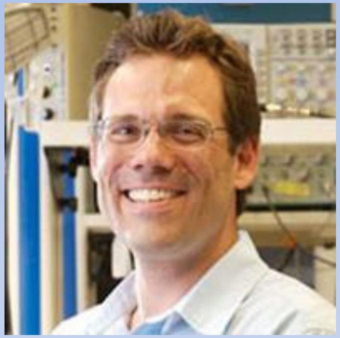
# How songbirds sing birdsongs?



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).

# The brain is a learning machine

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

# Vocal learning

**Mammals**:

- Humans
- Bats
- Dolphins/Whales
- Elephants
- Sealions

**Birds**:

- Parrots
- Hummingbirds
- Songbirds

Over 4000 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:

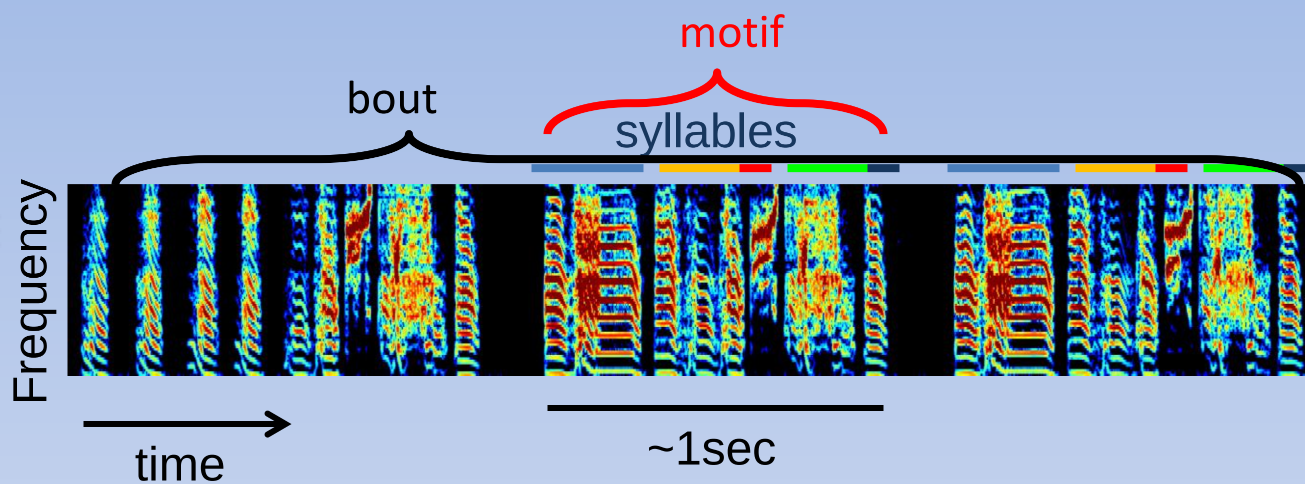
1. To attract a mate.
2. To establish and hold a territory.

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

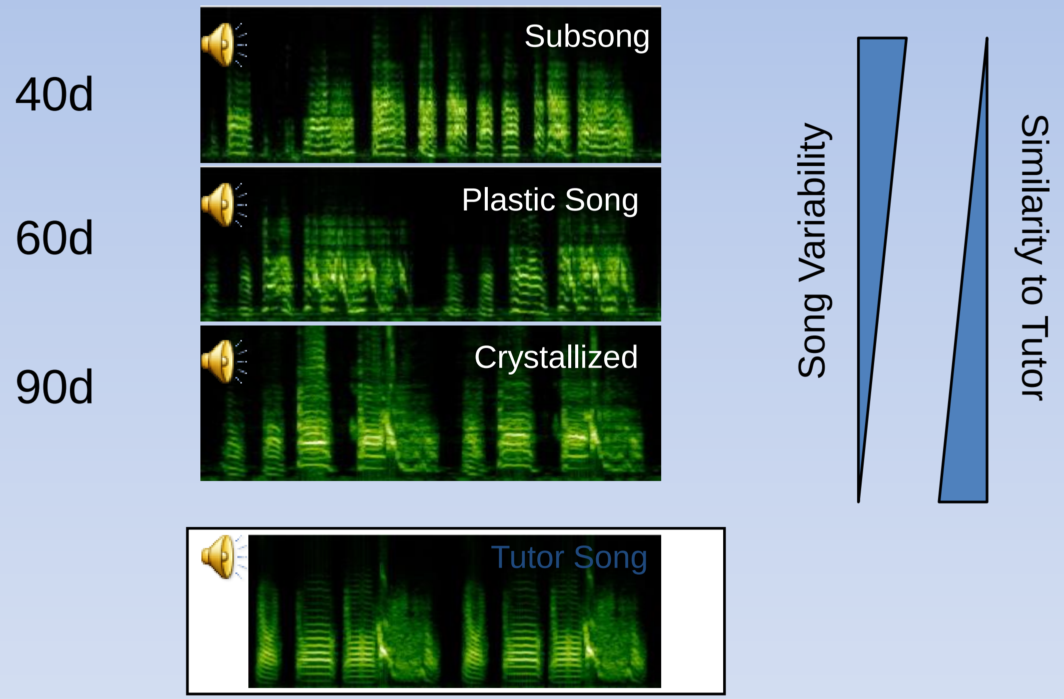


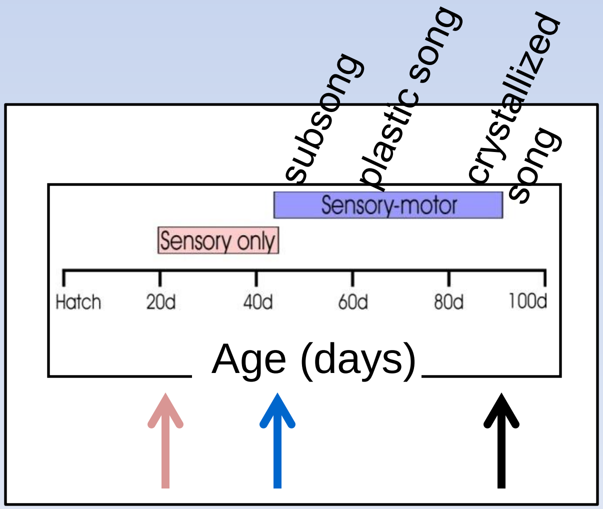

Ofer Tchernichovski's Lab (CUNY)

There are two modes of singing:

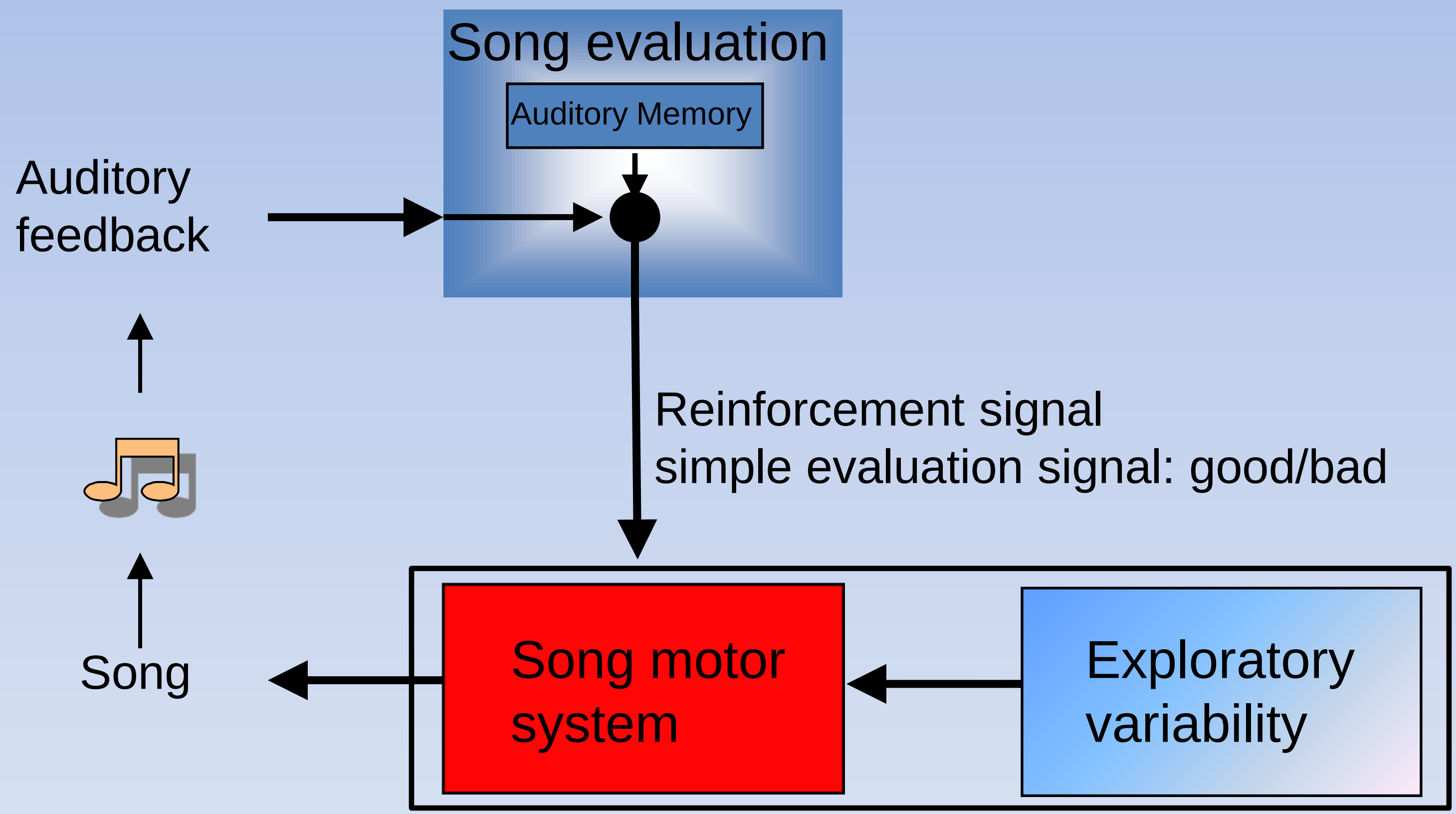
1) Directed singing
2) Undirected singing

# Songbirds learn to sing by imitating their tutor



# *Reinforcement learning model for song acquisition*

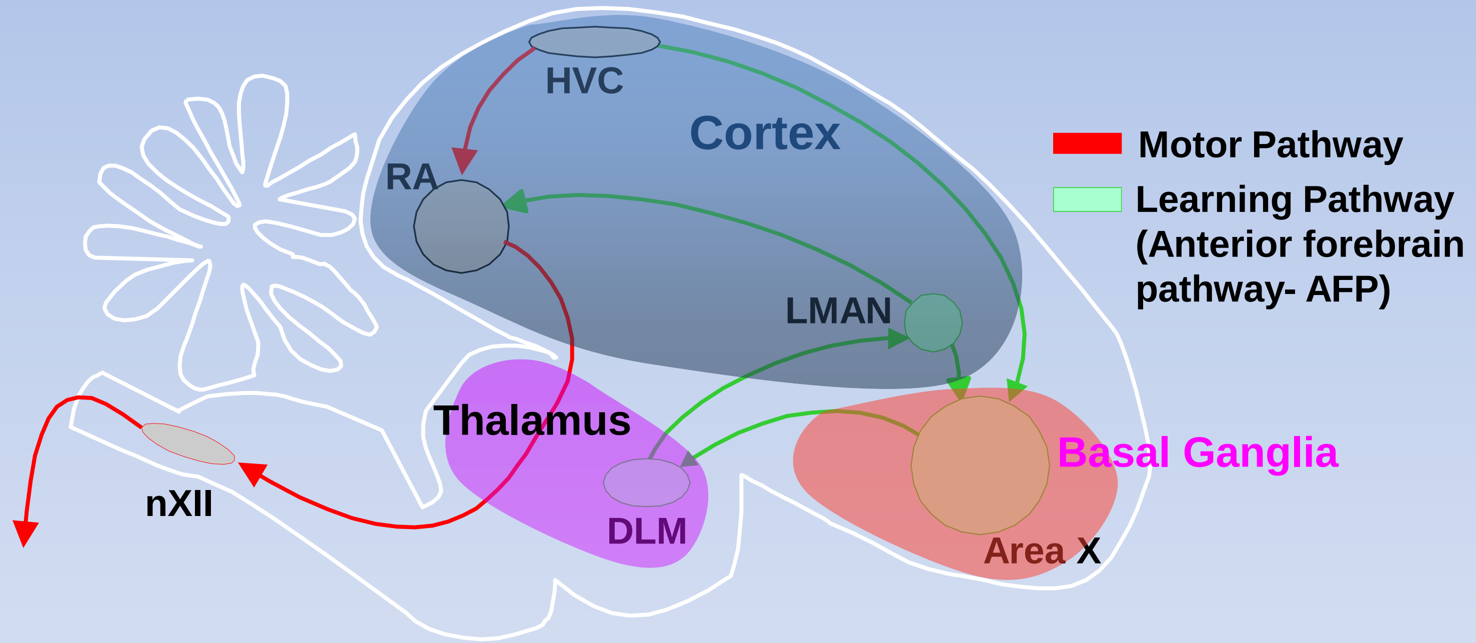



Konishi 1965; Marler 1970

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

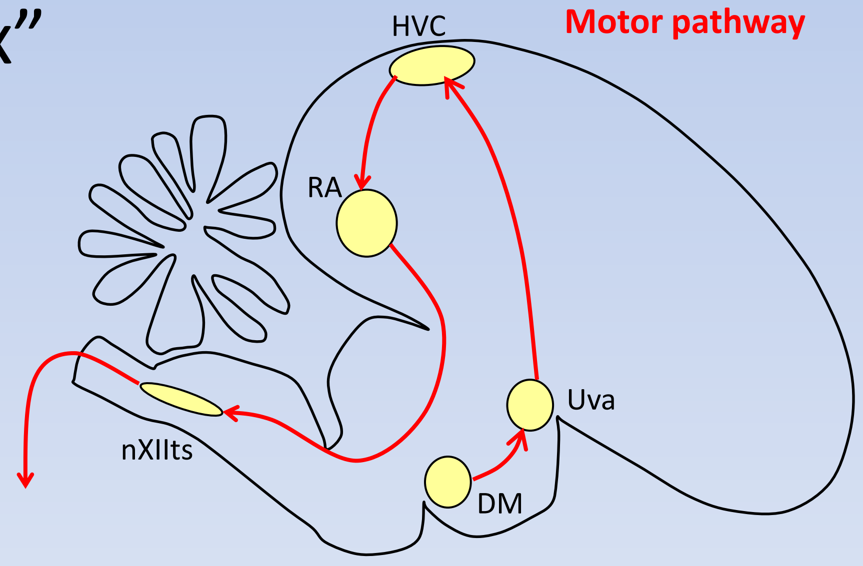
# Song system

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



# Motor pathway

- HVC (high vocal center)
- RA primary motor "cortex"
- brainstem motor areas
  - Muscles of the syranx
  - respiratory muscles



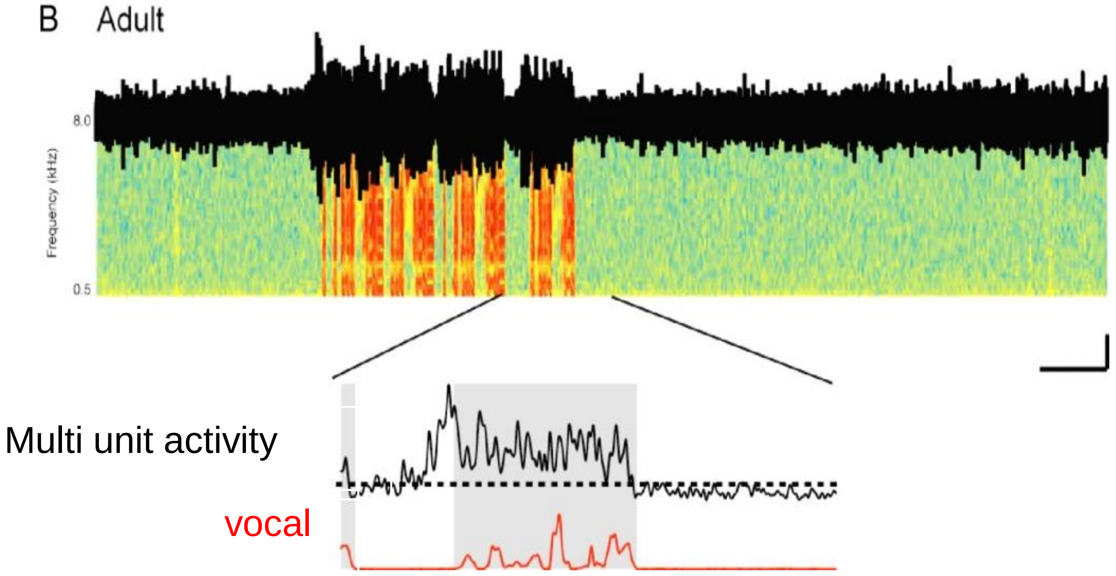

Nottebohm et al., (*J Comp Neurol*, 1976)

- **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

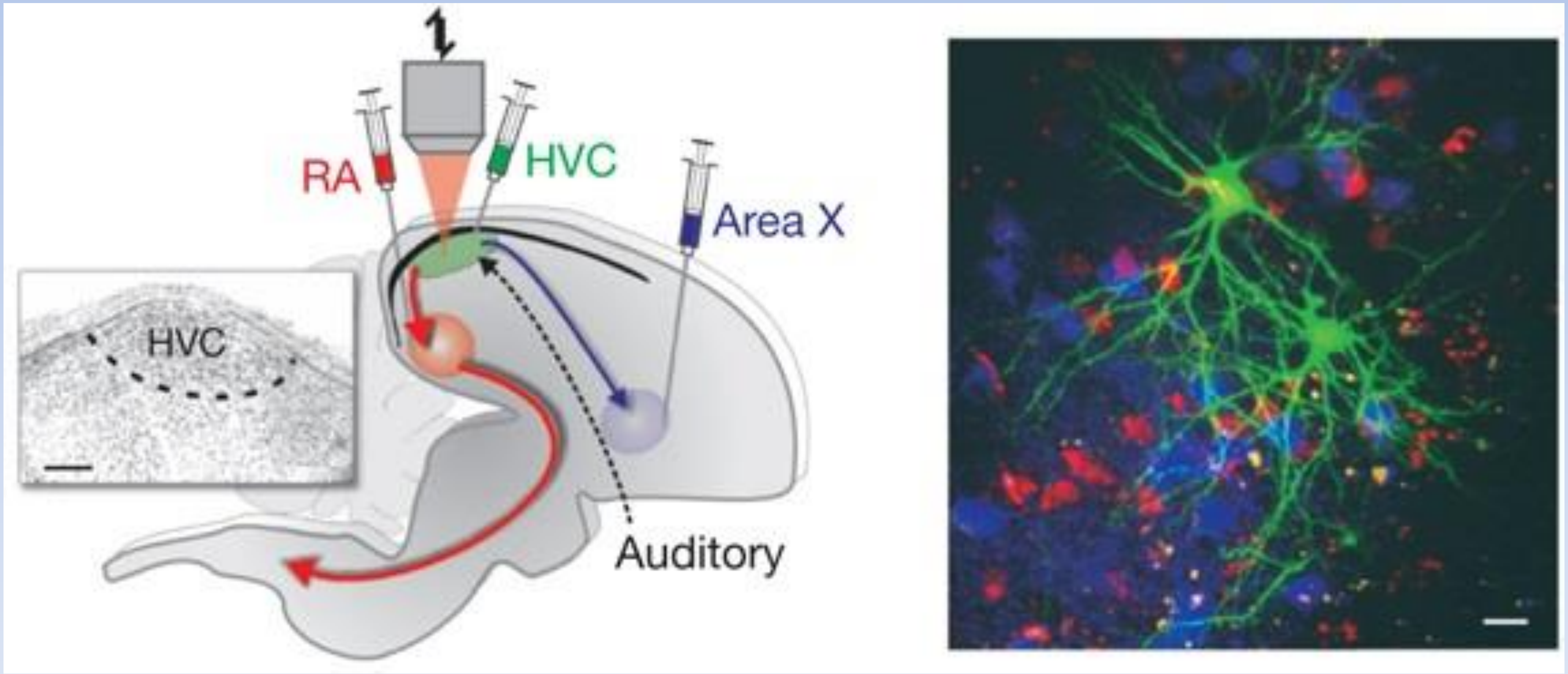



Recording from HVC showed a massive neuronal signal that was throughout the song- that was not well correlated with the song pattern.

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

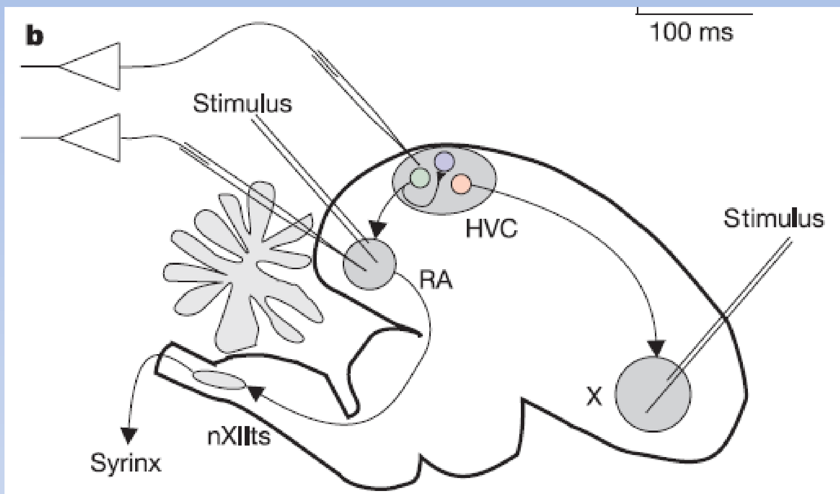
3 types of neurons in HVC –

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

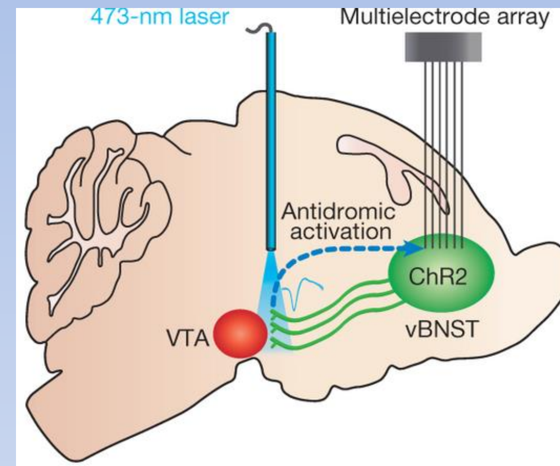


# How to identify neurons you are recording from?

- Antidromic stimulation (electrical or optogentic)

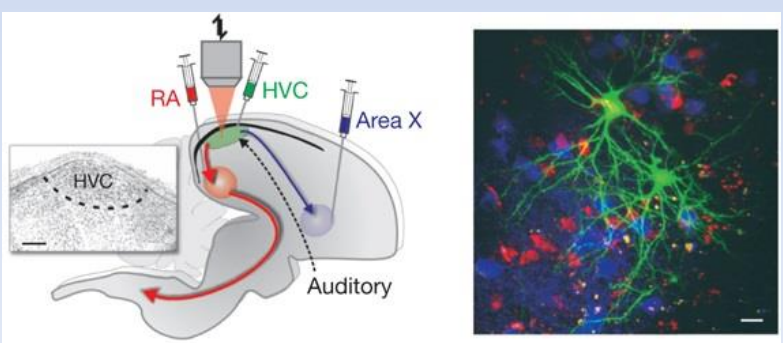



Hahnloser et al. (*Nature*, 2002)

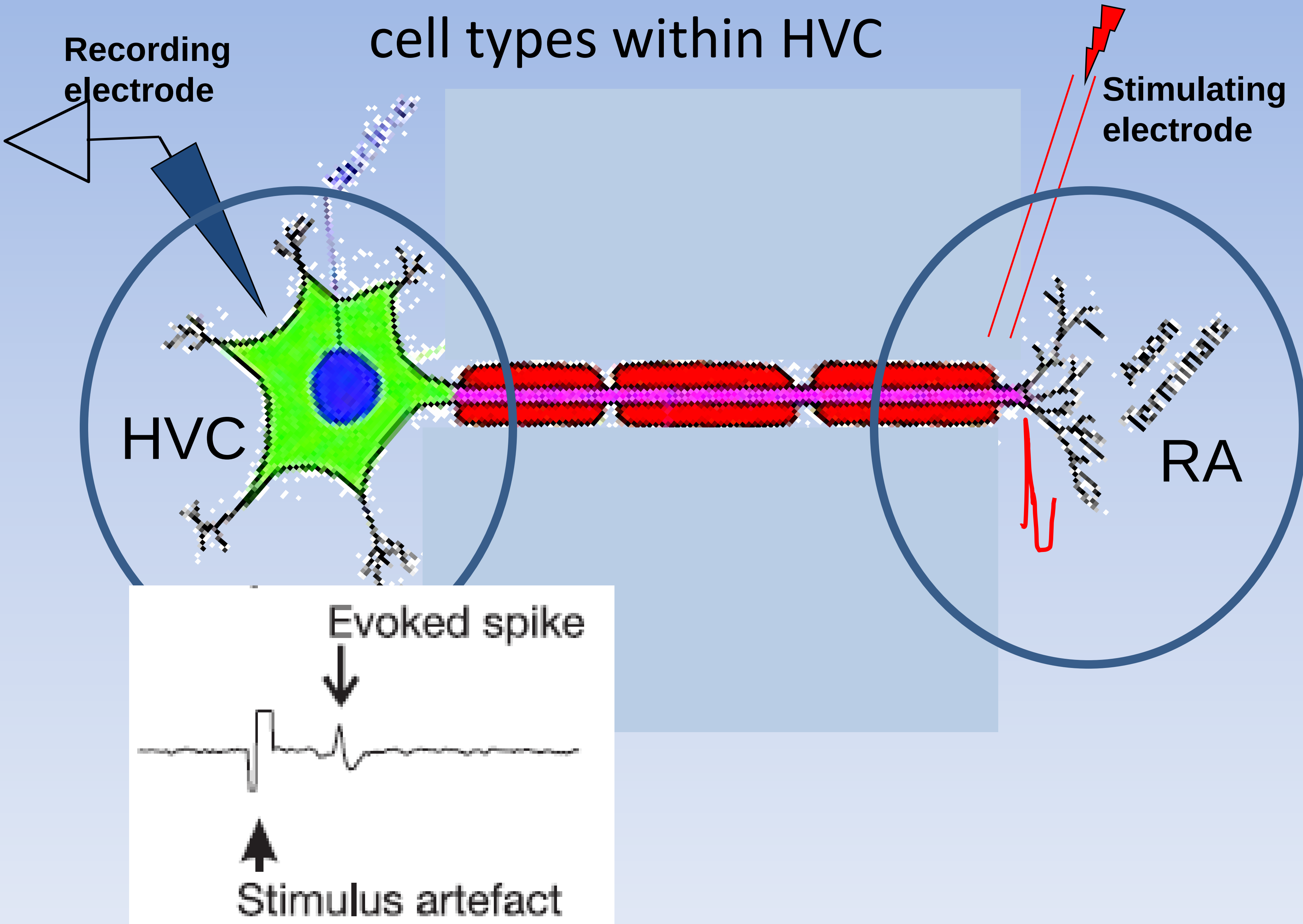



Distinct extended amygdala circuits for divergent motivational states (Joshua H. Jennings, Nature 2013)

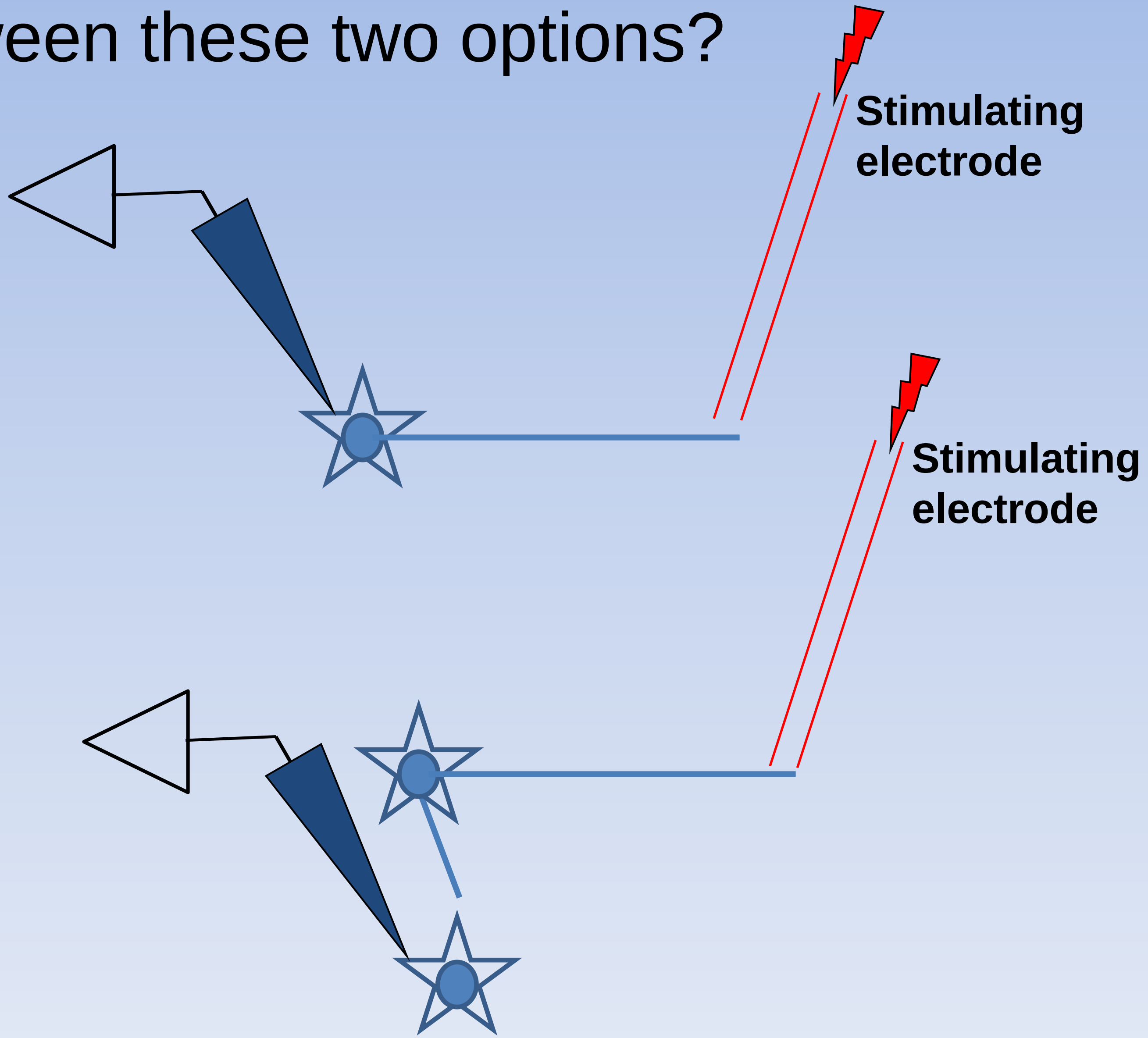
- Labeling (genetically or injections)



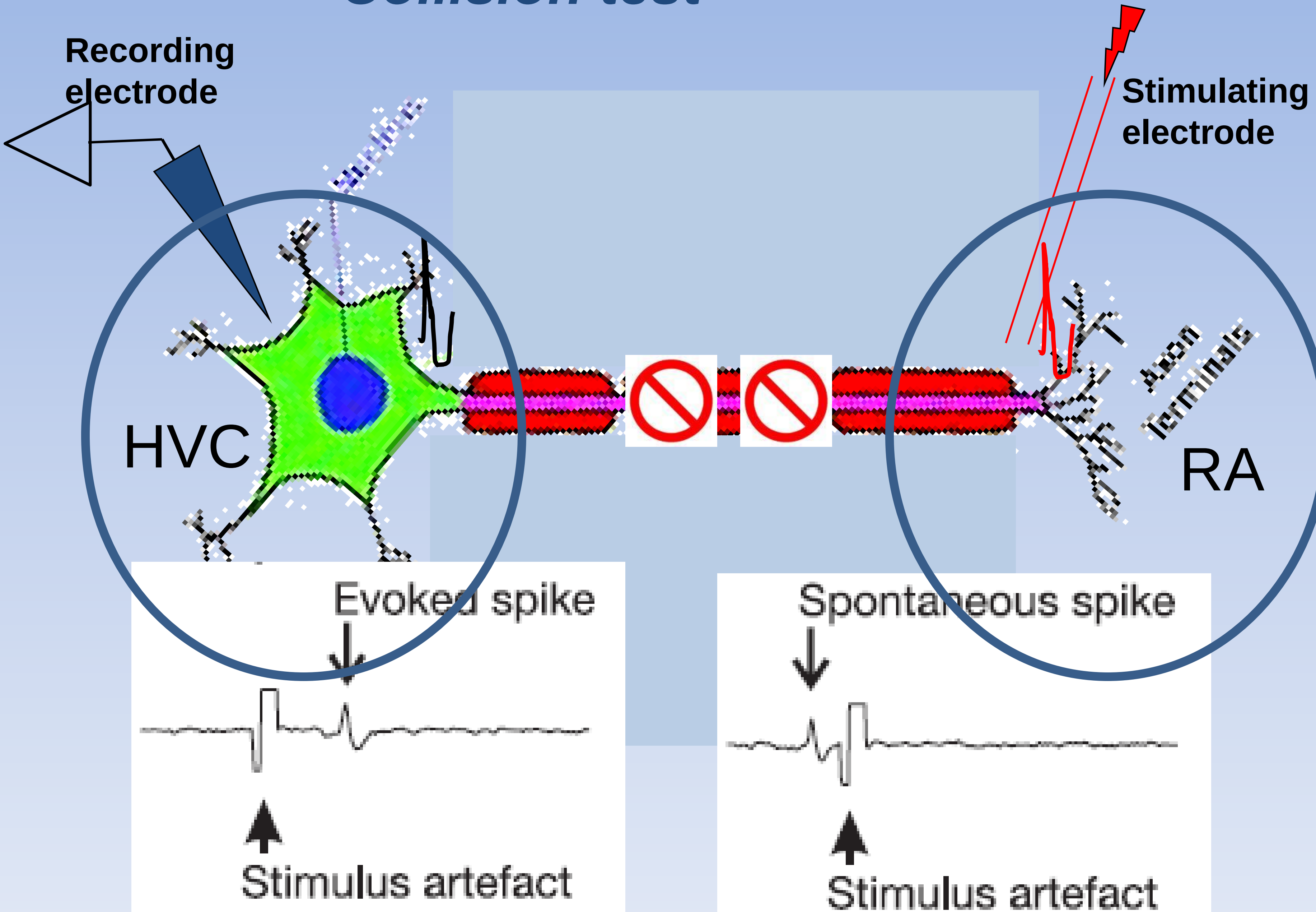
# Using antidromic stimulation to identify cell types within HVC



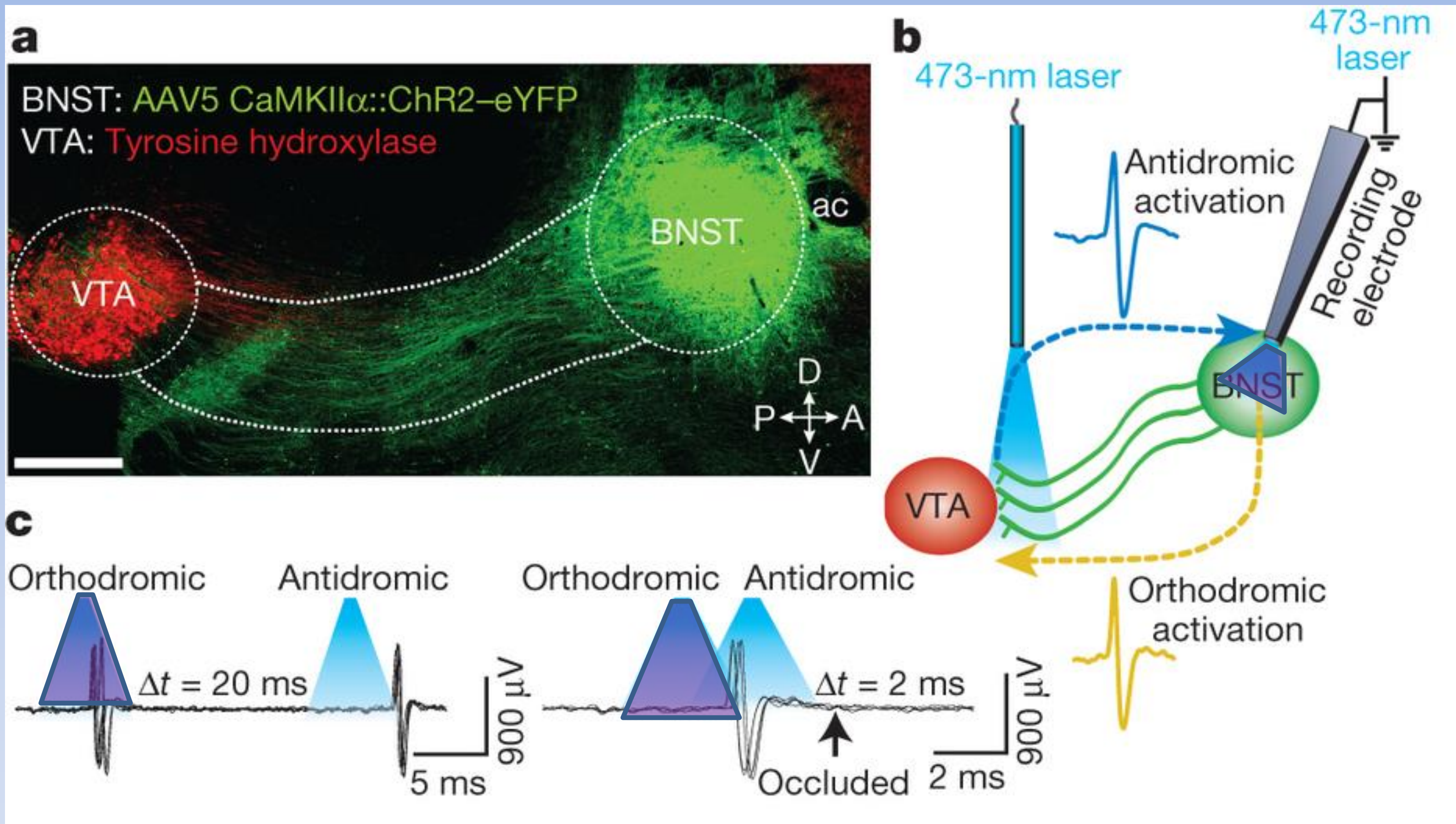
How do we differentiate between these two options?



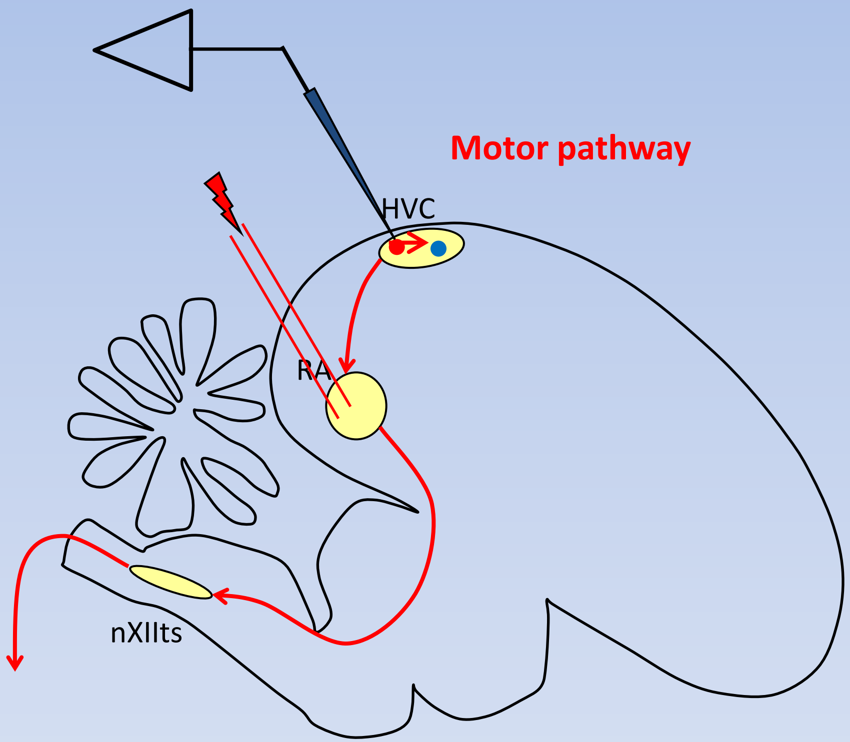
# *Collision test*

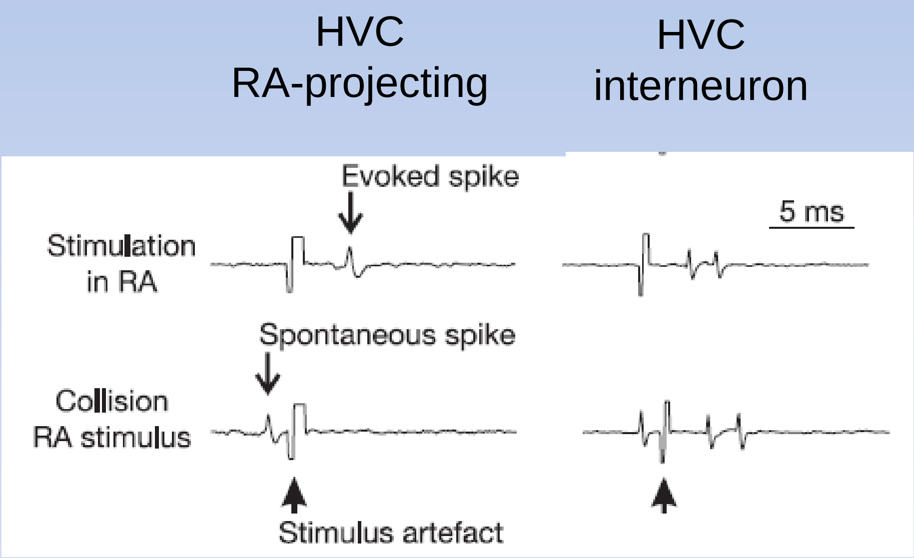


# *Collision test using optpgenetic setup*



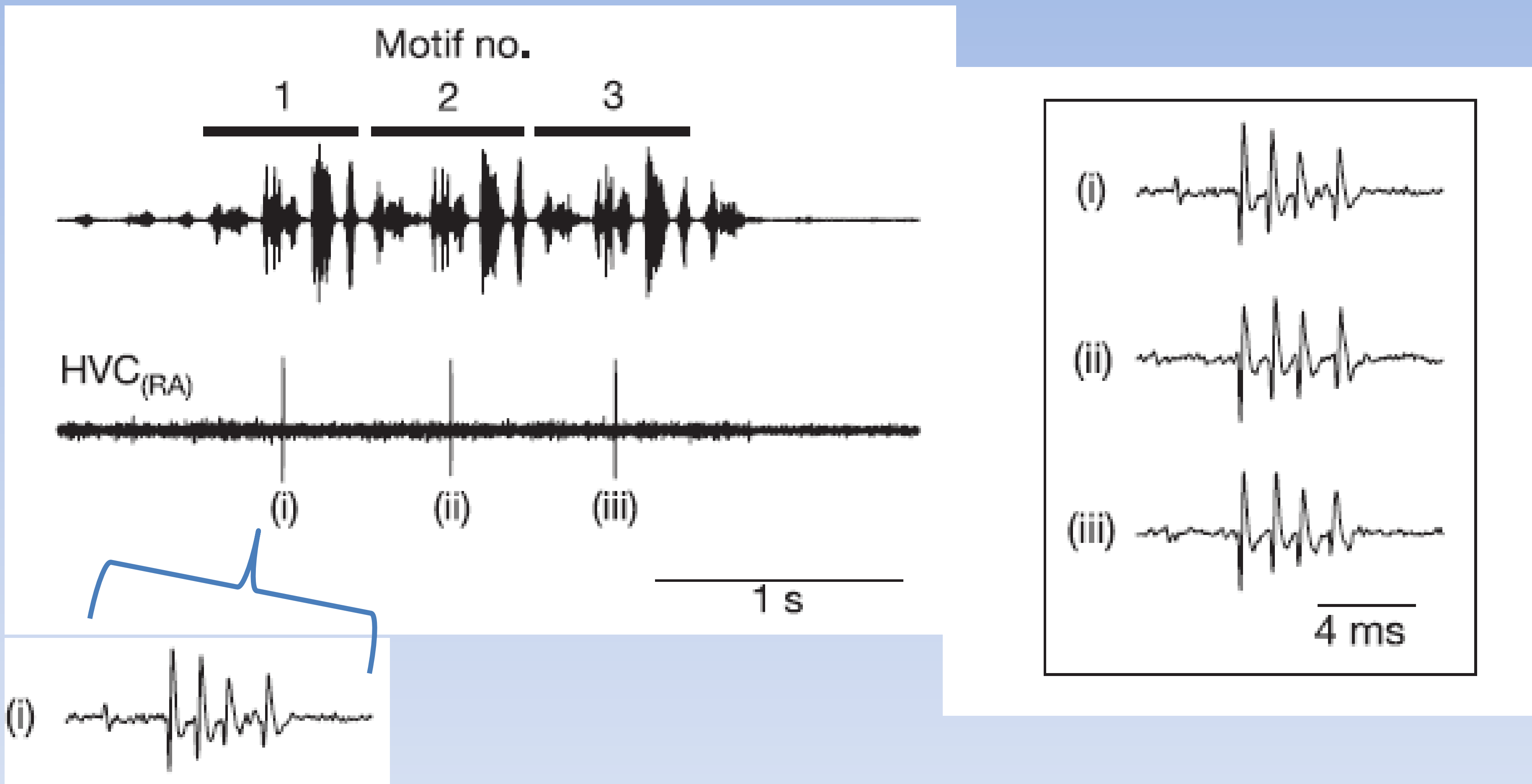
# *Using antidromic stimulation to identify cell types within HVC*



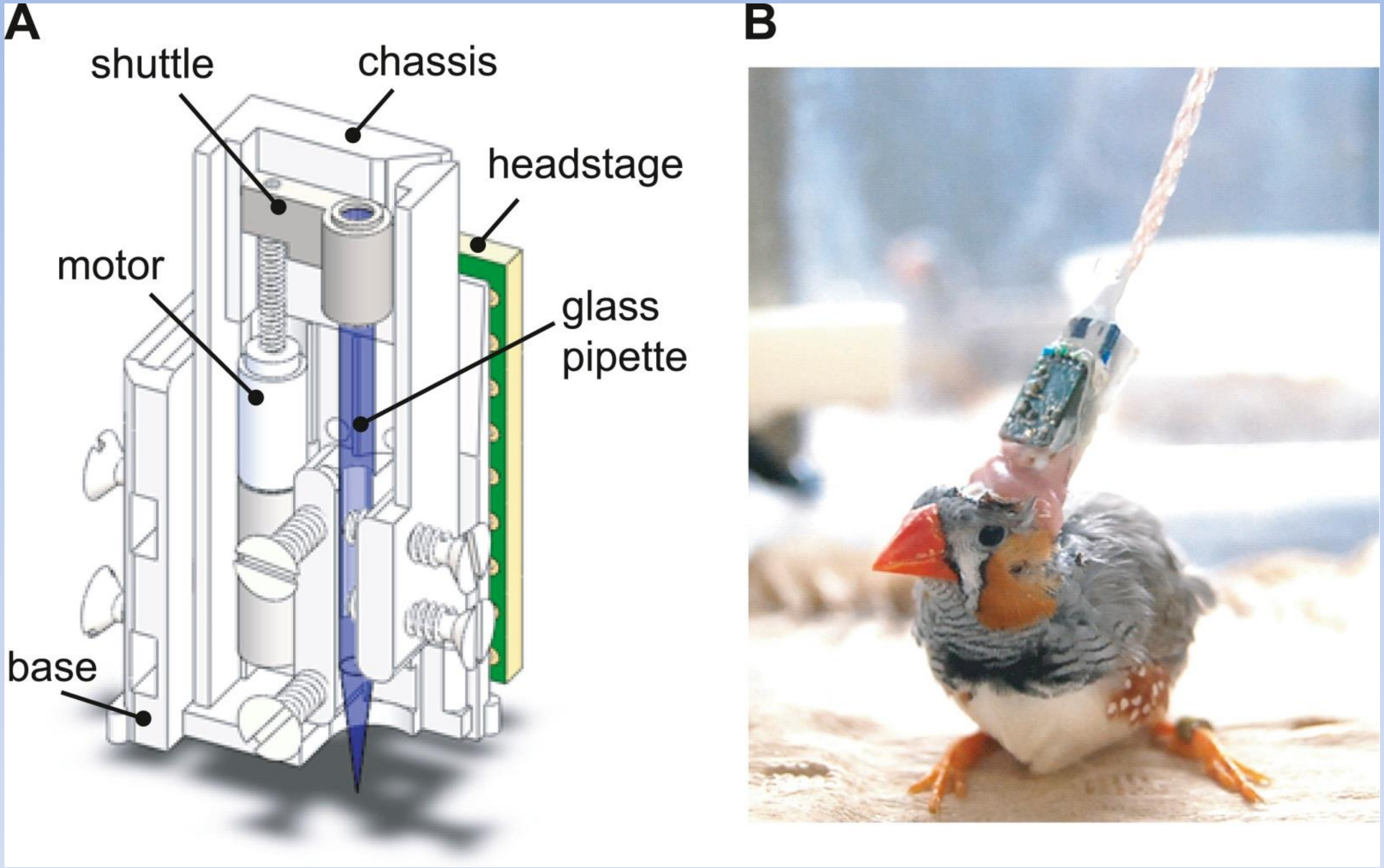



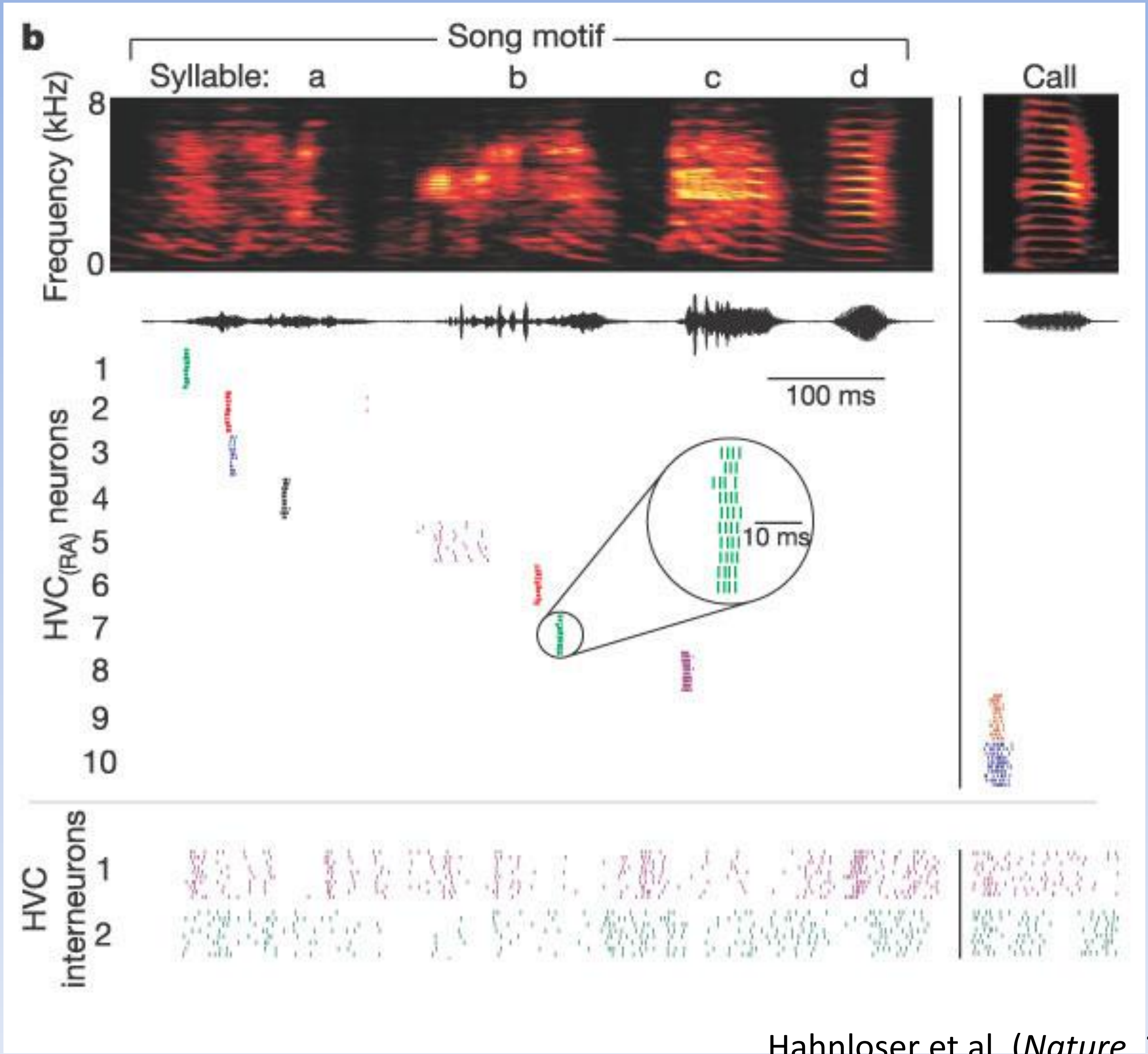

Hahnloser et al. (*Nature*, 2002)

# Activity of HVC-RA neurons during singing




Hahnloser et al. (*Nature*, 2002)

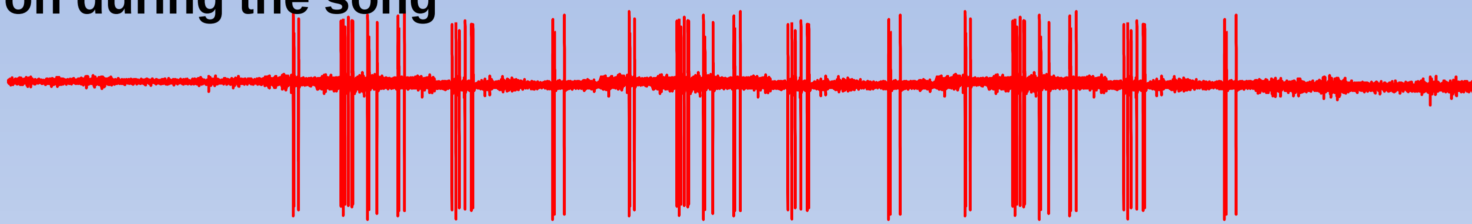

A
shuttle
chassis
headstage
motor
glass
pipette
base
B

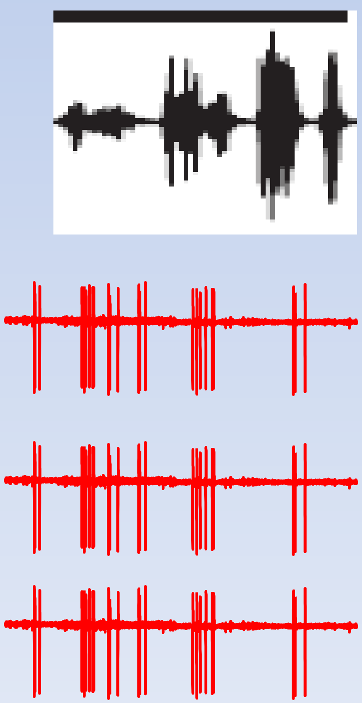


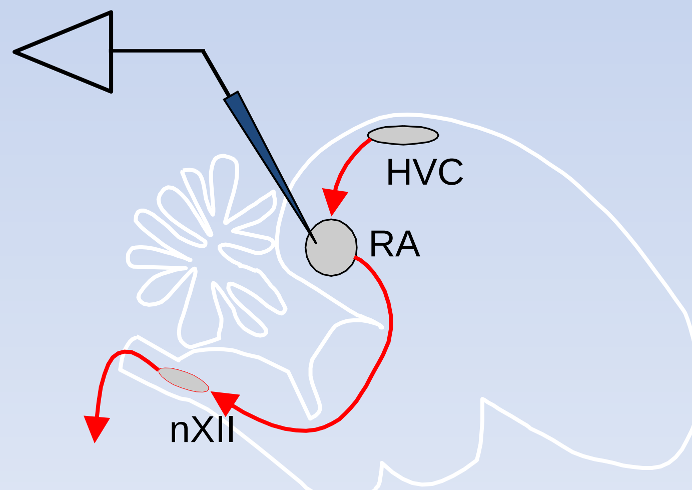

Hahnloser et al. (*Nature*, 2002)

The song

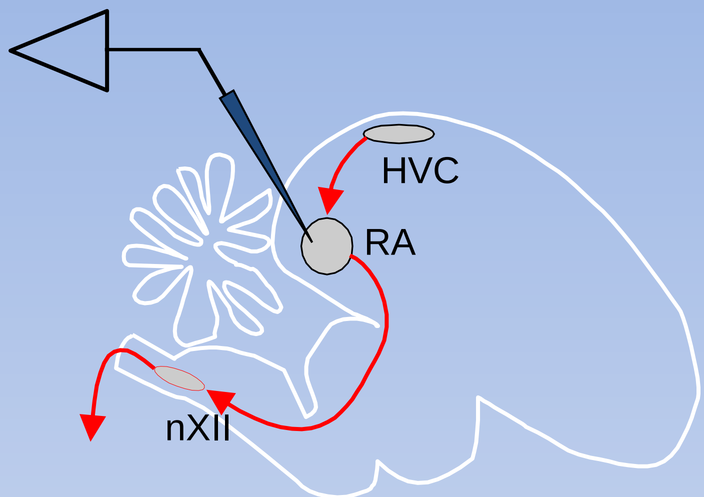

**Recordings from RA neuron during the song**



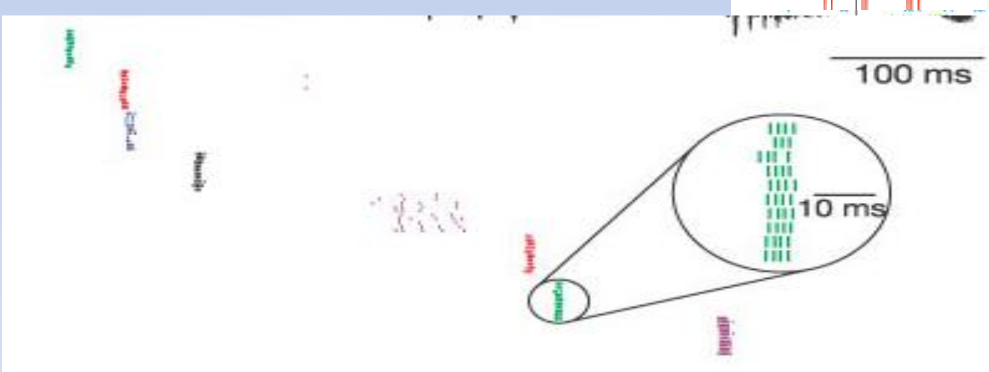


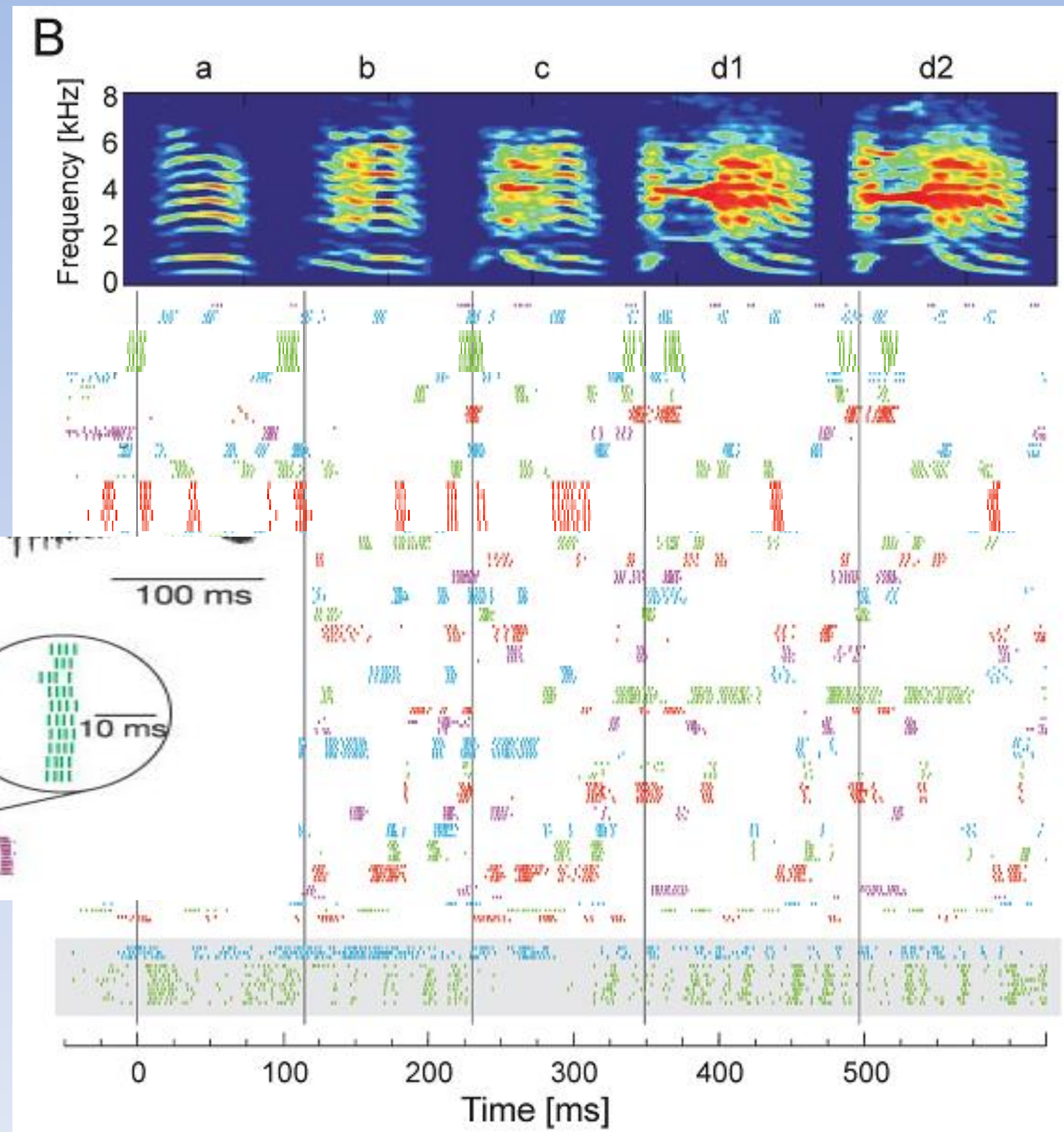


# RA activity during singing




HVC-RA neurons

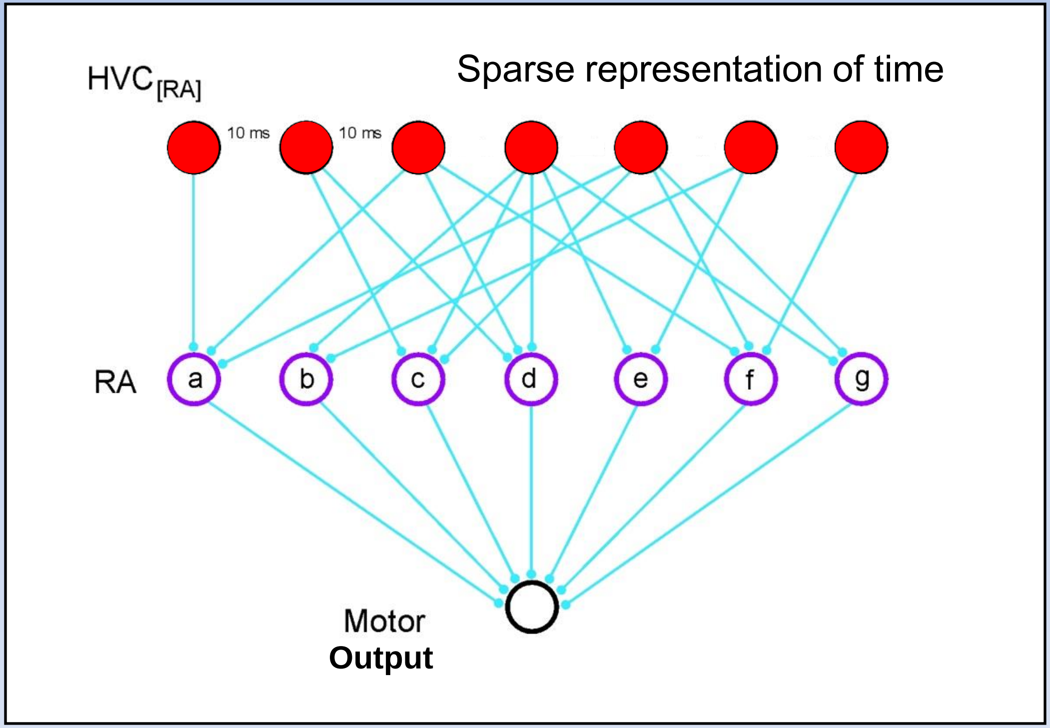


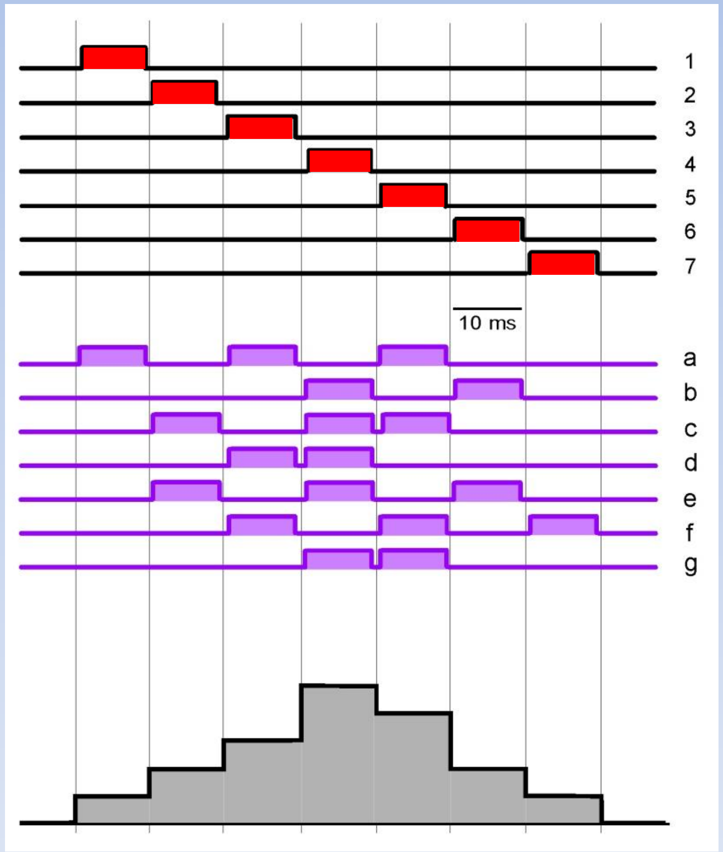




Leonardo & Fee (*J Neurosci*, 2005)

# Simple sequence generation circuit

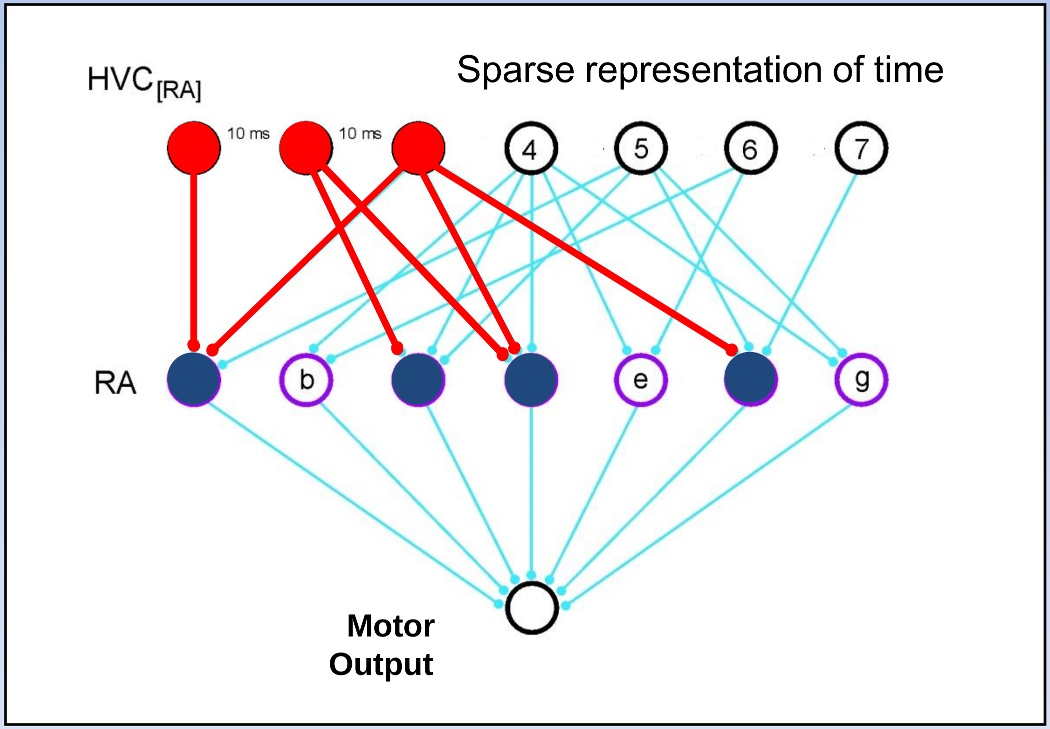
**Bursting activity propagates through a chain of synaptically connected $HVC_{RA}$ neurons (like falling dominoes), creating a timing signal that spans the entire motif.**

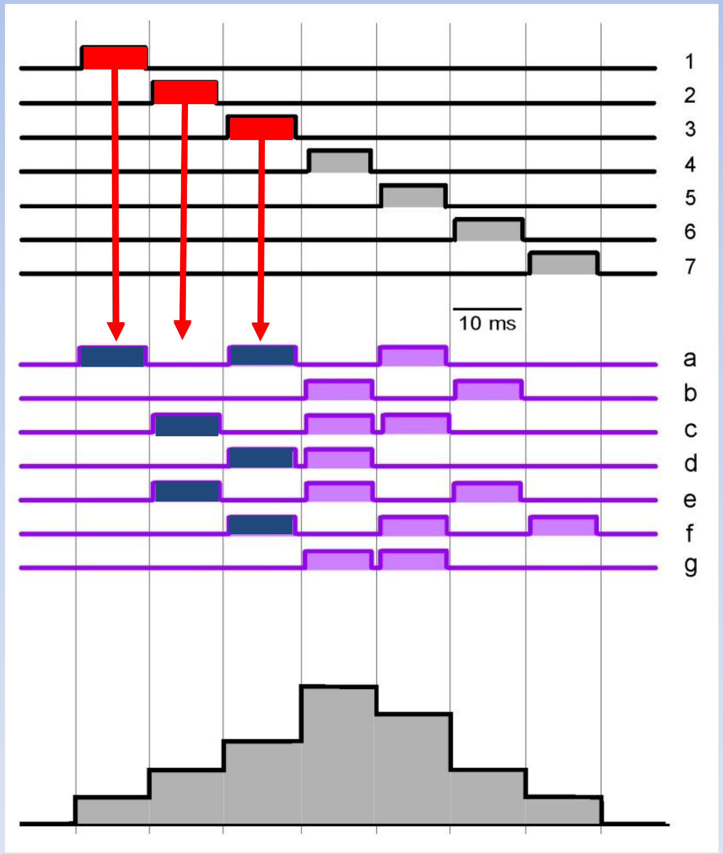



Leonardo and Fee, 2005

# *Simple sequence generation circuit*

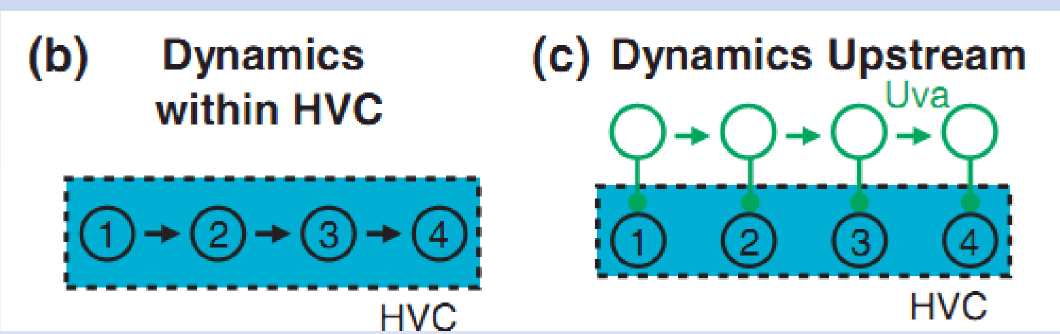


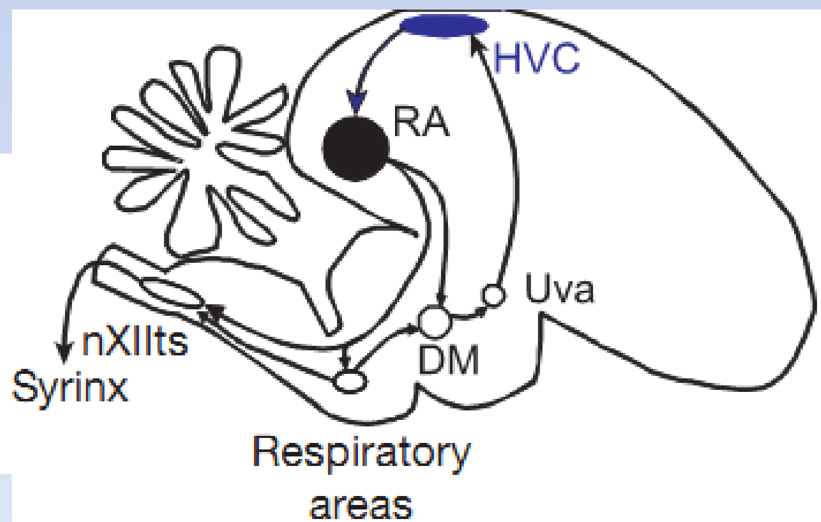




Leonardo and Fee, 2005

# 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?

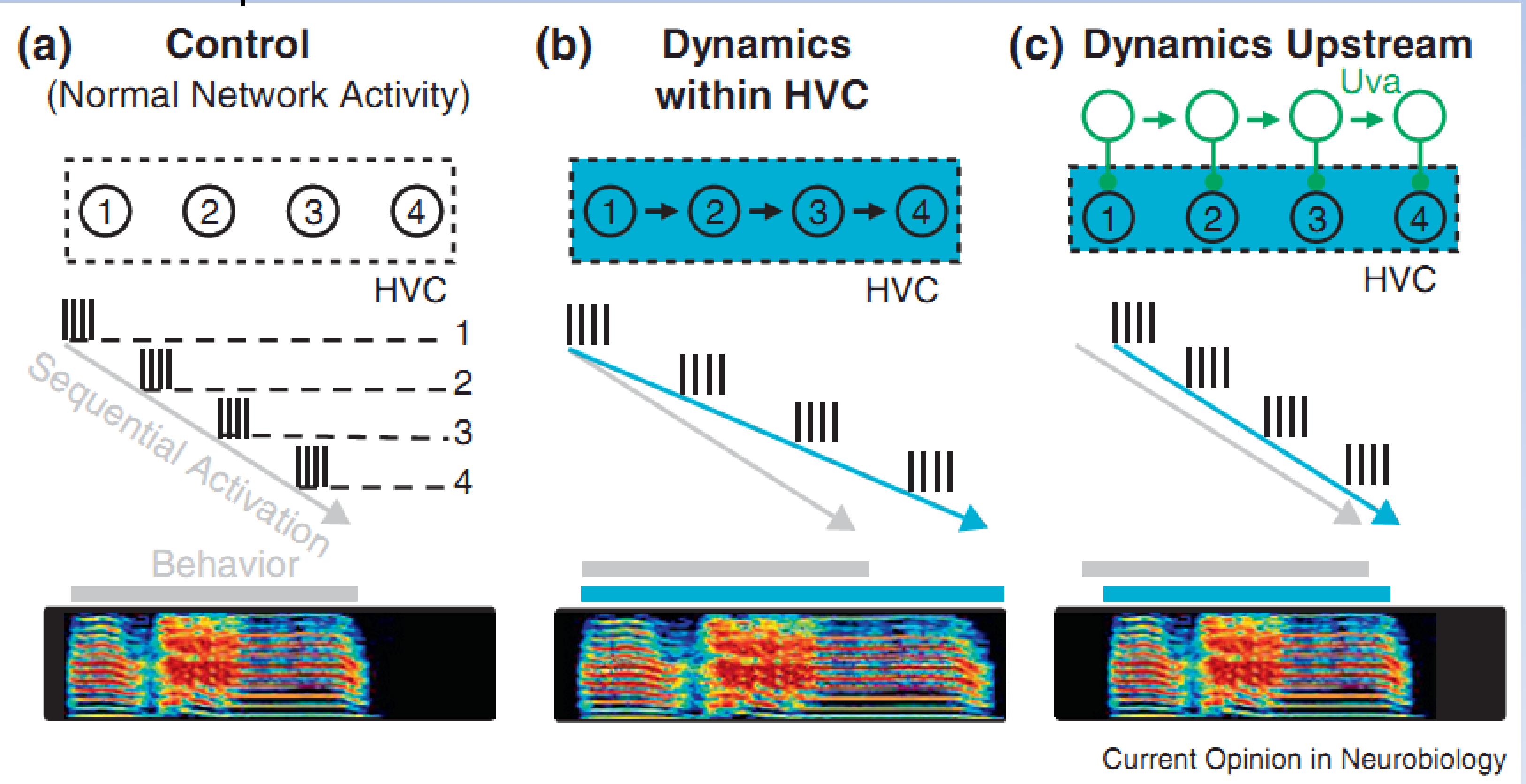


# What and where is the mechanism that determines tempo? Are the dynamics generated within HVC?

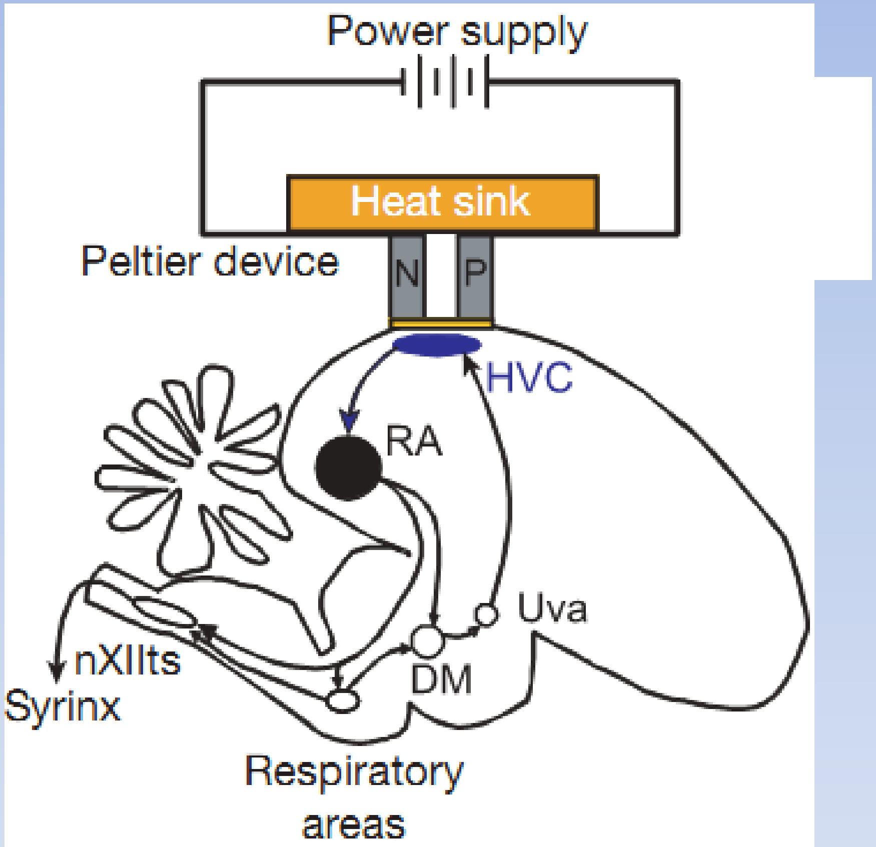
If song tempo is determined by the activity of the HVC local network, then song should slow as HVC is cooled.

Theoretical predictions



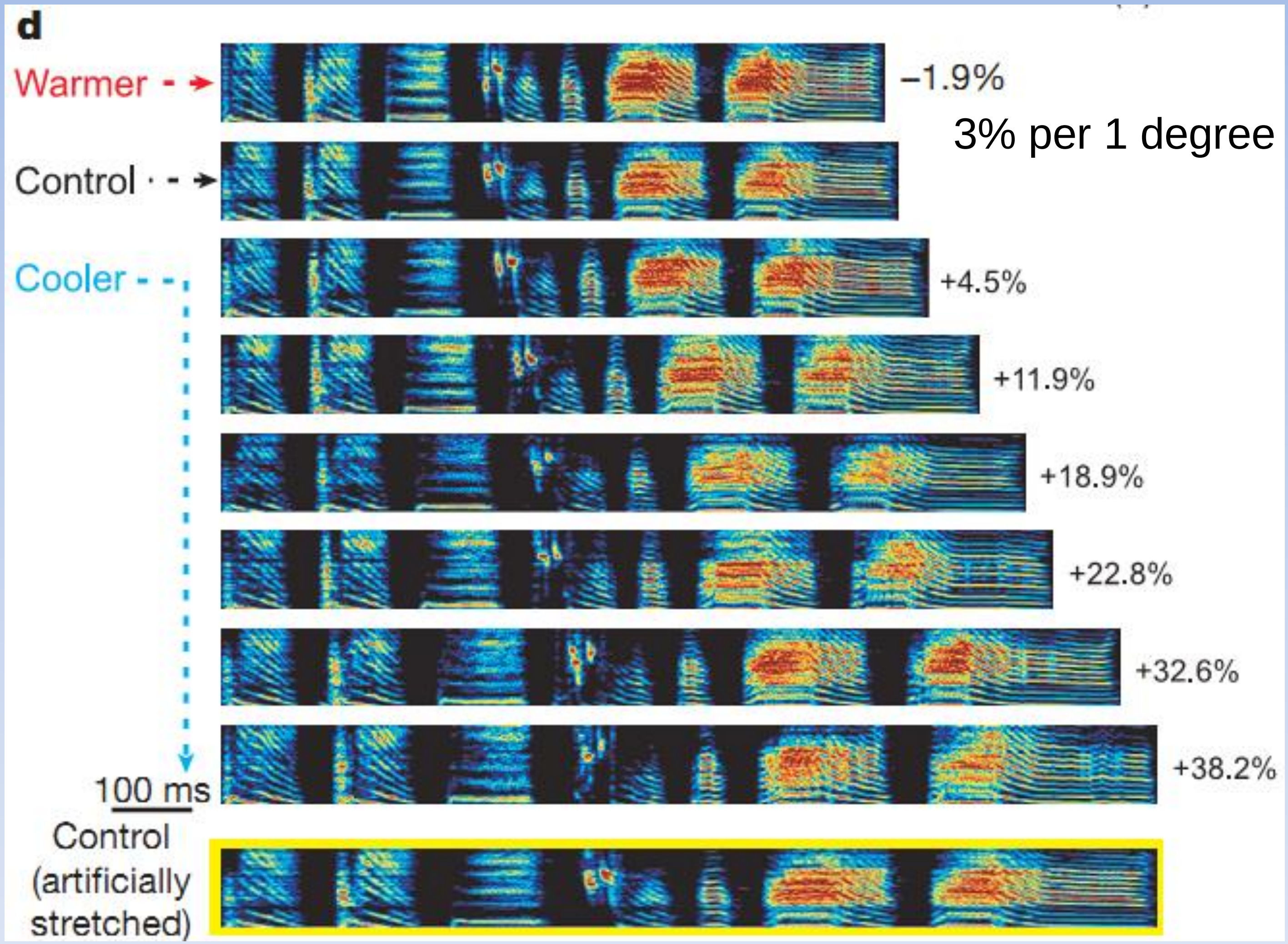

Fee & Long (*Curr Opin Neurobiol*, 2011)

# Local manipulation of brain temperature




Long & Fee (*Nature*, 2008)

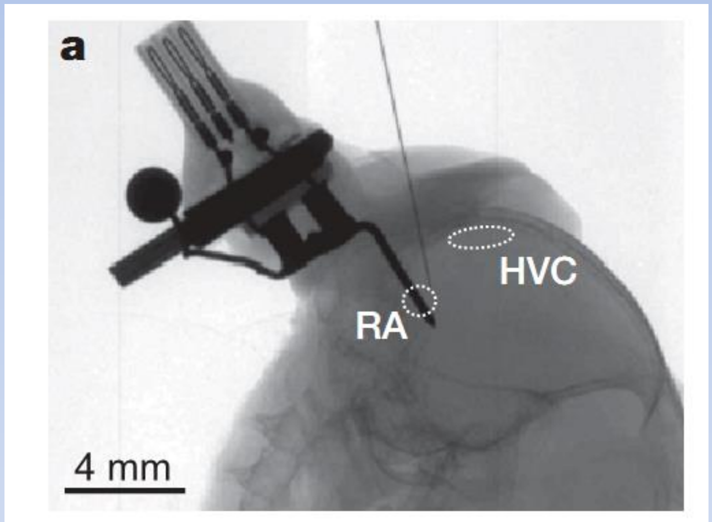
# Cooling of HVC: song tempo slowed similarly across all timescales: individual notes (~10 ms), entire motif (~1s) and the silent gaps

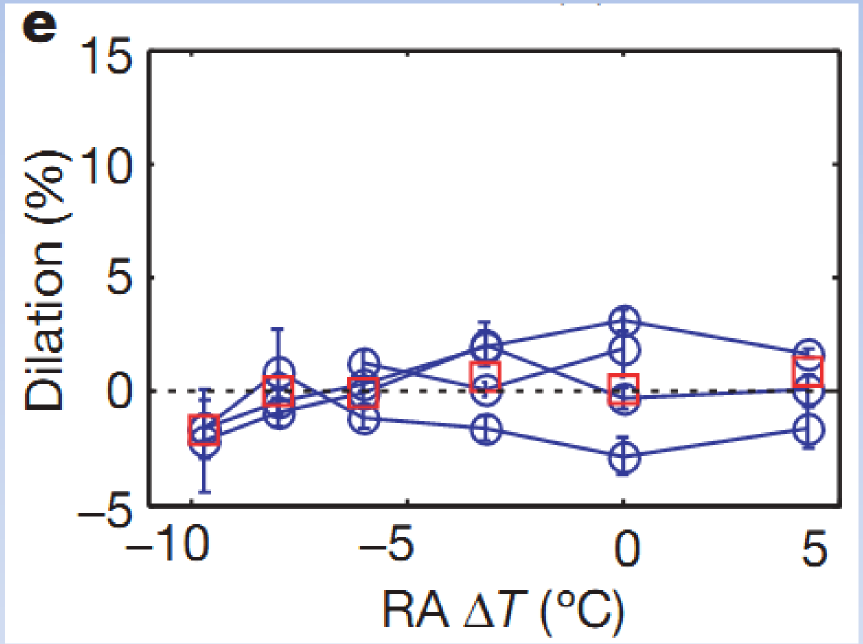



3% per 1 degree

Long & Fee (*Nature*, 2008)

# Cooling RA has no effect on song speed nor on the structure of the notes



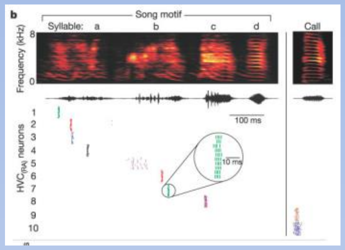



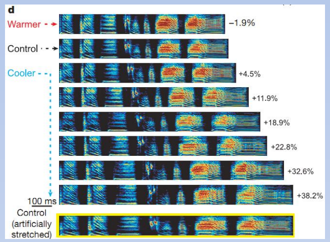

Long & Fee (*Nature*, 2008)

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

HVC:

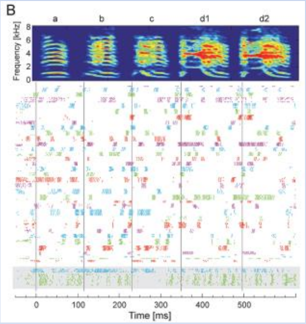
- HVC exhibits sparse bursts during singing.
- Song timing is controlled within HVC.



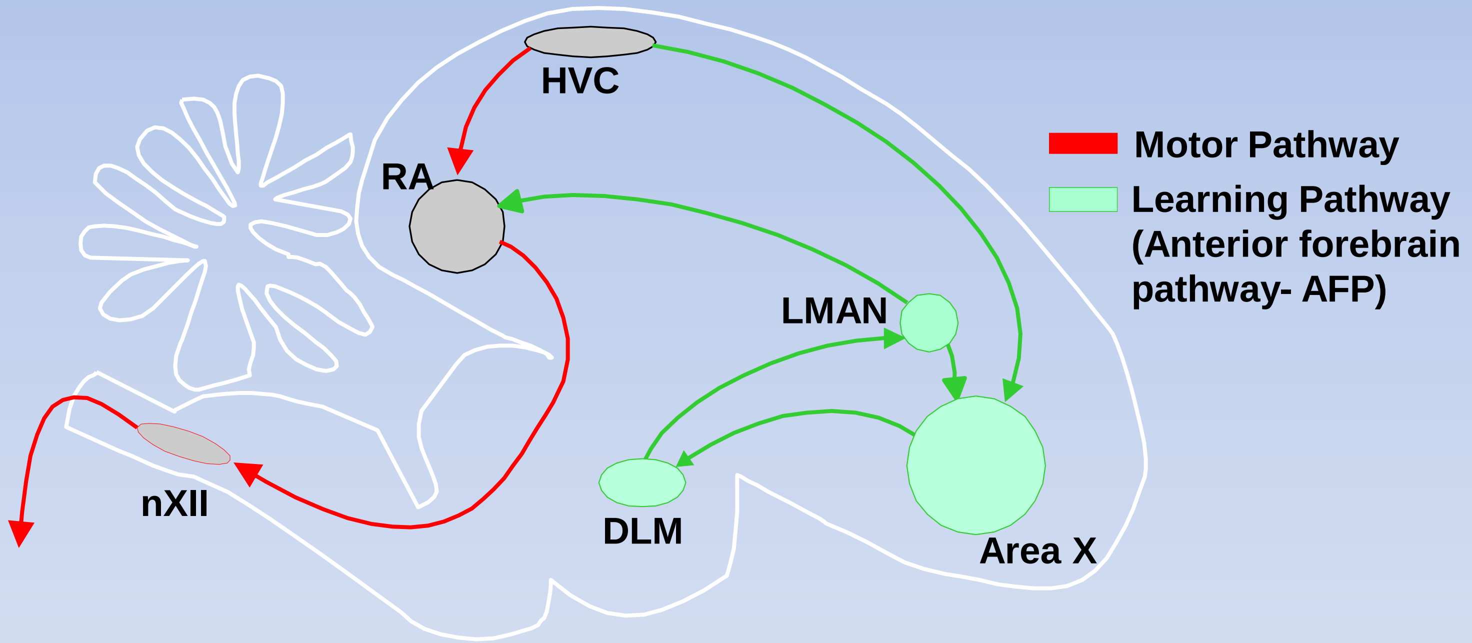




RA:

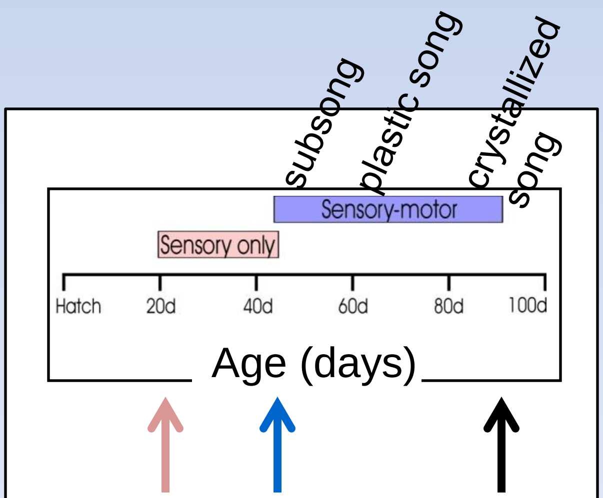
- RA transforms the sparse code into multiple bursts which then drive motoneurons.
- The configuration of the vocal organ (muscle activity) is determined by the convergent input from RA neurons on short time scale (~10 to 20 ms).

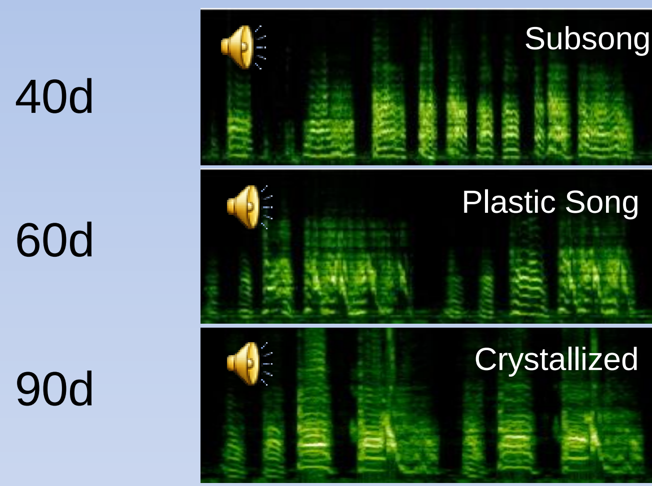


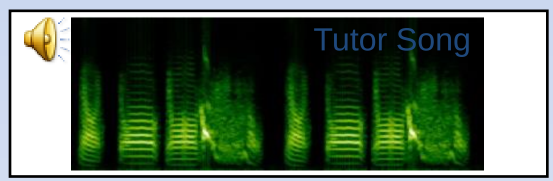
# RA also gets input from LMAN!
# What is the role of the learning pathway?

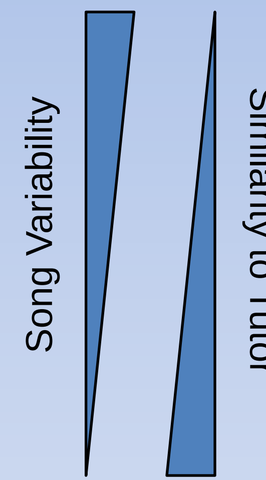


# Subsong (“babbling”) – i.e., the highly variable song in very young juveniles



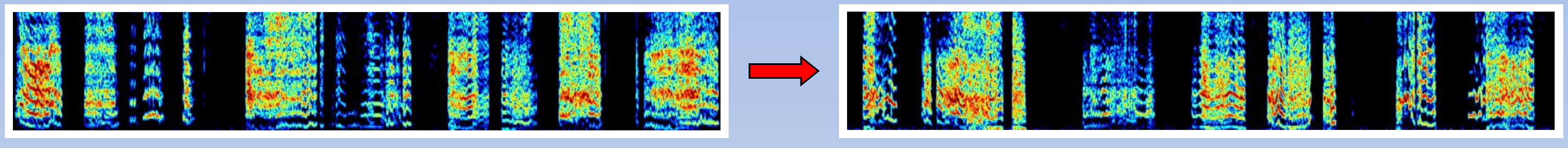
- **Question**:

What are the mechanisms that produce subsong ("babbling") – i.e., the highly variable song in very young juveniles?

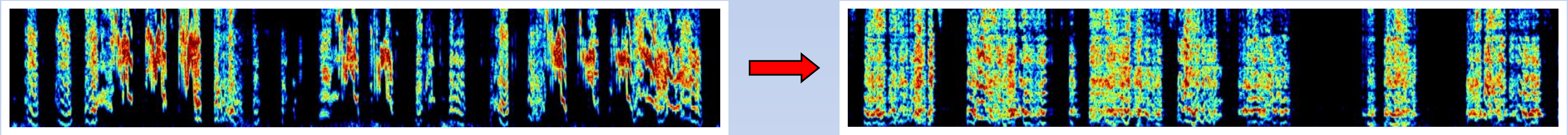
# HVC-lesioned birds could still produce subsong!

**Control** **no HVC**

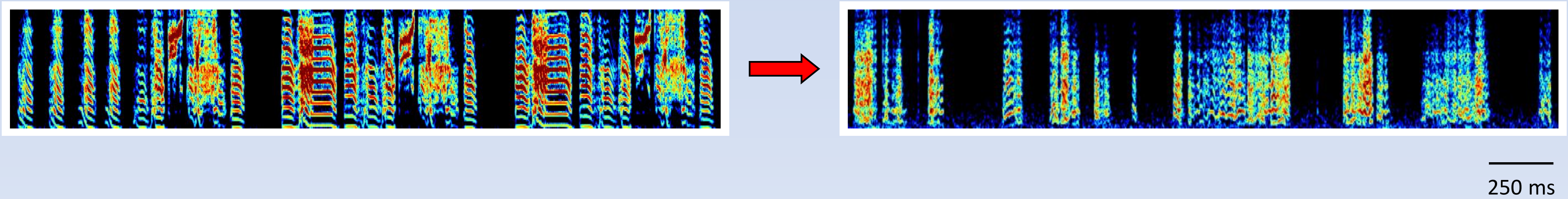
Subsong stage (37 dph)



Plastic song stage (50 dph)

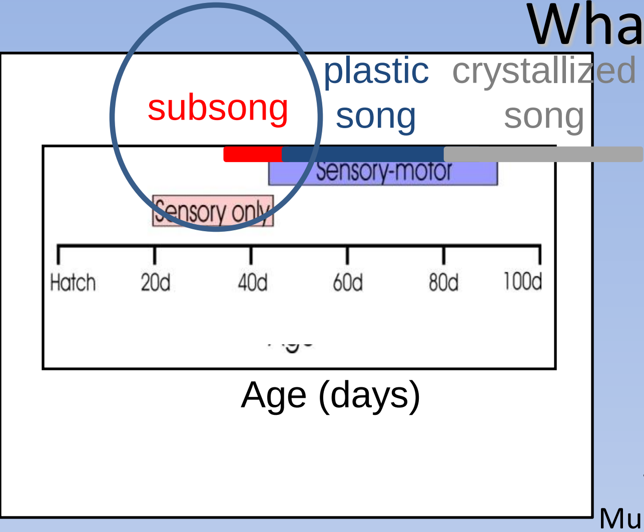


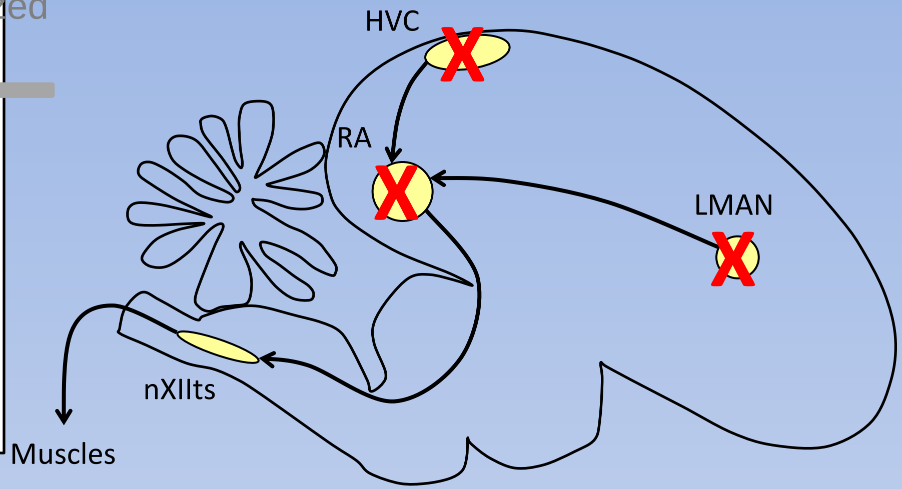
Adult

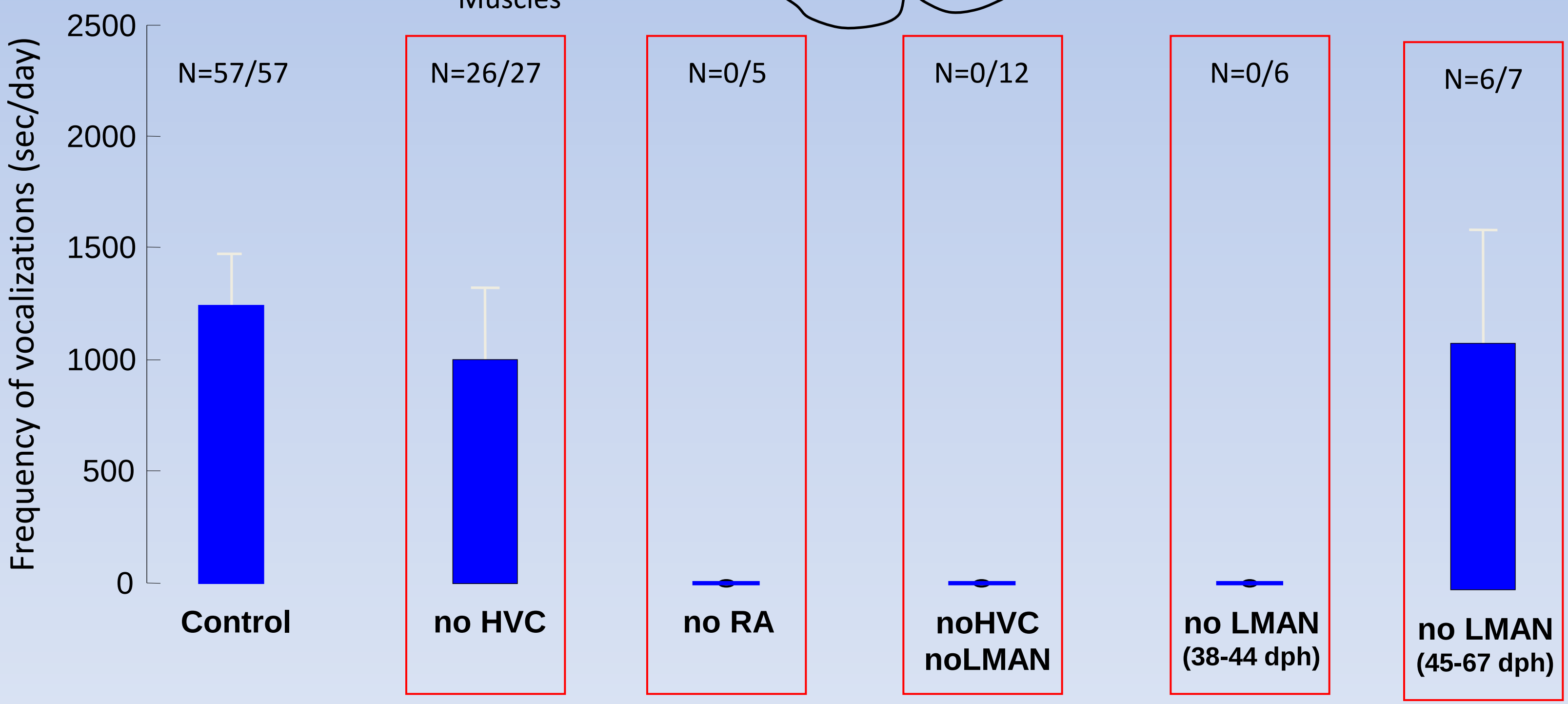



Aronov et.al. (2008) *Science*

# What drives subsong production?



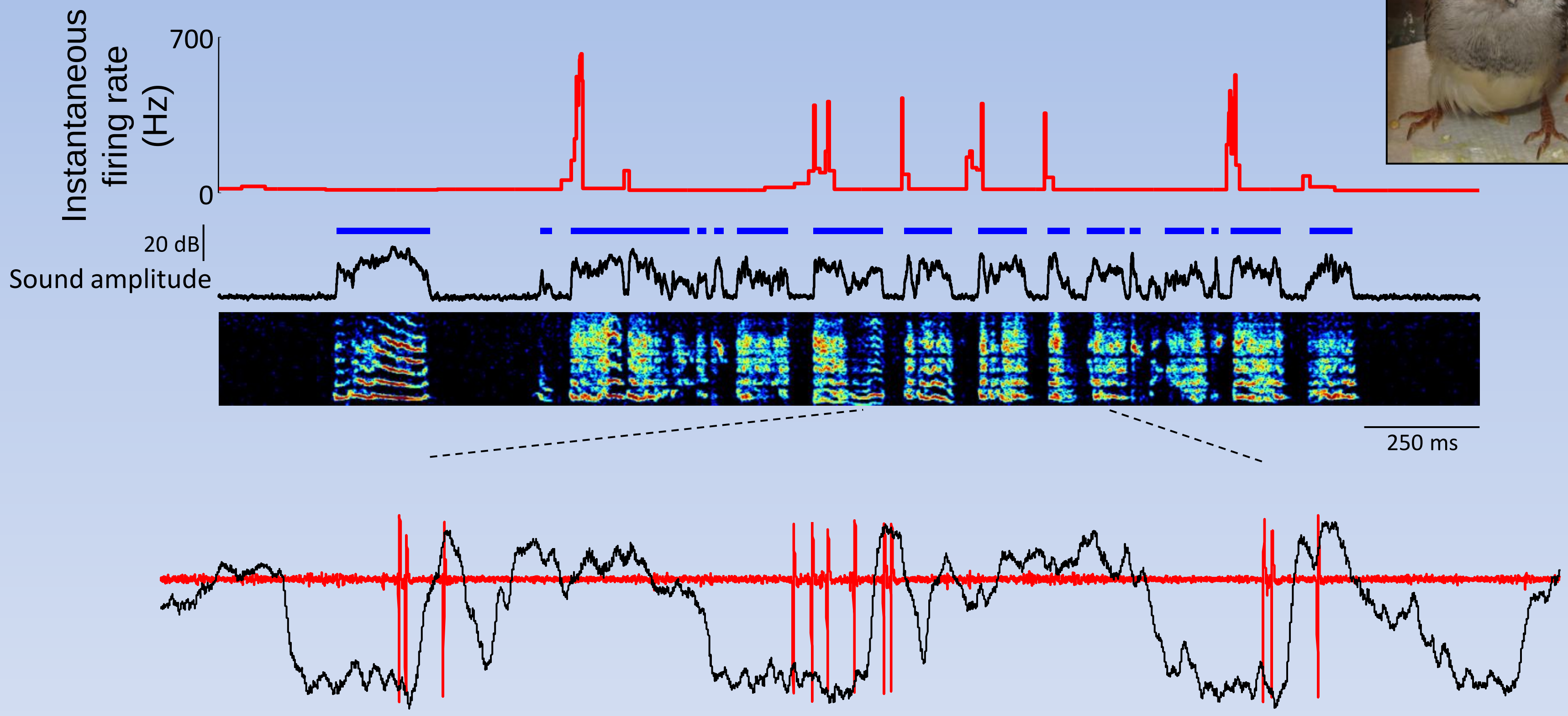




Aronov et.al. (2008) *Science*

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

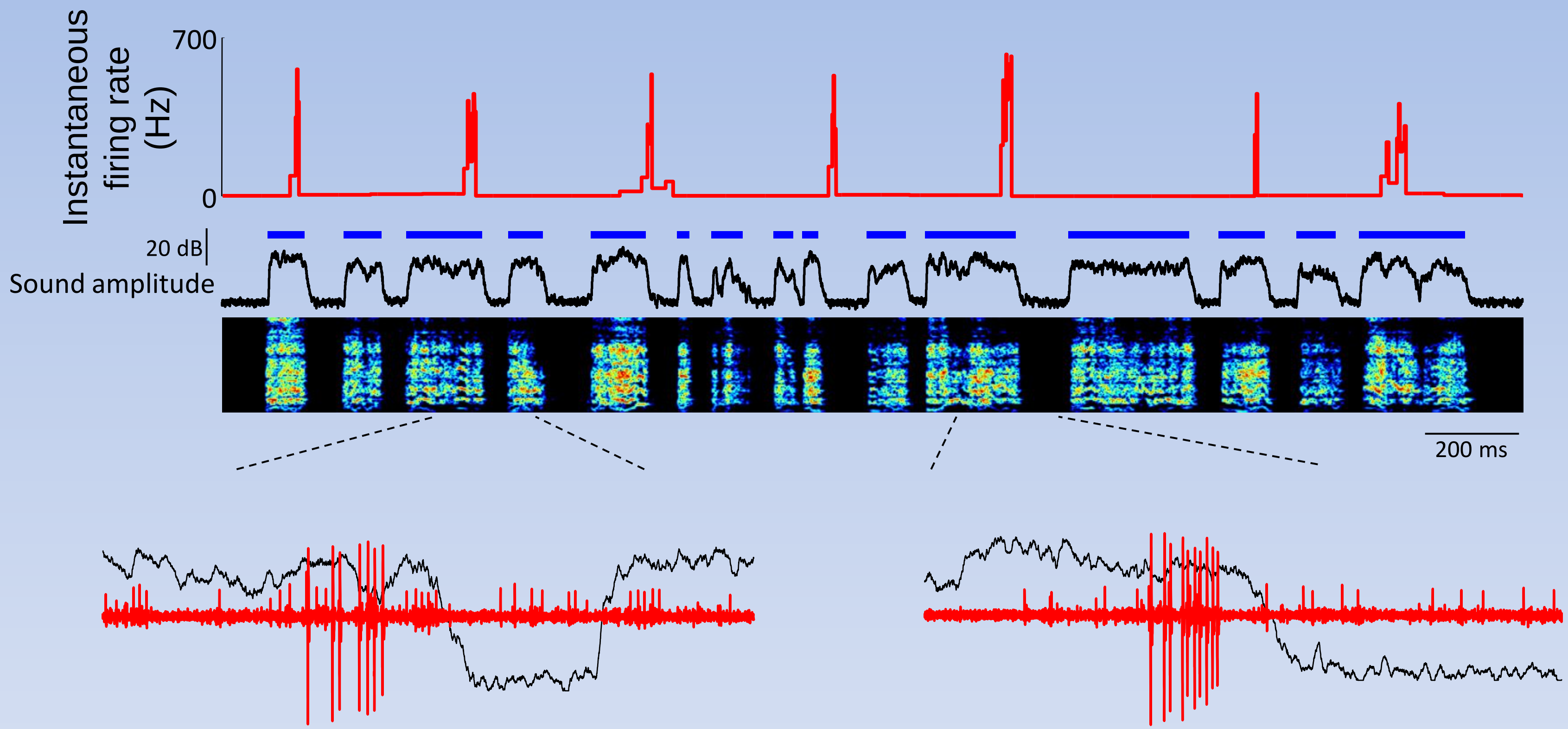
# LMAN-RA projecting neurons exhibit activity primarily prior to subsong syllable onsets




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*

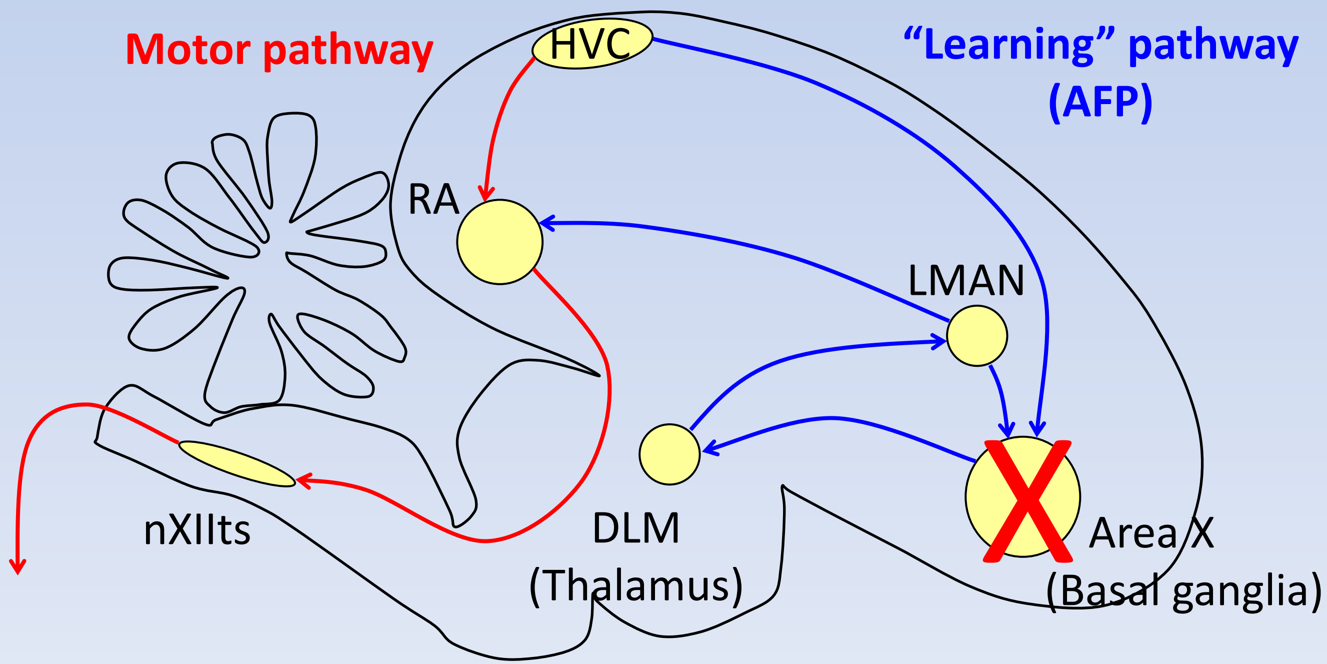
# LMAN neurons exhibit activity also prior to subsong syllable offsets



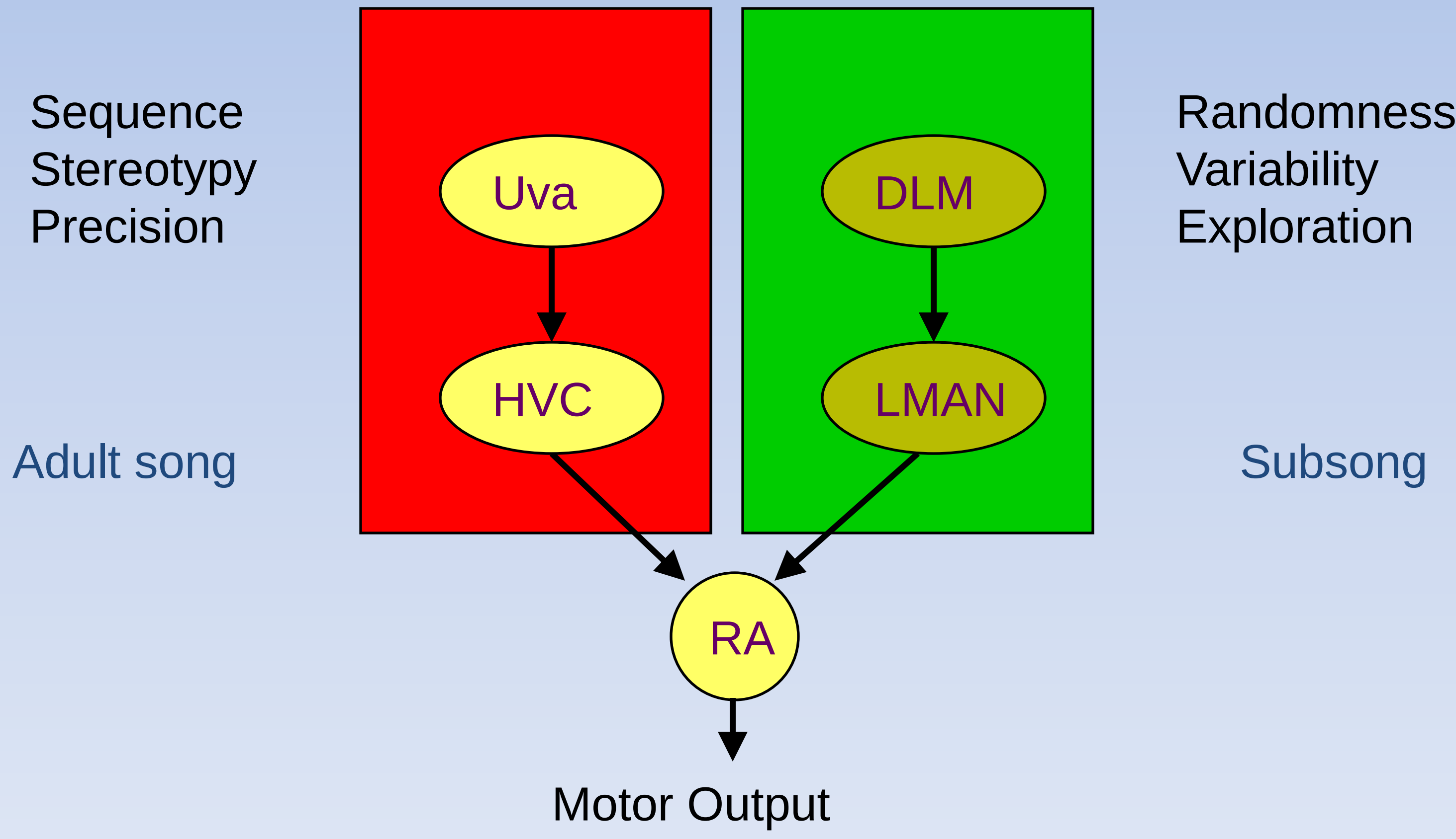

Aronov et.al. (2008) *Science*

- 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?

Lesion of area X does not lead to elimination of subsong.
DLM is necessary for the production of subsong.
LMAN → RA pathway cannot generate subsong like vocalizations independent of DLM.



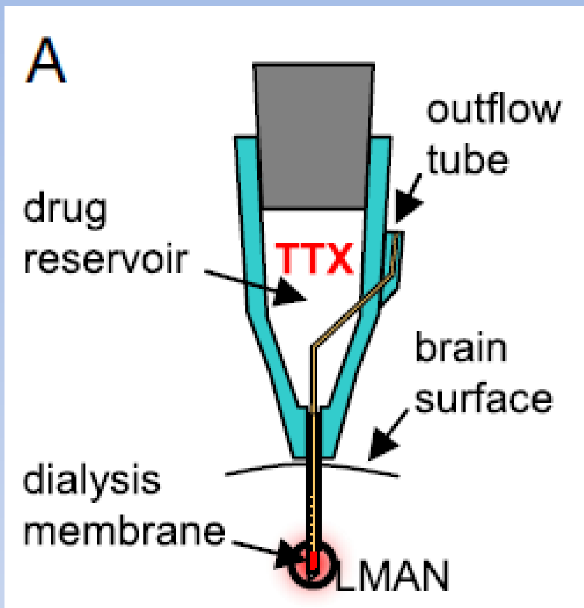
# Separate premotor pathways for stereotyped song and babbling



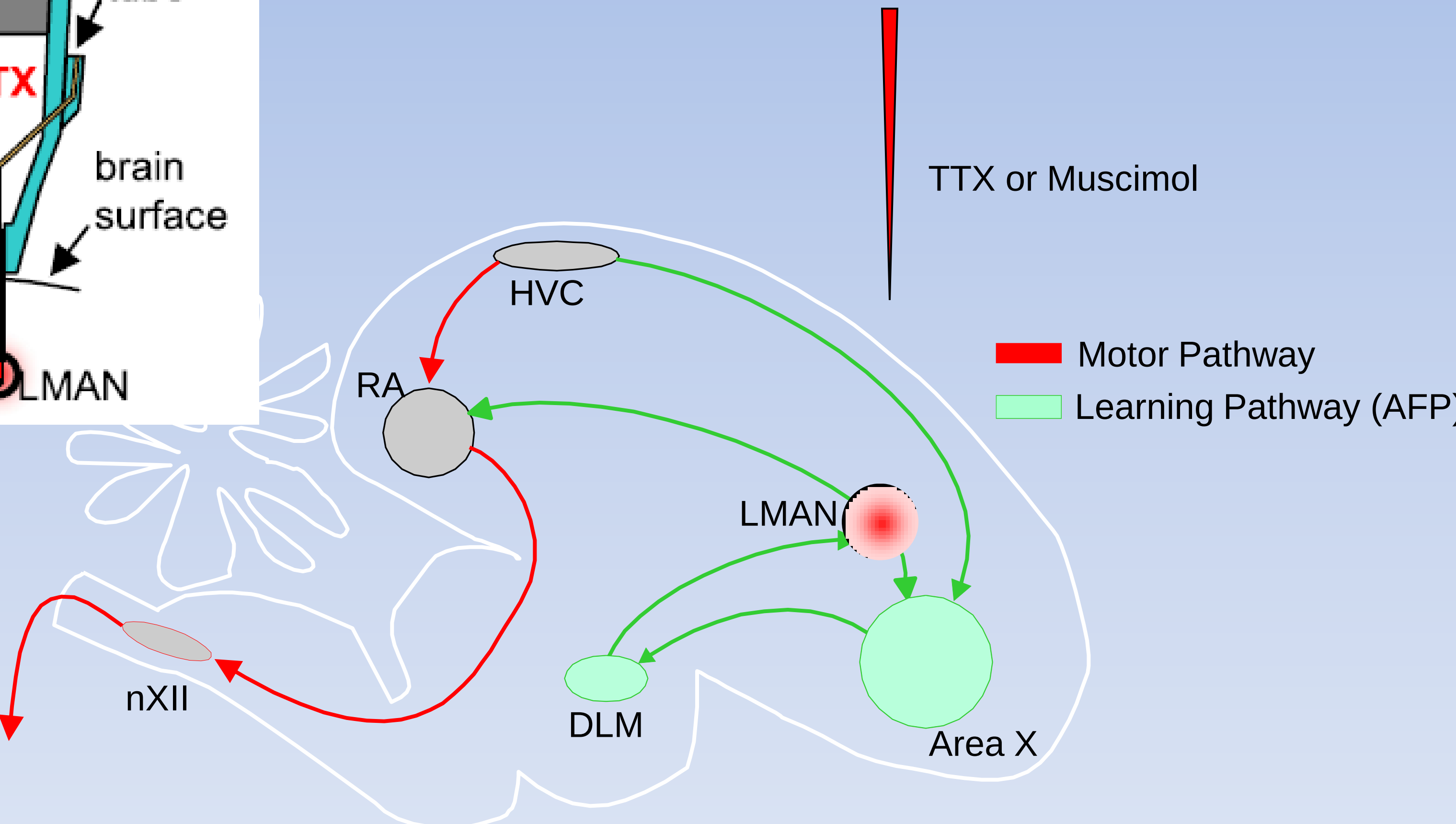
- **Question**:

  What is the role of LMAN in older juveniles?

# Role of LMAN in older juveniles



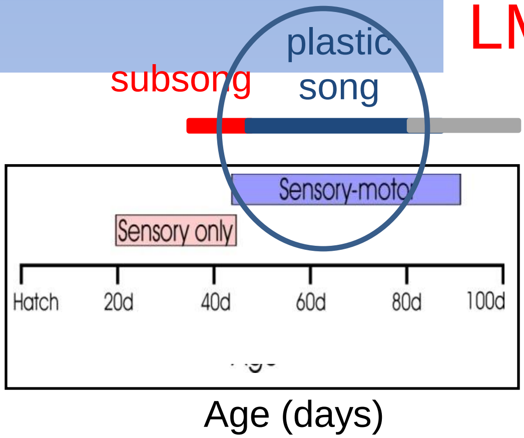


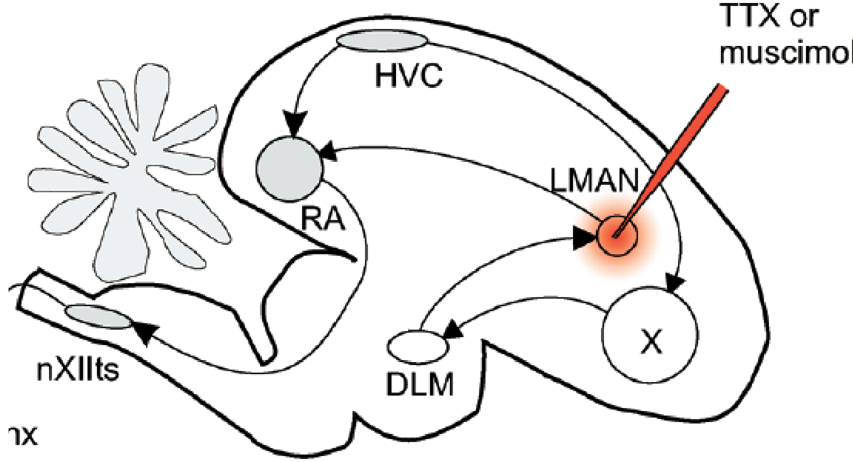


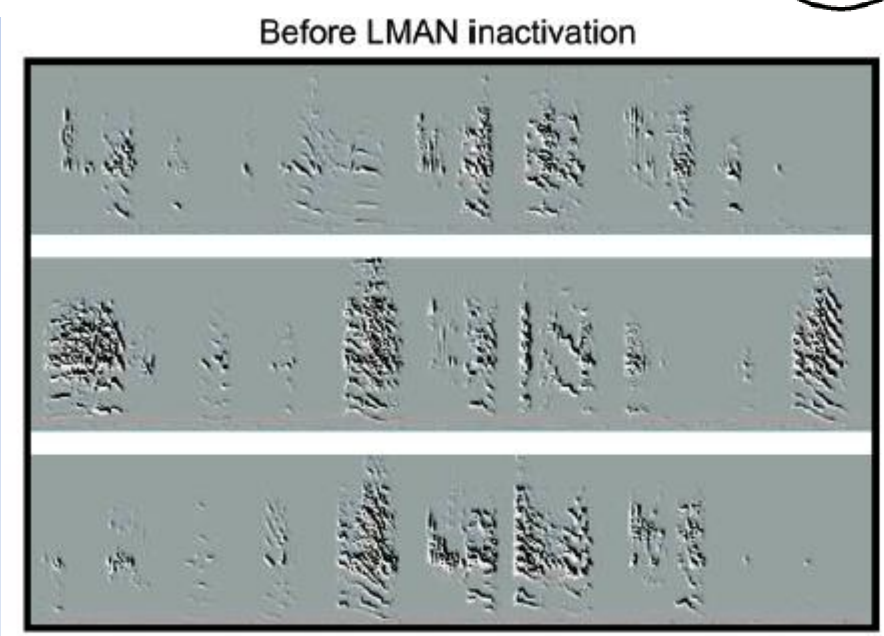

Ölveczky BP, Andalman AS, Fee MS (2005) Vocal Experimentation in the Juvenile Songbird Requires a Basal Ganglia Circuit. PLoS

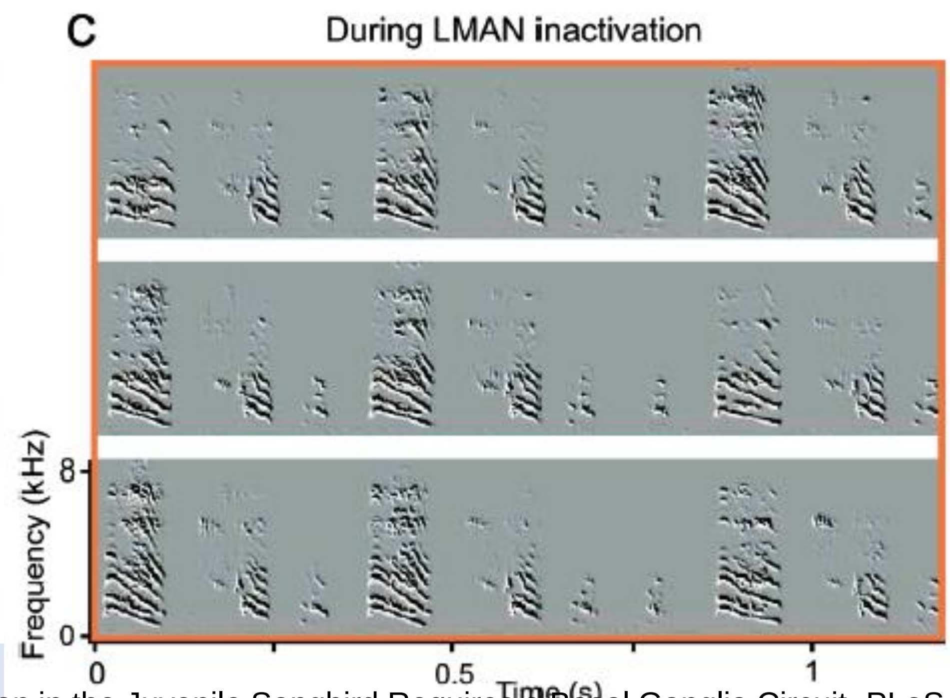
# *Role of LMAN in older juveniles*

## LMAN is a generator of variability



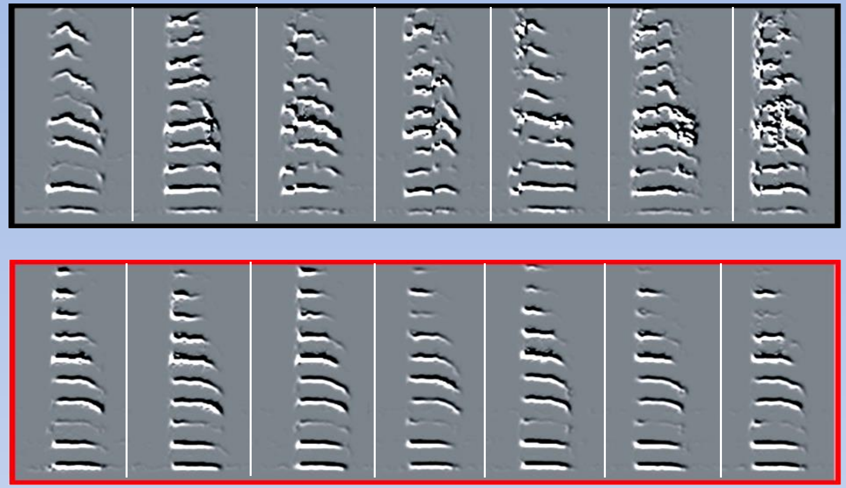




Ölveczky BP, Andalman AS, Fee MS (2005) Vocal Experimentation in the Juvenile Songbird Requires a Basal Ganglia Circuit. PLoS Biol 3(5): e153. doi:10.1371/journal.pbio.0030153

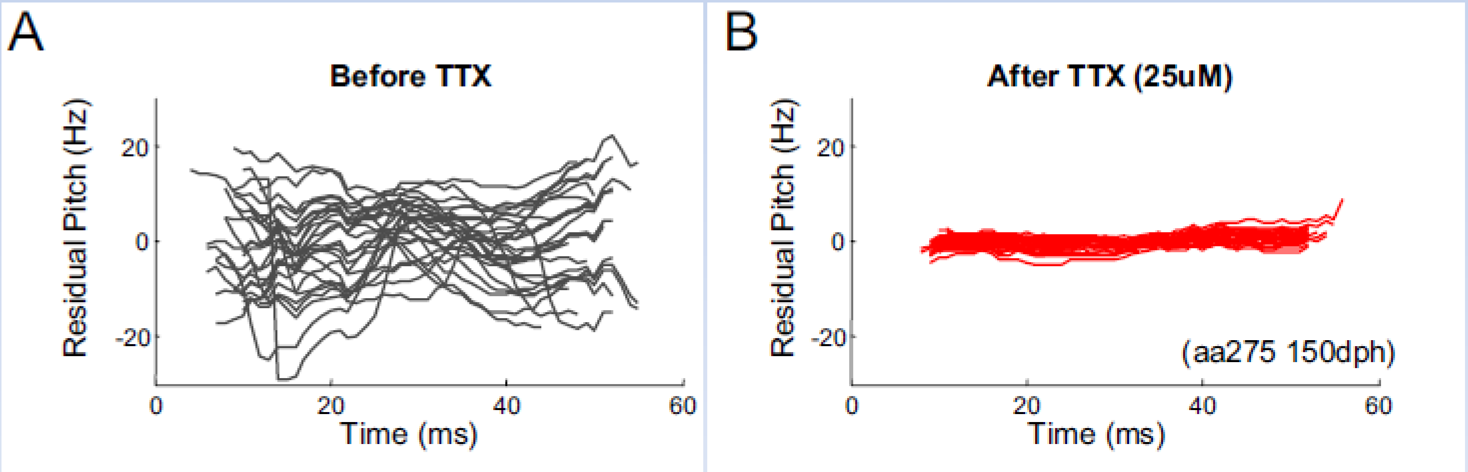
# Reduced variability in pitch after LMAN inactivation during crystalized song

**Control**

**LMAN inactivated**

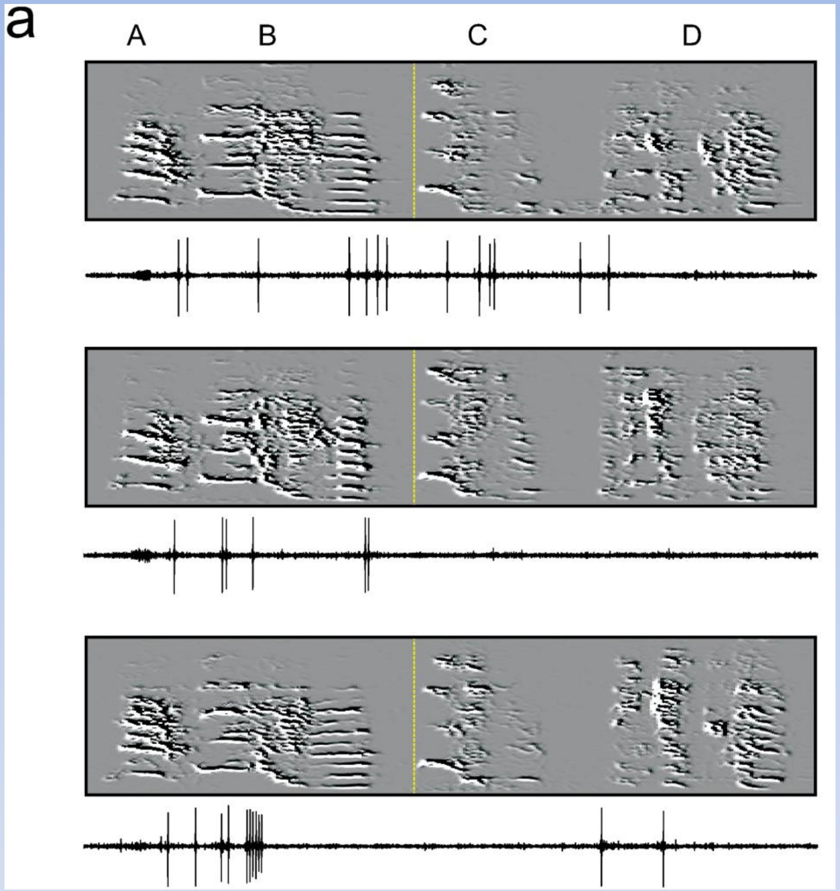


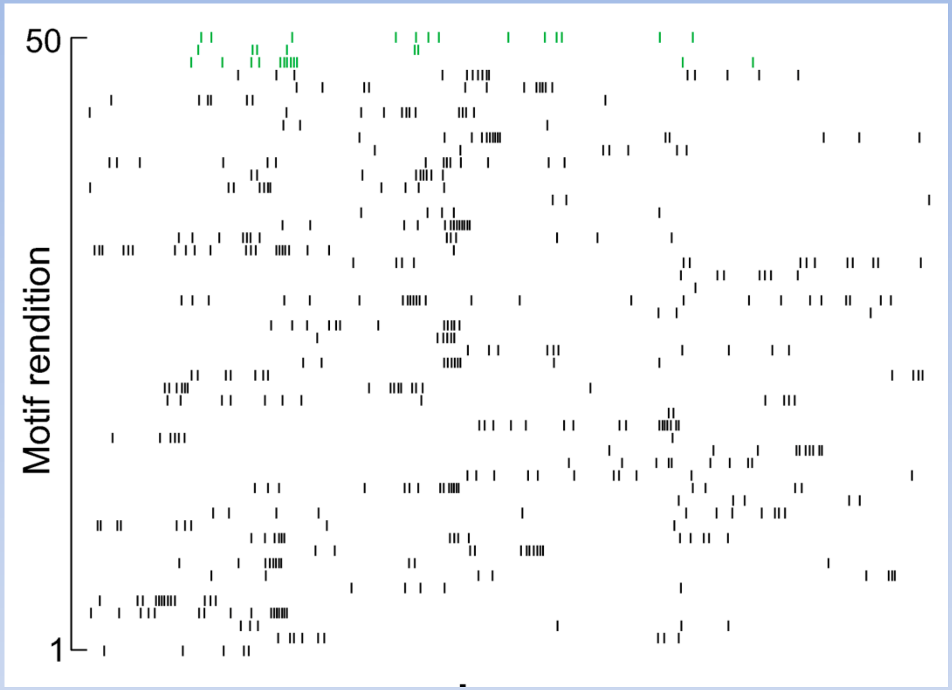
LMAN involves in injecting stochastic noise into the naive behavior so to have more variation on which to select the better performance.




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



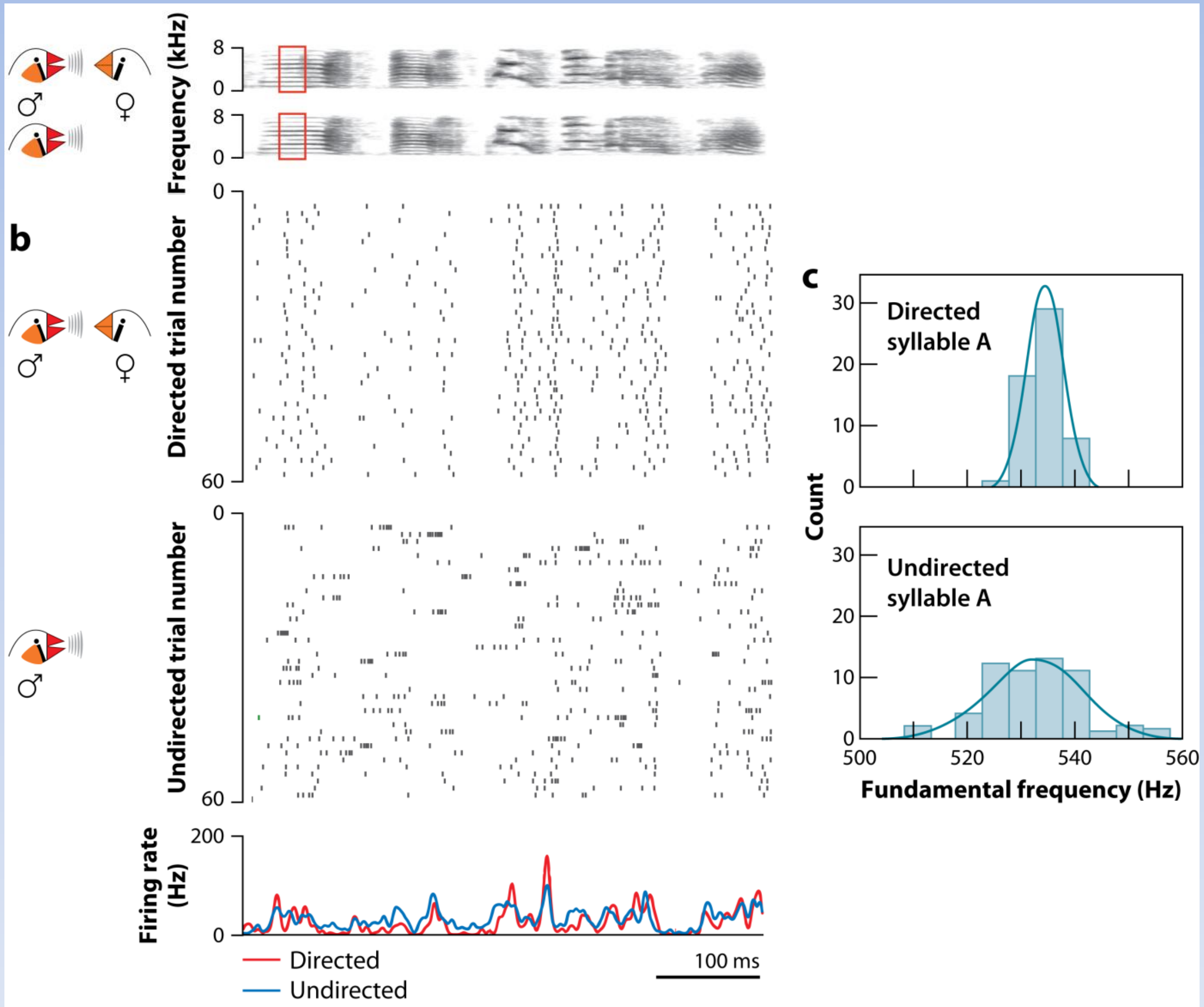

Ölveczky BP, Andalman AS, Fee MS (2005) Vocal Experimentation in the Juvenile Songbird Requires a Basal Ganglia Circuit. PLoS Biol 3(5): e153. doi:10.1371/journal.pbio.0030153

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

Directed singing

Undirected singing

rehearsal?



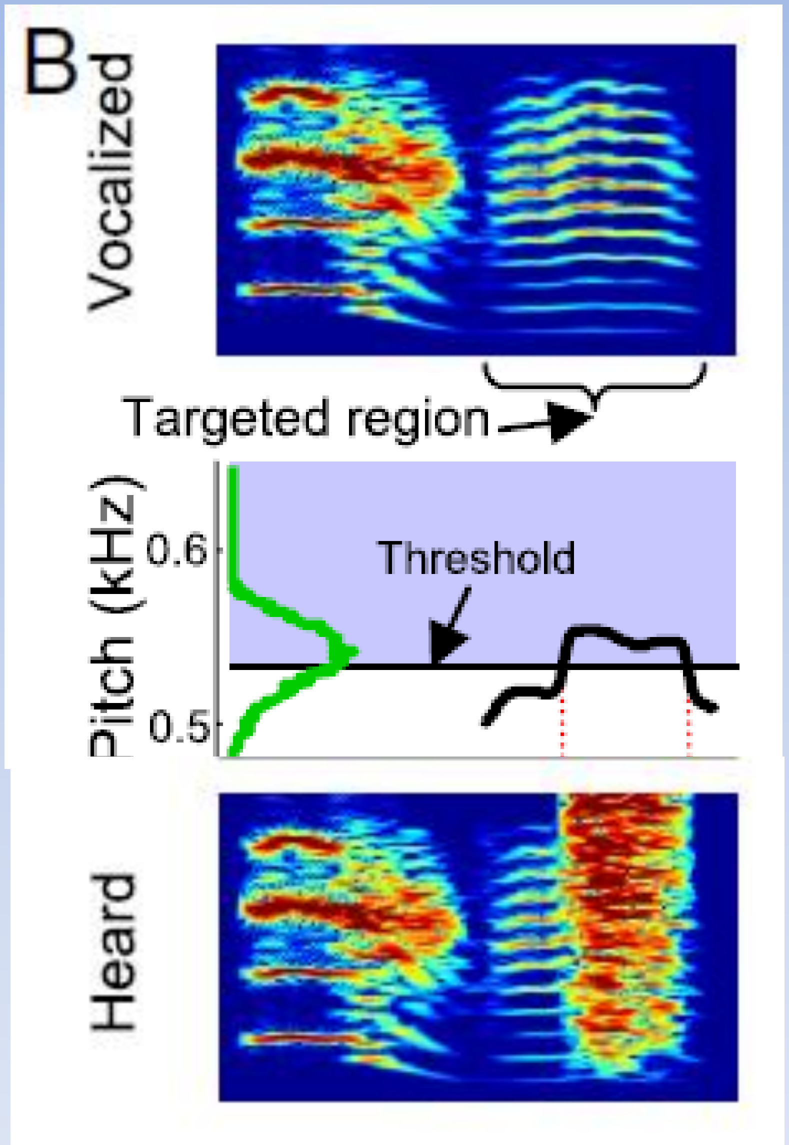

Brainard MS, Doupe AJ. 2013. Annu. Rev. Neurosci. 36:489–517

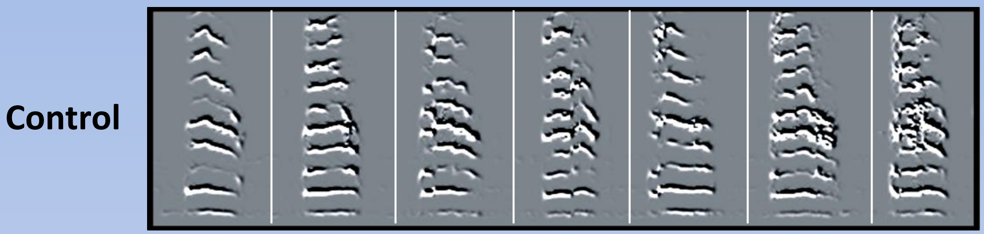
Kao et al. (Journal of Neuroscience, 2008)

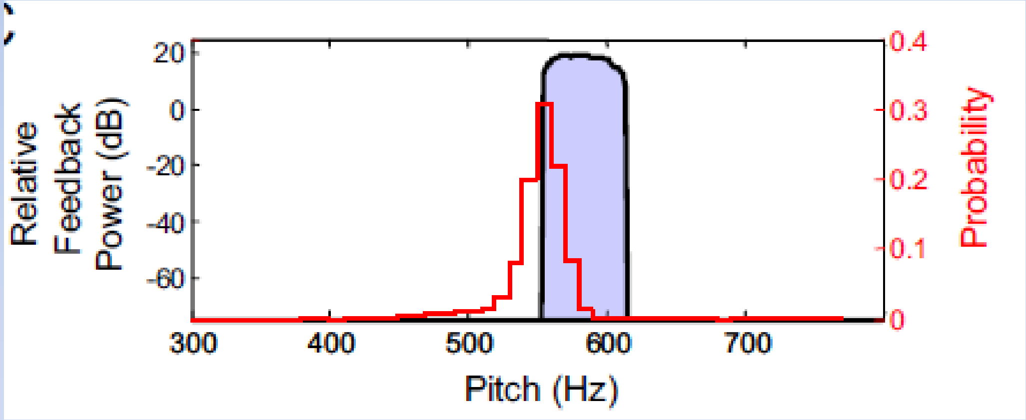
# Role of LMAN in learning

- 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

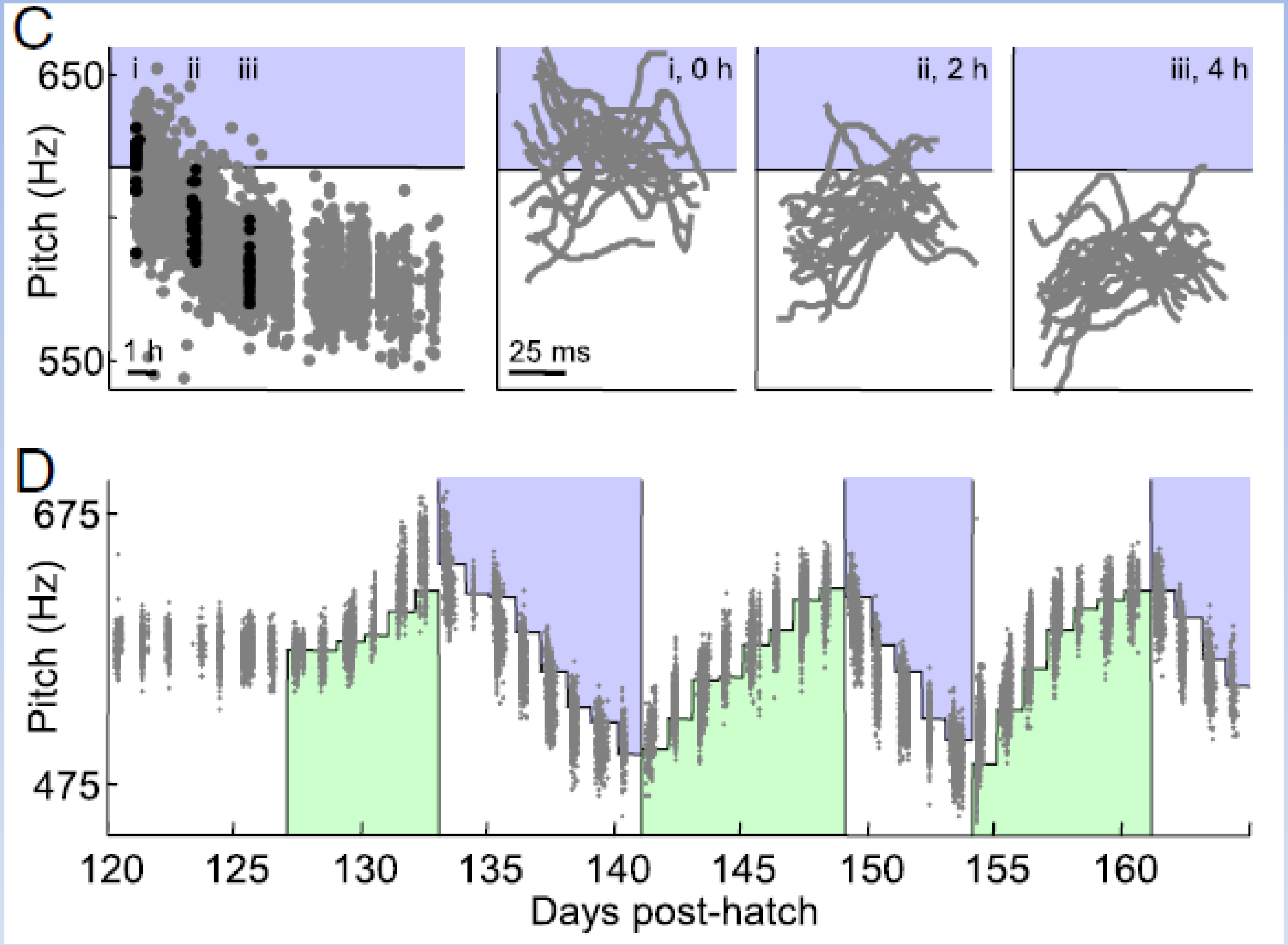



Tumer & Brainard (Nature 2007)
Andalman & Fee (PNAS, 2009)

# Behavioral results




Andalman & Fee (PNAS, 2009)

# Two possibilities

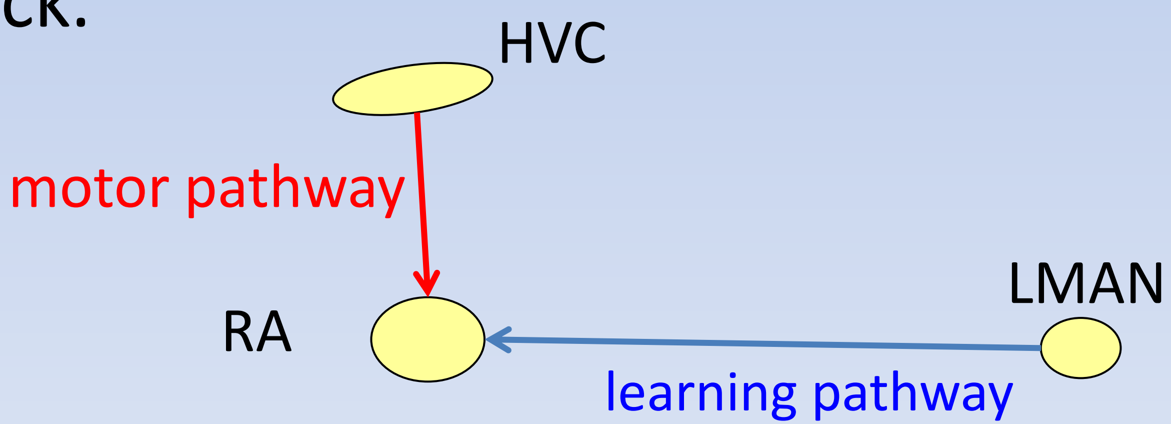
Where does the learning take place?

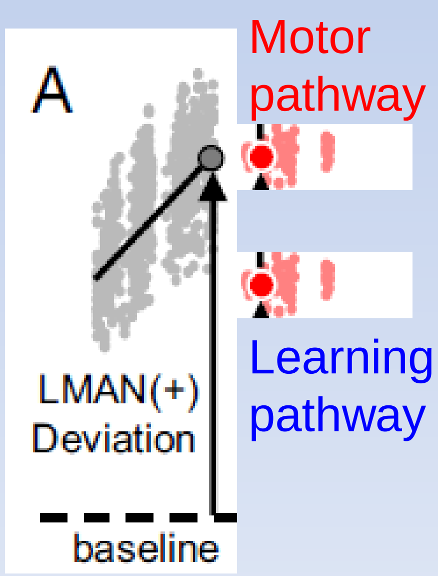
- Hypothesis 1: motor pathway

  → LMAN inactivation will not change the pitch.

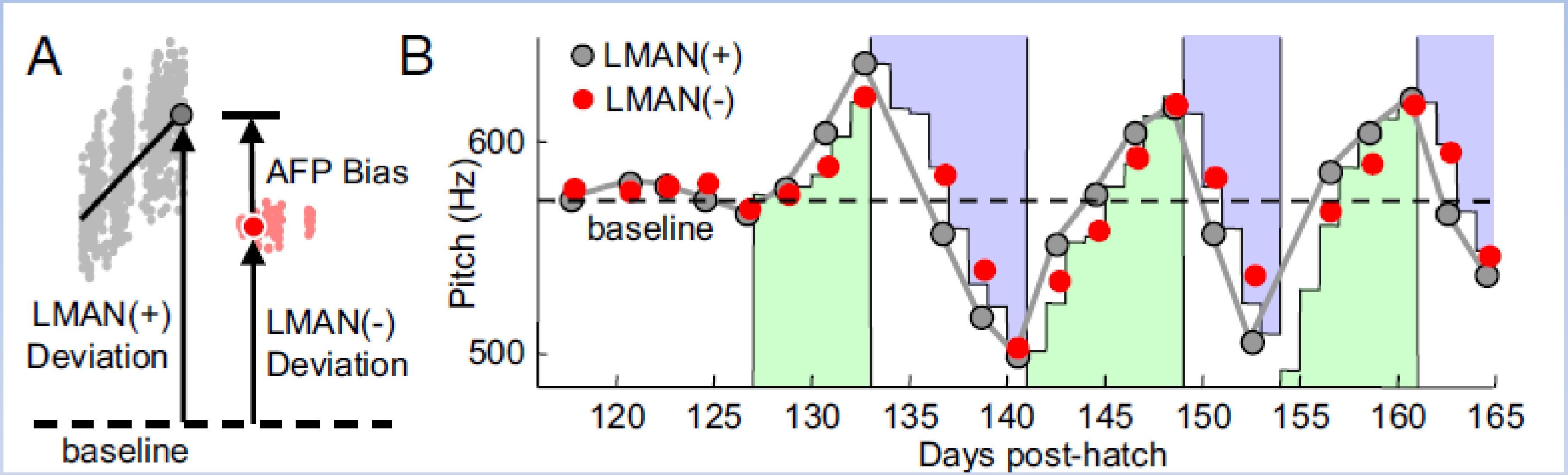
- Hypothesis 2 : learning pathway

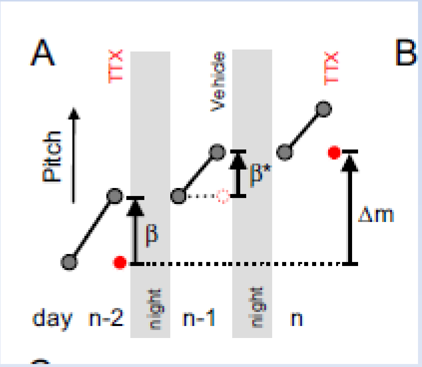
  → LMAN inactivation will change the pitch to go back.



# 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

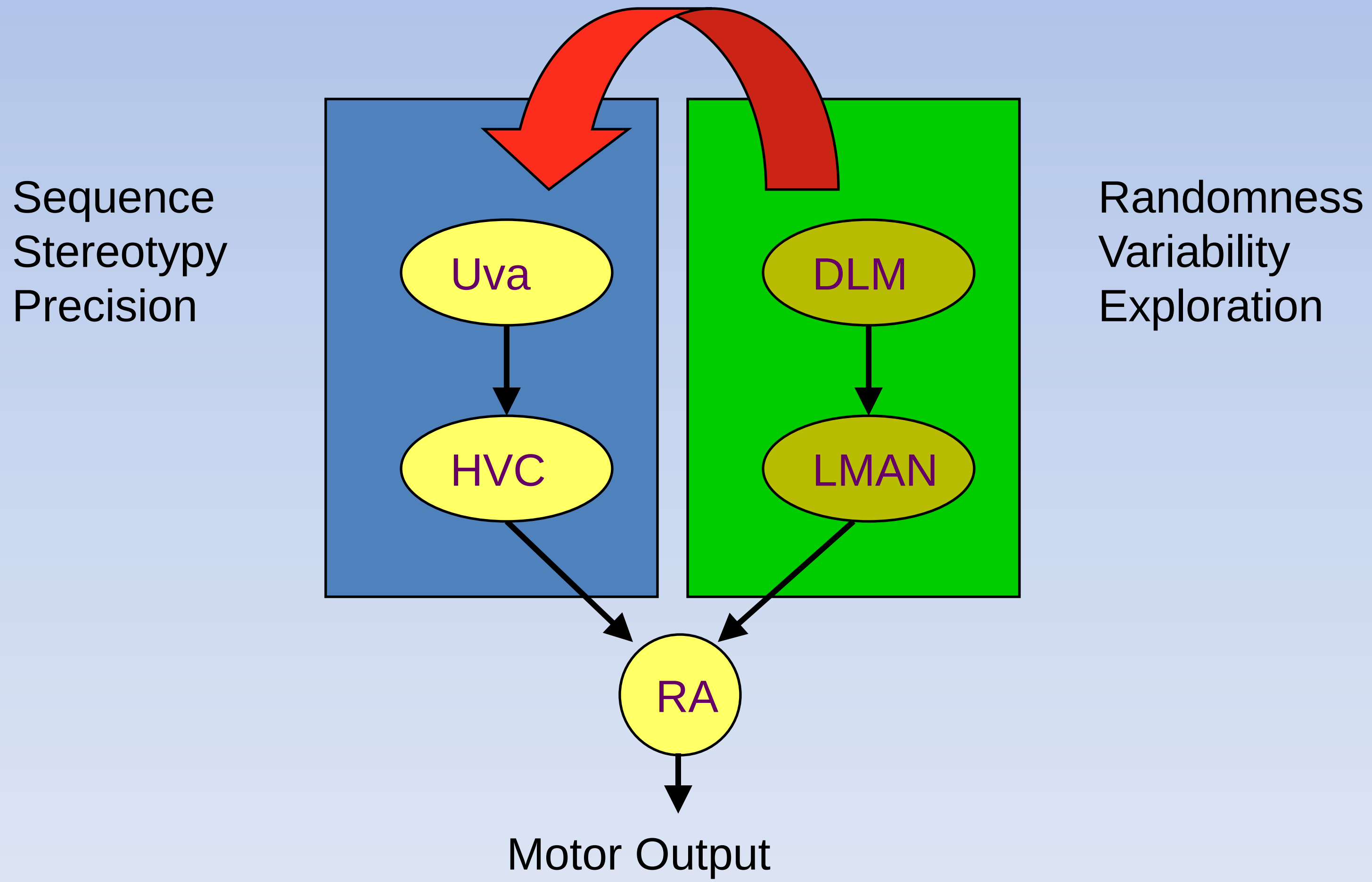
Andalman & Fee (PNAS, 2009)

# LMAN is a generator of variability

- LMAN is the essential premotor nucleus for the earliest ‘babbling’ vocalizations (Aronov and Fee, 2008).

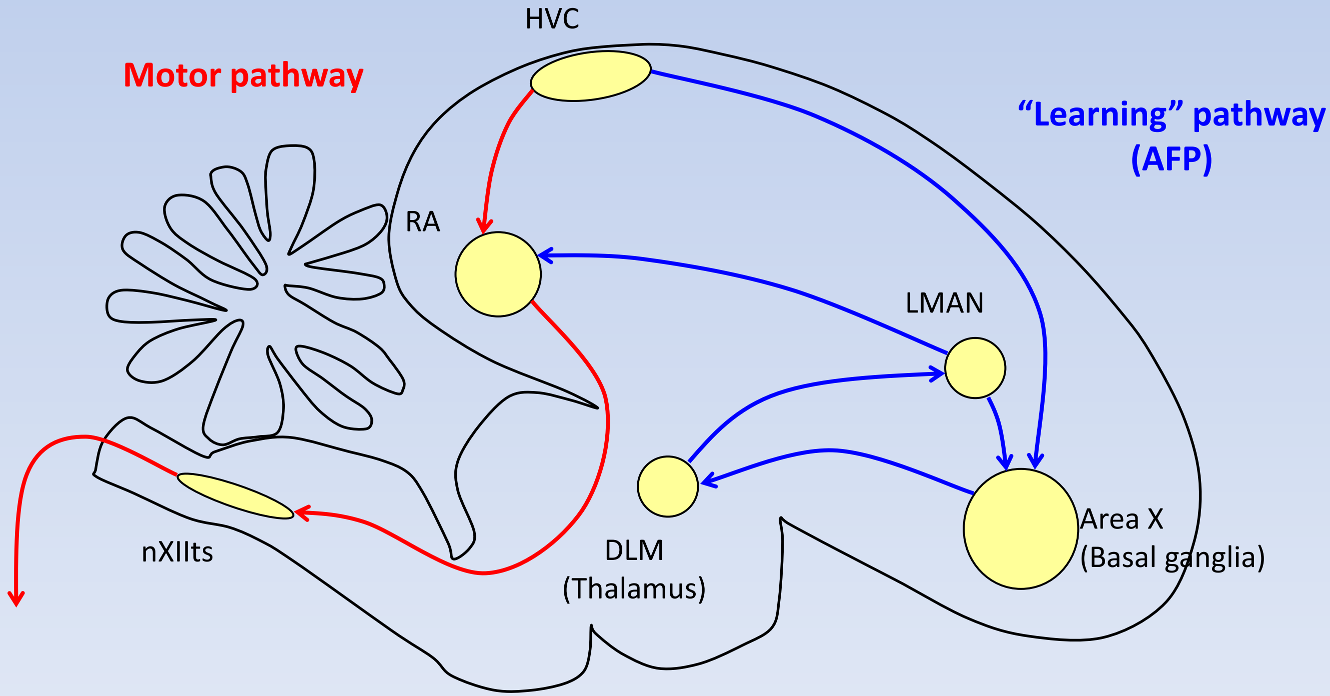
- LMAN may serve an essential role in song learning by driving variability: in subsong, plastic song, and even in adult song (Kao et al, 2005).
- LMAN adds variability to enable exploration. This variability produced by the learning pathway is not purely random, but instead biased.
- This bias is consolidated in the motor pathway after one day delay.

# Separate premotor pathways for stereotyped song and babbling



# Summary

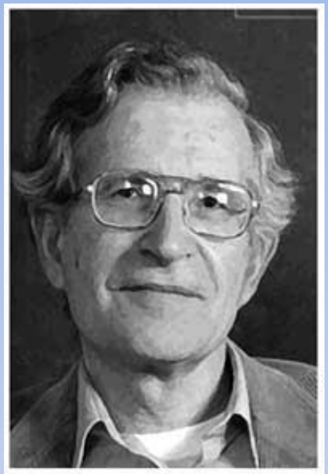
- Activity of the motor pathway is stereotyped.
- Activity of the learning pathway is variable.
- These two signals are combined at RA.



# Innate versus learned

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

Chomsky



## Chomsky's linguistic theory

Noam Chomsky proposed in the 60s that structure of language (syntactic), is biologically determined, namely genetically transmitted.

He argued that all humans share the same underlying syntactic, irrespective of socio-cultural difference.

In other words: genes constrain language diversity.

The City University of New York

**Ofer Tchernichovski:**
**Do genes constrain song diversity?**

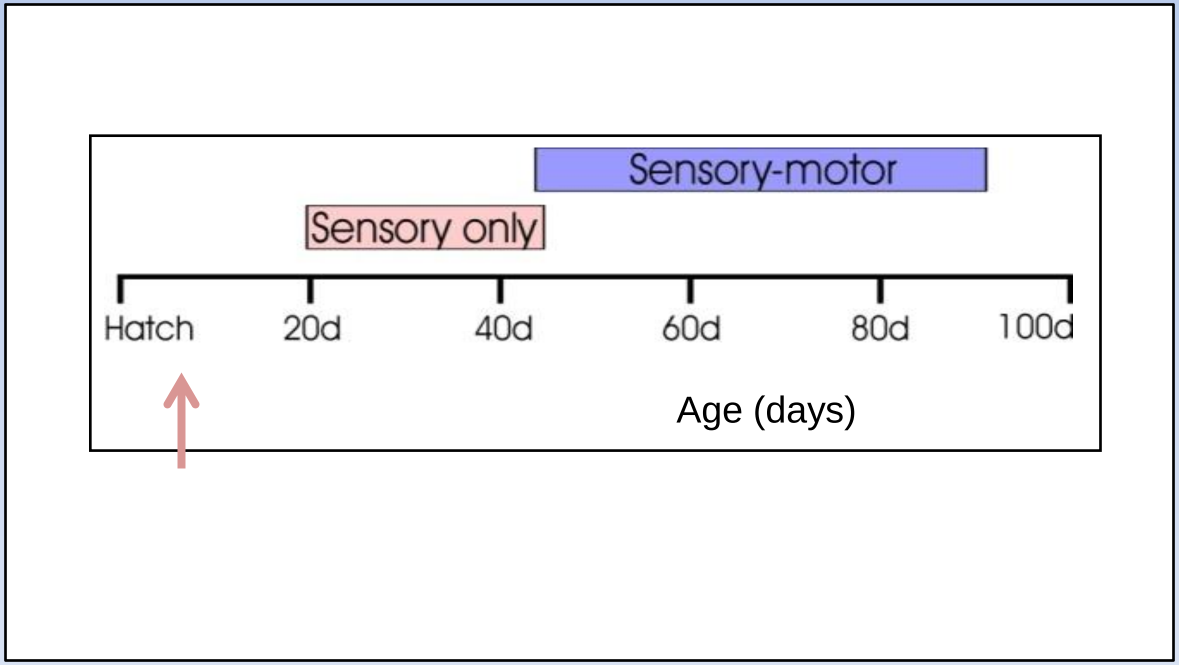
# Song culture in birds

- Some songbirds provide biologically tractable models of culture:

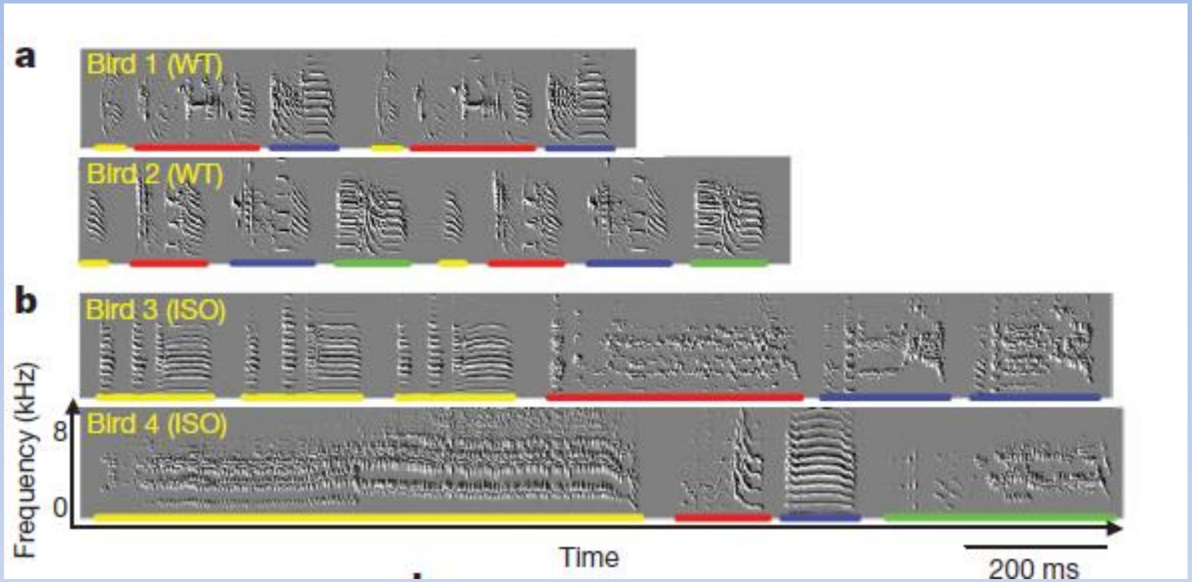
geographically separated groups have local song dialects- just like humans.

- 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?



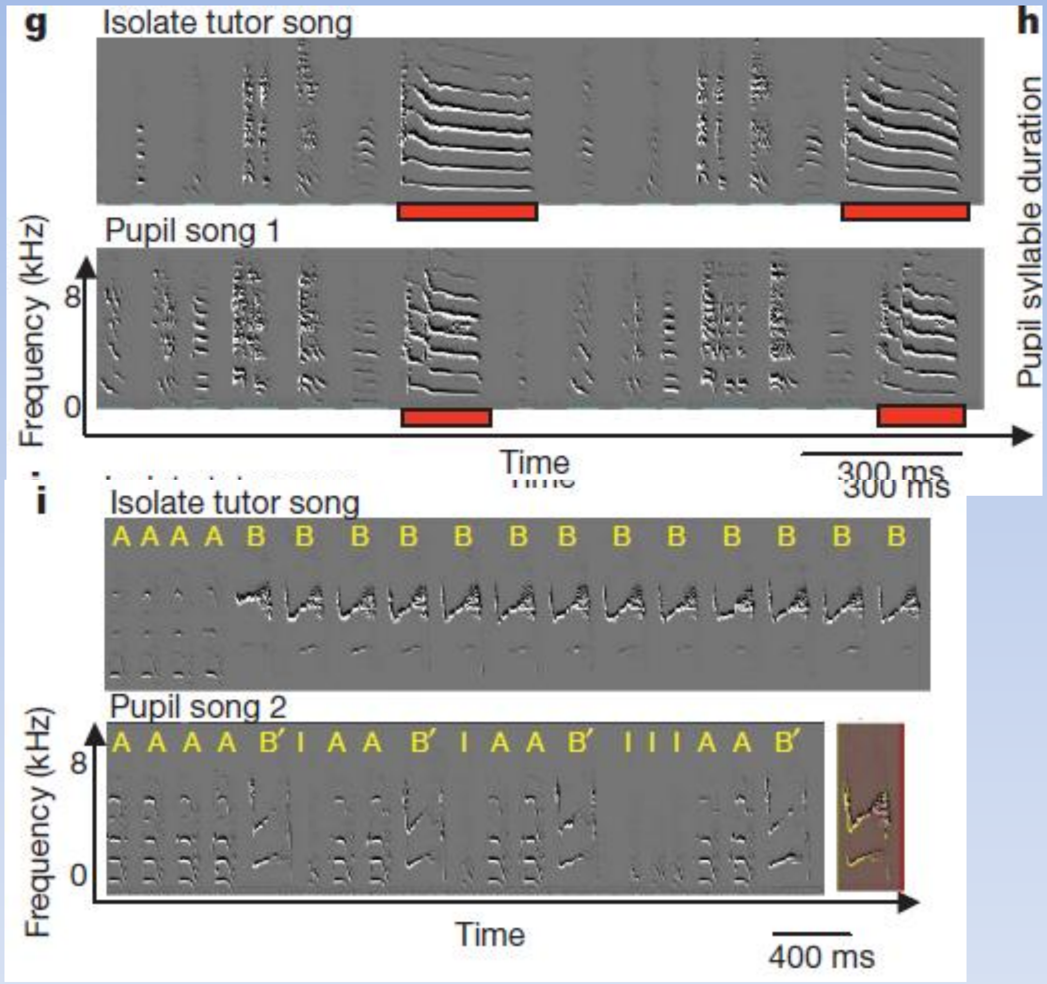

Isolated birds are establishing a 'song template' very early, during the sensory period.

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

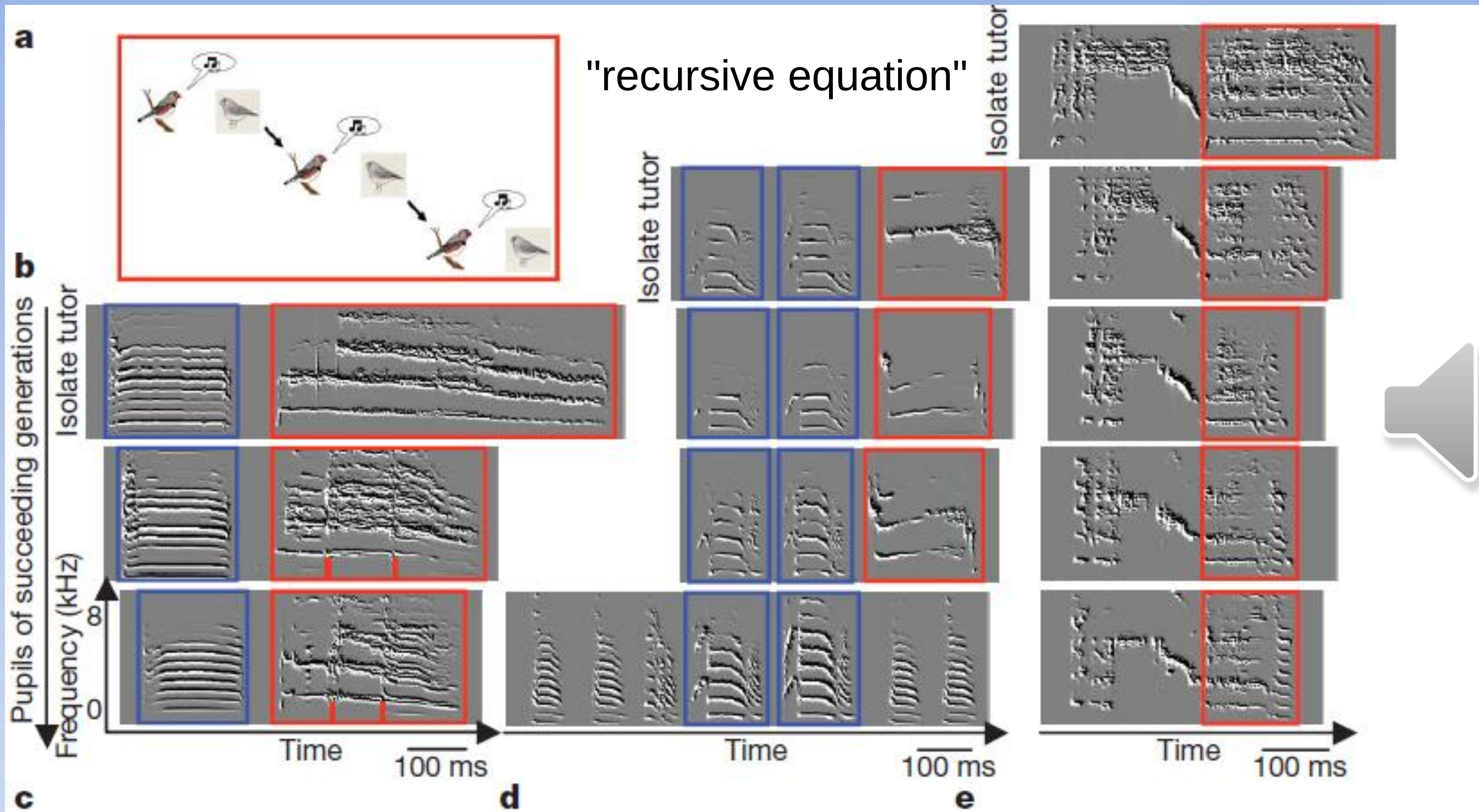
Konishi 1965; Marler 1970

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