The expression of autism gene FOXG1 in the zebra finch brain during vocal development

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Introduction

Rett Syndrome and FOXG1 Syndrome

- Rett syndrome (RTT) is an X-linked neurodevelopmental disorder classified as an autism spectrum disorder.
- It typically affects young females and causes progressive impairment and loss of motor skills and language.
- Forkhead box protein G1 (FOXG1) gene mutations are said to cause FOXG1 syndrome, which overlaps with Rett syndrome symptoms.
- Children with FOXG1 syndrome have severe physical and cognitive disabilities such as intractable seizures, movement disorders, cortical vision impairment, and language difficulties.

MeCP2 Gene

- Mutations in the X chromosomes encoding the methyl-CpG-binding protein 2 (MeCP2) gene have been found to cause Rett syndrome.

Zebra Finch Songbird Model

- The zebra finch bird is an optimal research design as its song learning process mimics language development in children.
- The song learning circuitry of the zebra finch includes Area X, a basal ganglia nucleus within the anterior forebrain pathway (AFP), which provides insight into human neurodevelopmental disorders.

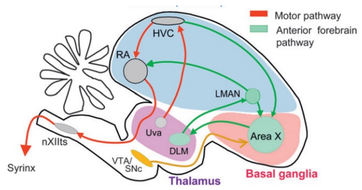

Figure 1. Song circuitry of the zebra finch songbird brain

Figure 2. Zebra finch songbird model

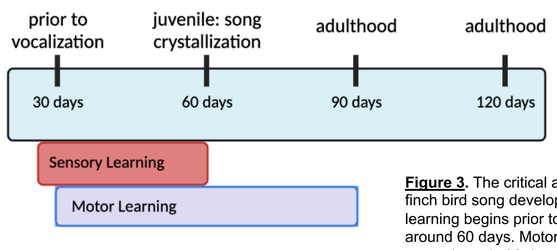

Figure 3. The critical age points in zebra finch bird song development. Sensory learning begins prior to 30 days and ends around 60 days. Motor learning last from approximately 30 days to 90 days.

Objectives

- We are seeking to examine the expression of the FOXG1 and the MeCP2 genes in the zebra finch song circuitry, specifically in Area X as this region is required for song learning.
- We are also looking for signs of developmental regulation with these two genes.

Materials and Methods

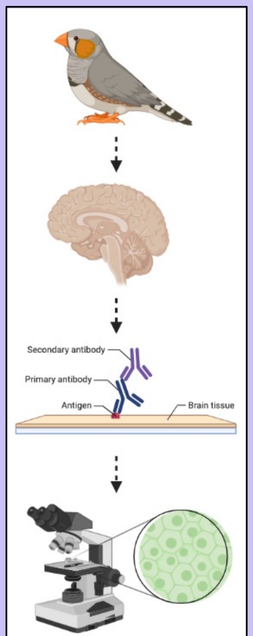

Figure 4. Stepwise experimental procedure

1. Zebra finch bird perfusion
- Birds were sacrificed and perfused at specific age points: 60 days and adulthood.
- Brains from male zebra finch birds were collected as the females do not sing.

2. Brain Cutting
- The brains were cut at 40-50 microns per brain tissue section.
- The tissue sections were placed into PBS.

3. Immunohistochemistry Staining
- Staining was conducted with either MeCP2 or FOXG1 primary antibody.
- The secondary antibody was composed of FITC for MeCP2 and CY3 for FOXG1.

4. Imaging through Fluorescence microscopy
- Imaging was performed using 10x and 40x lenses with the DAPI, FITC, and CY3 channels on a fluorescent microscope.

Results I

1. MeCP2 Expression in Area X

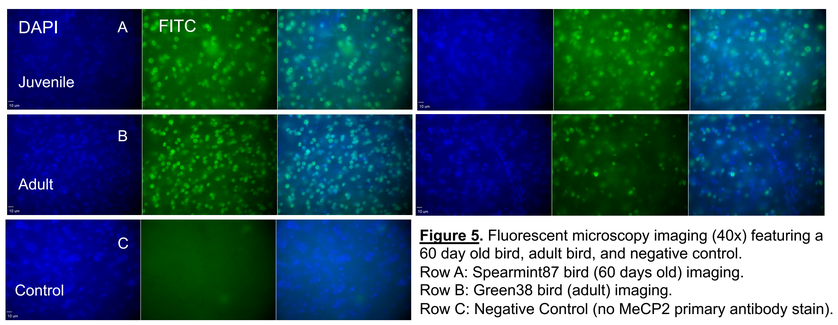

Figure 5. Fluorescent microscopy imaging (40x) featuring a 60 day old bird, adult bird, and negative control.
Row A: Spearmint87 bird (60 days old) imaging.
Row B: Green38 bird (adult) imaging.
Row C: Negative Control (no MeCP2 primary antibody stain).

2. FOXG1 Expression in Area X

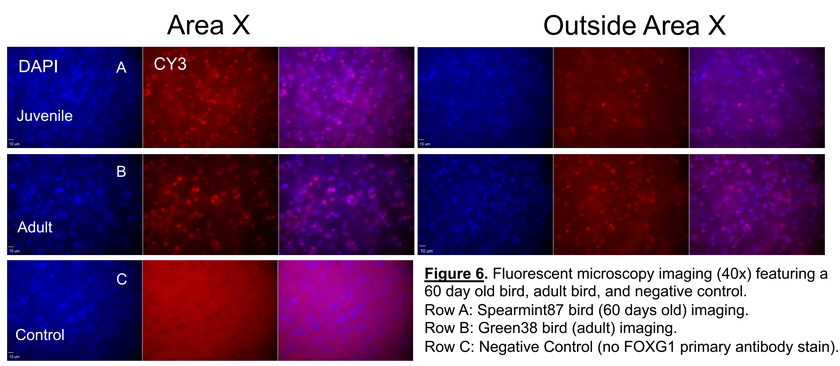

Figure 6. Fluorescent microscopy imaging (40x) featuring a 60 day old bird, adult bird, and negative control.
Row A: Spearmint87 bird (60 days old) imaging.
Row B: Green38 bird (adult) imaging.
Row C: Negative Control (no FOXG1 primary antibody stain).

Results II

3. FOXG1 expression is developmentally regulated

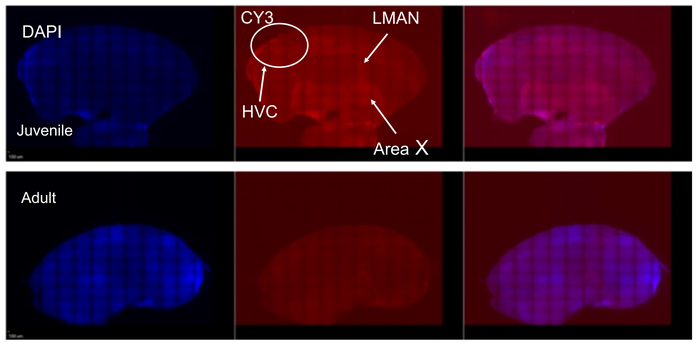

Figure 7. Imaging featuring 12x11 Montage using 10x lens
Top row: Spearmint87 (60 day) zebra finch brain showing higher levels of FOXG1 expression in the high vocal center (HVC), LMAN, and Area X through the CY3 channel.
Bottom row: Green38 (adult) brain showing lower levels of FOXG1 expression across the tissue sample in the CY3 channel.

4. FOXG1 Expression in HVC

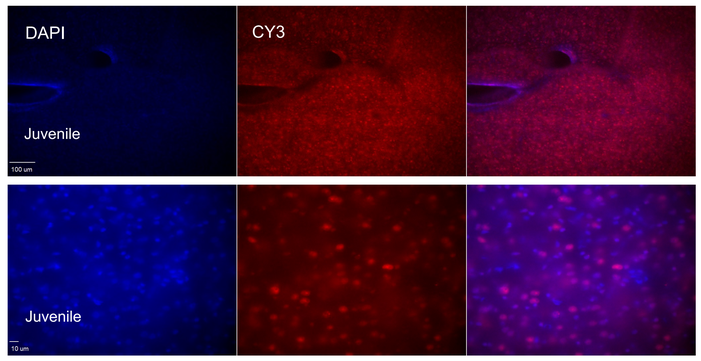

Figure 8 Top row: Spearmint87 (60 day) zebra finch brain HVC seen through 10x lens.
Bottom row: Spearmint87 (60 day) brain HVC seen through 40x lens.

5. FOXG1 Expression in LMAN

Figure 9. Spearmint87 (60 day) zebra finch brain LMAN region seen through 10x lens.

Conclusions

- MeCP2 gene expression was found in certain zebra finch songbird brain regions, such as Area X.

Three major conclusions were drawn from FOXG1 data:
1. FOXG1 appears to be developmentally regulated.
2. FOXG1 is differentially expressed between different brain regions (i.e. cortical, sub-cortical, and striatal).
3. FOXG1 gene is enriched in the song nuclei, specifically in LMAN, HVC, and Area X.

- Studies with further staining are ongoing and we are continuing to gather results.

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