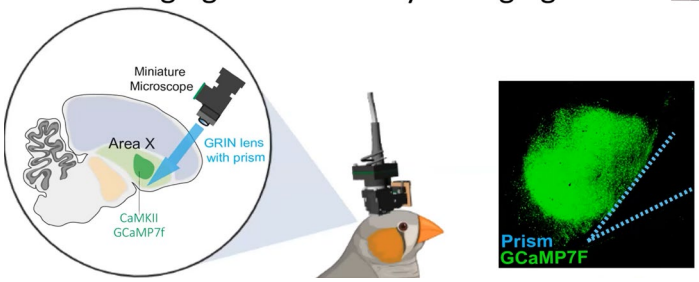


Brainard MS, Doupe AJ. 2013.
Annu. Rev. Neurosci. 36:489–517

song learning ***by driving variability in all stages:*** in subsong, plastic song, and even in adult song.

Neu
Nat

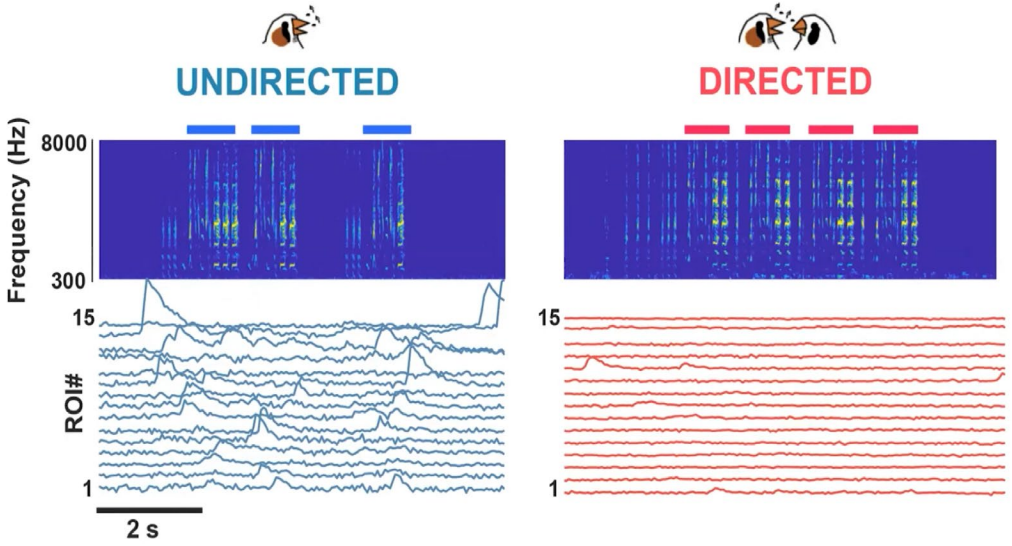
Imaging neural activity in singing birds



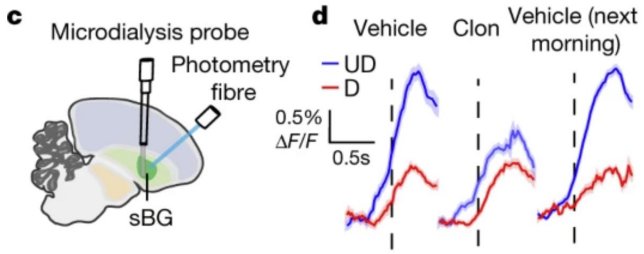
Ensemble activity of Spiny neurons in area X

During practice mode: is song-specific and highly variable

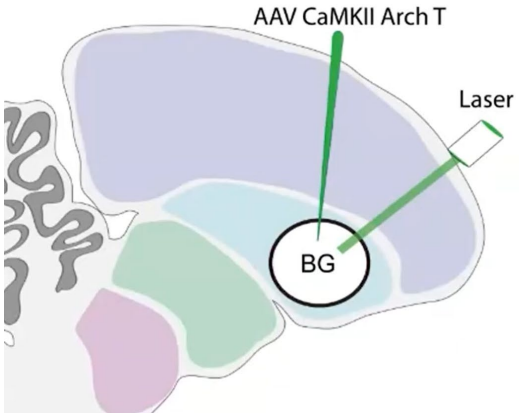
During directed song: activity is suppressed

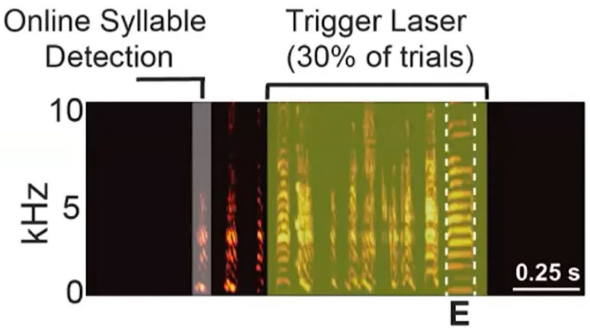


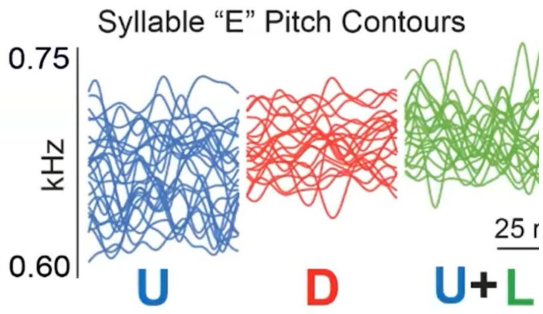
Noradrenergic signalling reduces vocal variability by directly suppressing SN activity



Optically silencing spiny neurons reduces variability seen in undirected song







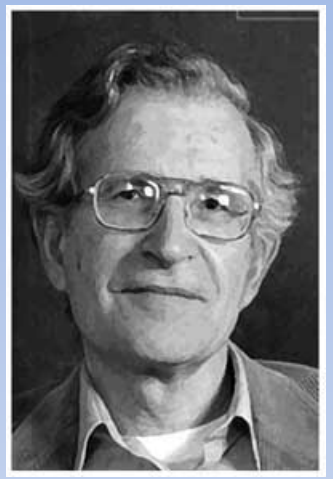
Innate versus learned

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

https://www.youtube.com/watch?v=fOIM1_xOSro

Chomsky's linguistic theory

Chomsky



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.

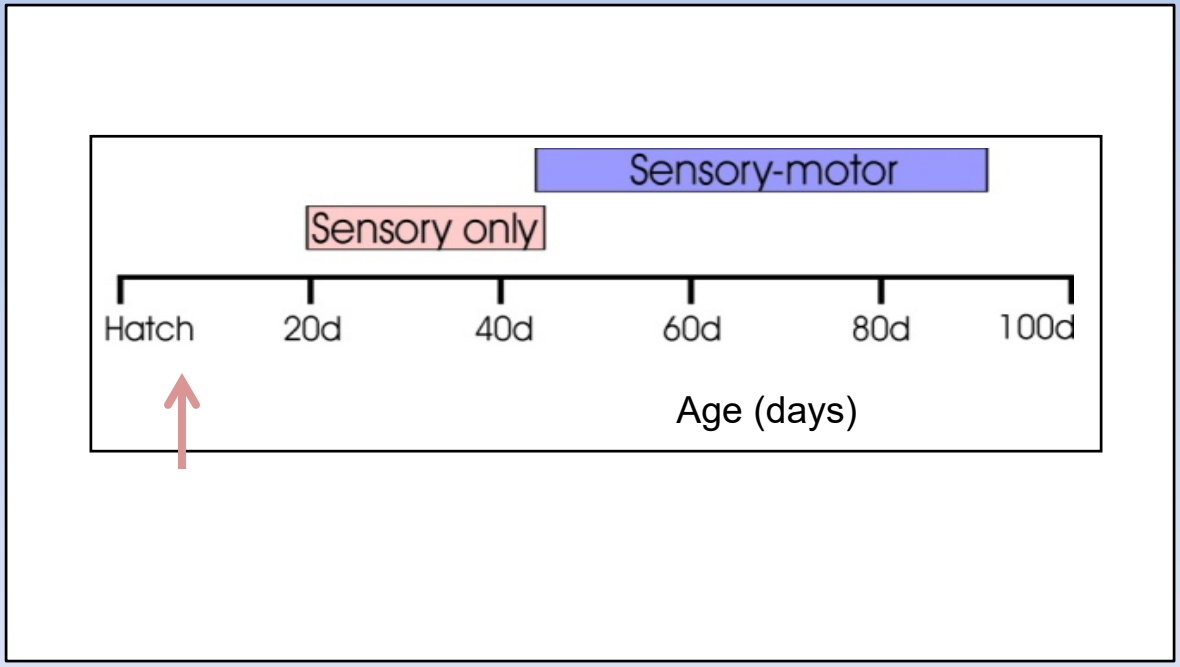
Ofer Tchernichovski:
Do genes constrain song diversity?

The City University of New York

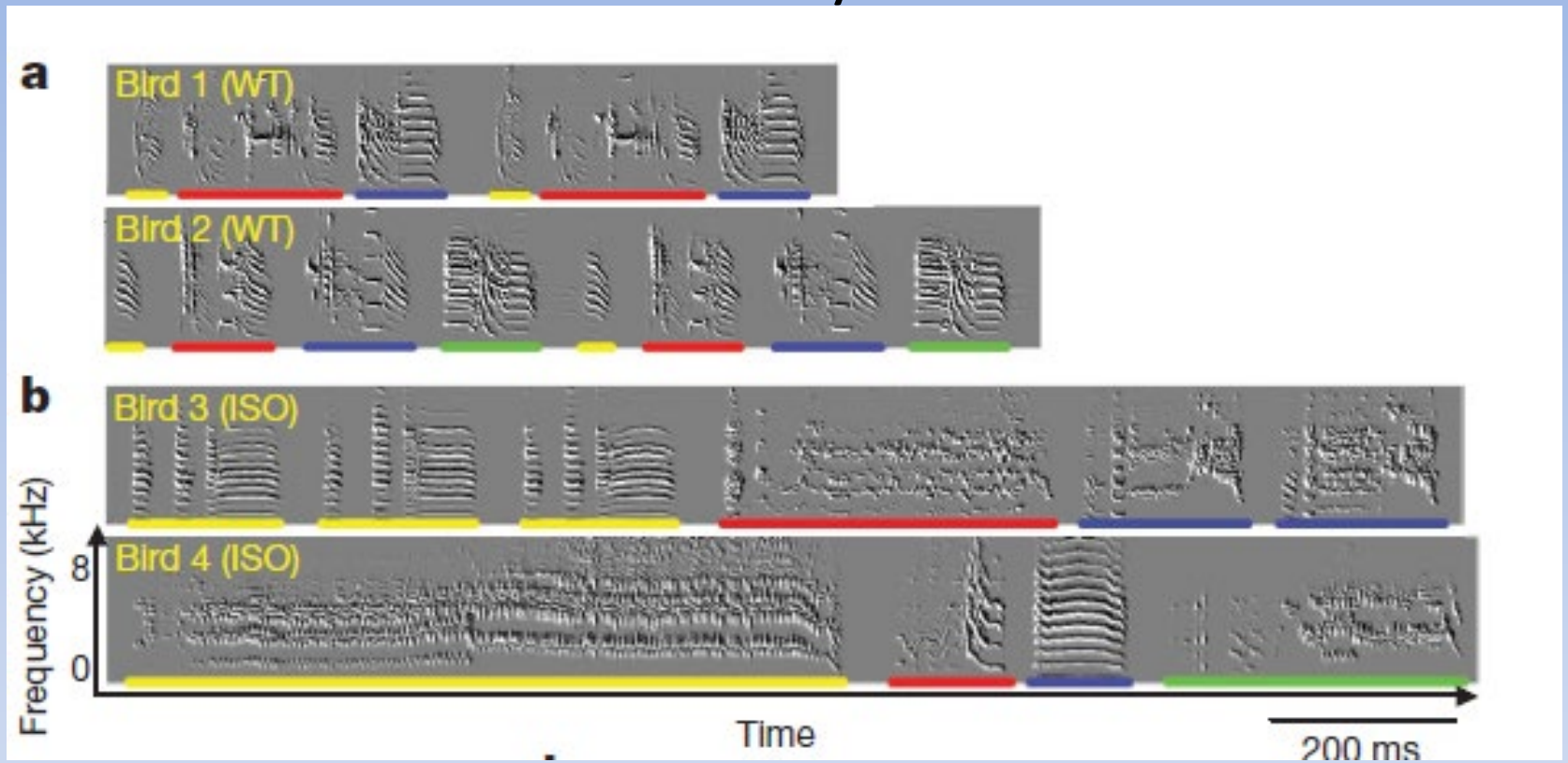
Song culture in birds

- 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?

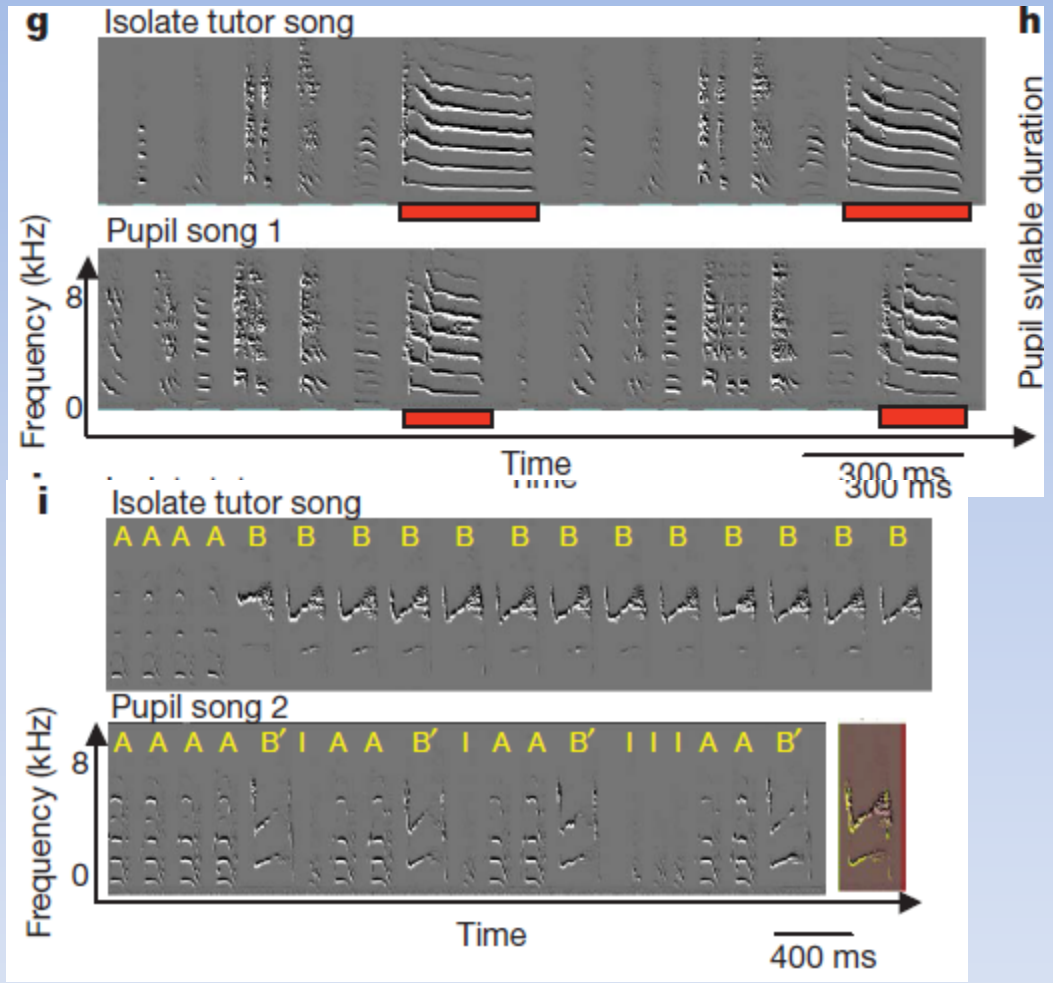


The experiment: to determine whether normal wild-type song culture might emerge over multiple generations in an isolated colony founded by isolates.

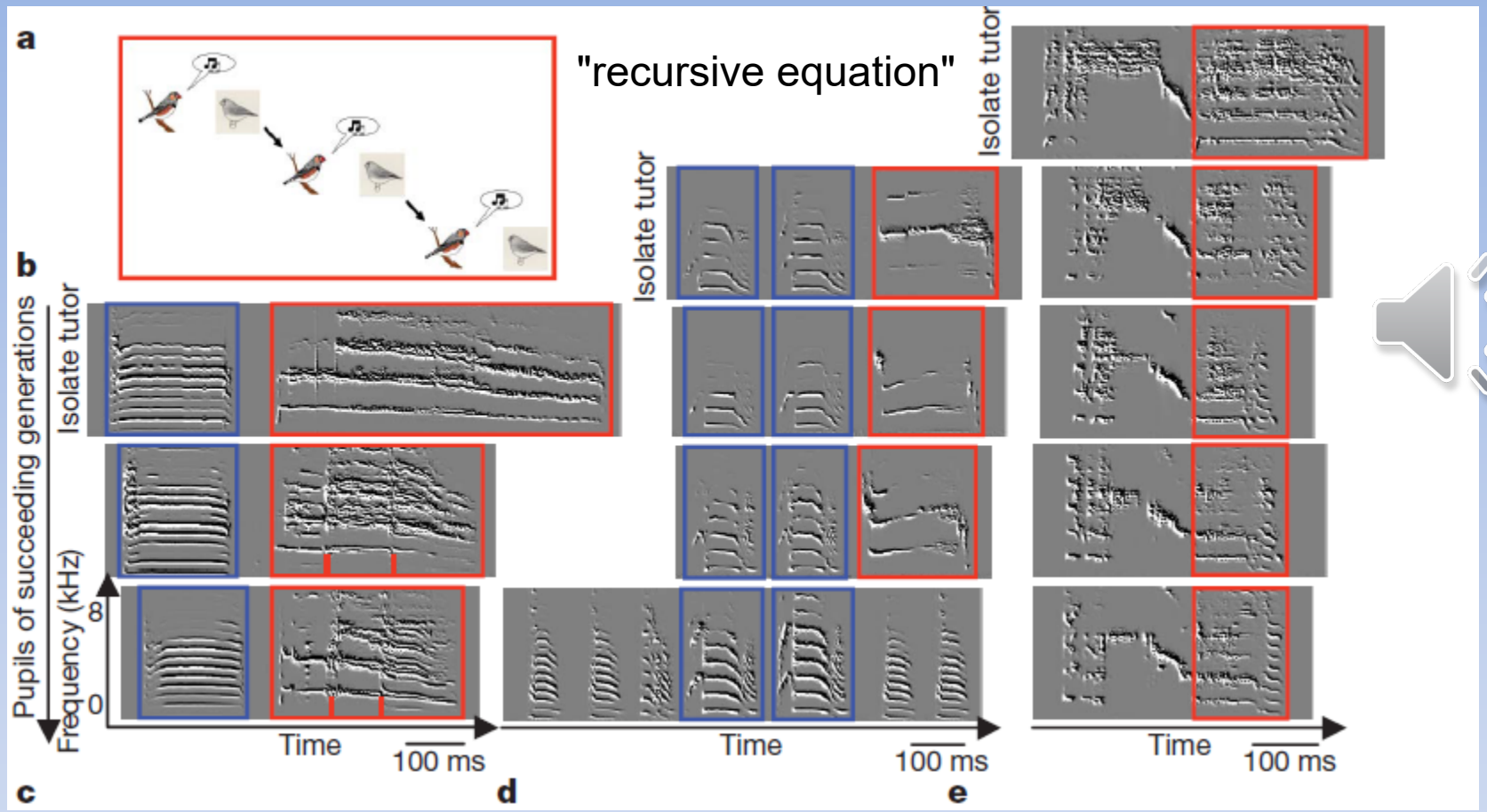
Konishi 1965; Marler 1970

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

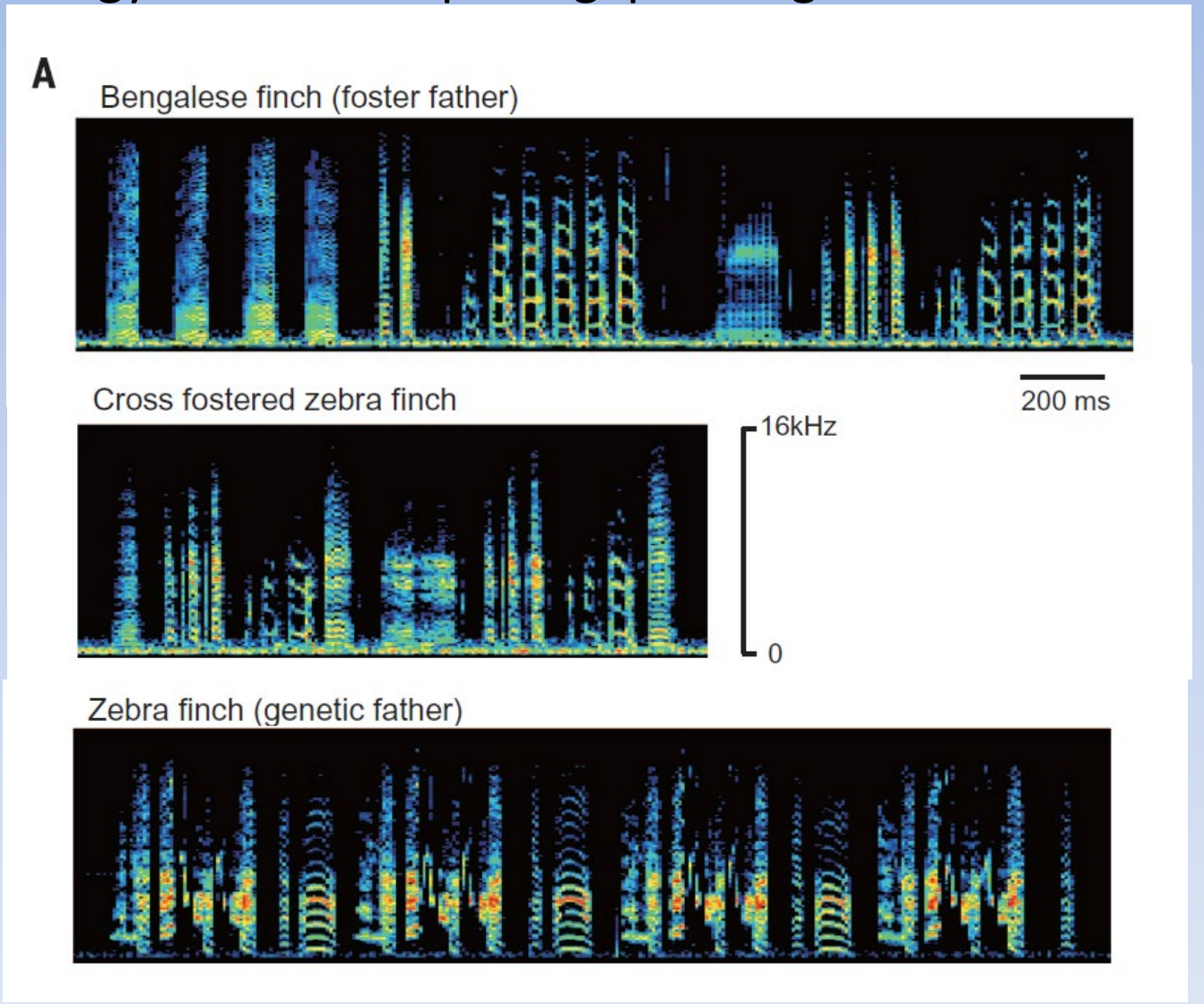


Song evolved towards the wild-type in three to four generations. Thus, species-typical song culture can appear *de novo*.

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.

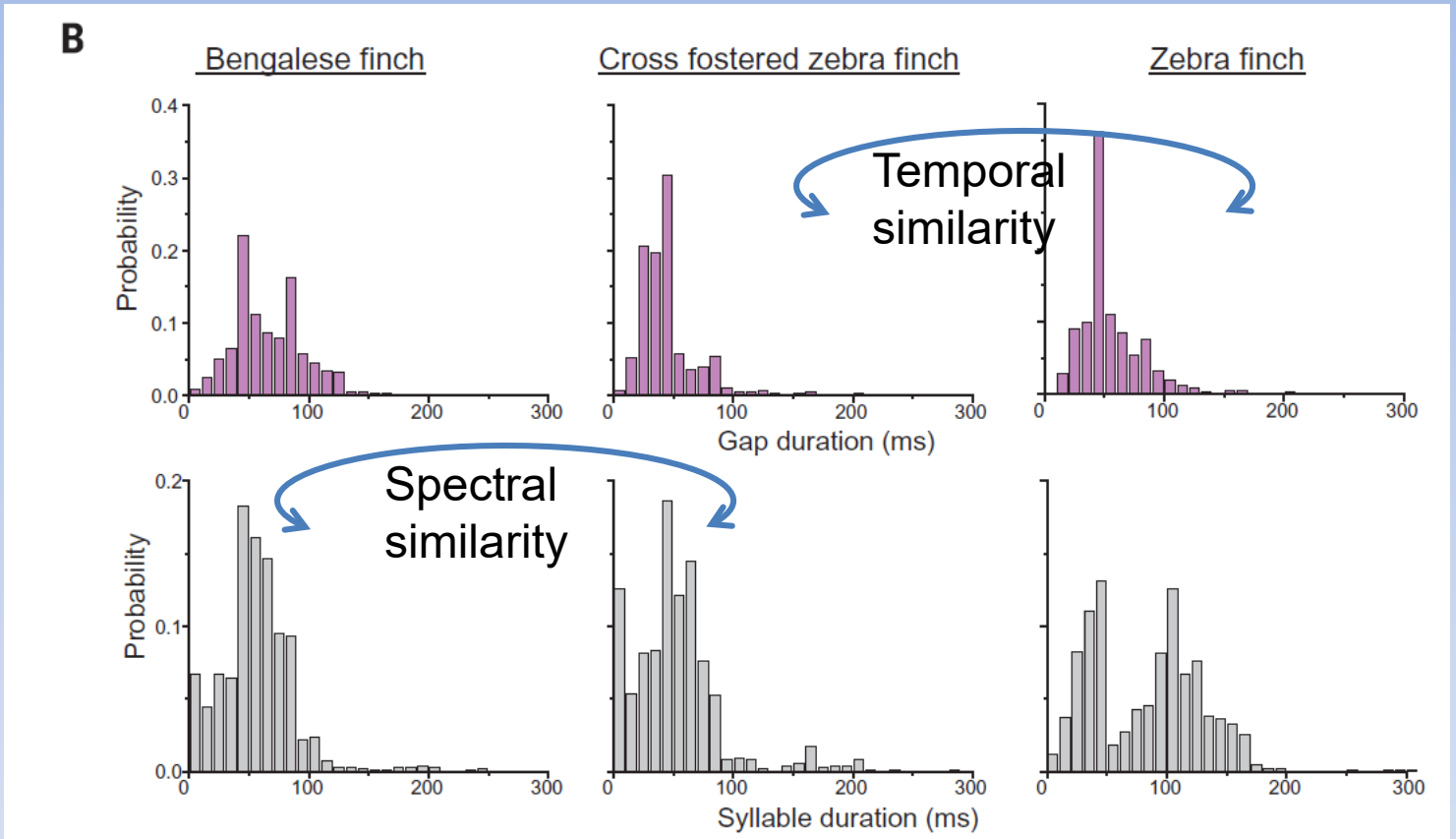
Temporal gap coding is innate, whereas syllable coding is experience dependent.



Mind the gap: Neural coding of species identity in birdsong prosody

Makoto Araki, M. M. Bandi, Yoko Yazaki-Sugiyama

Cross fostered zebra finches has similar temporal structure to zebra finch song but the spectral was of Bengalese



Chomsky: the ***structure of language*** (**syntax**) **are** biologically determined and hence ***genetically transmitted***

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 (syntax) is innate, whereas syllable coding is experience dependent.