Neural Basis of Vocal Communication in Songbirds

Raphael Pinaud

Department of Brain and Cognitive Sciences
University of Rochester
Rochester, NY, USA
Outline of Talk

Part I:
- Why songbirds?
- Vocal learning: auditory processing and auditory memories.
  - The songbird NCM:
  - Song selectivity, auditory discrimination and perception.

Part II:
- Proteomics screening: song-regulated proteins.

Conclusions and Acknowledgements
Songbirds As a Model for Vocal Learning and Auditory Processing

**Vocal Learners**
- Independent gains
- Independent losses
- Everybody has it to varying degrees
Songbirds As a Model for Vocal Learning and Auditory Processing

A

Zebra Finches (*Taeniopygia guttata*)
Vocal Learning and Maintenance of Learned Songs Requires Auditory Feedback
Cross Fostering Illustrates Vocal Learning

Syntax and Phonology is Copied
Songbirds As a Model for Vocal Learning and Auditory Processing

Zebra Finches (Taeniopygia guttata)

Sensory, sensorimotor & motor processes
Auditory Processing is Required for:
- Vocal Learning
- Song Maintenance
- Perceptual Processing of Songs
  - mate selection
  - individual recognition
  - territorial defense
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NCM:
- Greater selectivity for complex stimuli
- Preference for conspecific songs
- Appears to be involved in discrimination of birdsong and maybe the formation of auditory memories.
zenk

A

B

C

D

NCM

auditory input

vocal output

cochlea

n.XII

HVC

MLD

RA

ciHV

X

Nif

L

CMHV

Para-

HVC

Gv

shelf

HVC

MLAN

LPO

Hp

Cb

H

zenk
Frequency-Dependent Organization in NCM as Revealed by ZENK Expression
Discrimination of Natural Stimuli in NCM
NCM Neurons are Selective to Conspecific Songs and Calls

Electrophysiological responses
NCM Habituate and Respond Robustly to Novel Songs

Role in Auditory Discrimination?
Auditory Memory Formation?
Neuronal Activation in NCM is Correlated with Strength of Learning

Diagram showing the timeline of events involving hatch (D 0), exposure to playback of tutor song, critical period for vocal learning, songs recorded, re-exposure to tutor song (D 250), and removed from tutor’s cage (D 7). Graphs illustrate the relationship between neuronal activation and the fraction of shared elements for experimental and control conditions.
Lesions in NCM Impact Tutor Song Preference, but not Production
Conclusions – Part I
NCM as a key site involved in auditory discrimination and the formation of auditory memories

-Auditory stimuli drive activation of NCM neurons.

-These responses are selective to species-specific stimuli, habituate and are vigorously reinstated upon presentation of novel songs.

-Activation of neurons in NCM is positively correlated with strength of song learning.

-Lesions of NCM impact discrimination of species-specific songs (possibly formation of auditory memories).

What are molecular mechanisms underlying these processes?
Proteomic Analysis – 2D-DIGE

1. Protein Extraction, Labeling with Different CyDyes
2. Mix and Run on A Single 2-D Gel
3. 2-D gel Analysis
4. Protein Identification by Mass Spec
Proteomic Analysis – 2D-DIGE

SAMPLE LABELING
Samples are labeled with specific fluorescent dyes.

SAMPLE FRACTIONATION BY 2DE
Mix Labeled extracts

GEL IMAGING
Typhoon 9410 Imager

IMAGE ANALYSIS
Differential Analysis

SPOT PICKING
PROTEIN DIGESTION

MS/MS
Peptide Mass Finger-printing
Experimental Design
Representative Gels and Protein Identification

5 min

1 hr

3 hr

5 proteins

19 proteins

18 proteins
Conclusions – Part II

Calcium-regulated biochemical and gene expression programs in NCM may underlie long-term changes in neural circuitry required for song discrimination and the formation of auditory memories.

- High throughput proteomics screening reveals a complex network of proteins regulated by auditory experience in NCM.

- The identified proteins belong to multiple functional classes and are present in several cell compartments.

- The MAPK pathway appears to be highly regulated by auditory stimuli in NCM.
Acknowledgments

Liisa Tremere
Ernest Nordeen
Kathy Nordeen
Thomas Terleph
Oscar Alzate
Erich Jarvis
Cristina Osorio
Robert Buechler