

# Identifying expression patterns of genes MeCP2 and FOXP1 in the zebra finch songbird neural song network

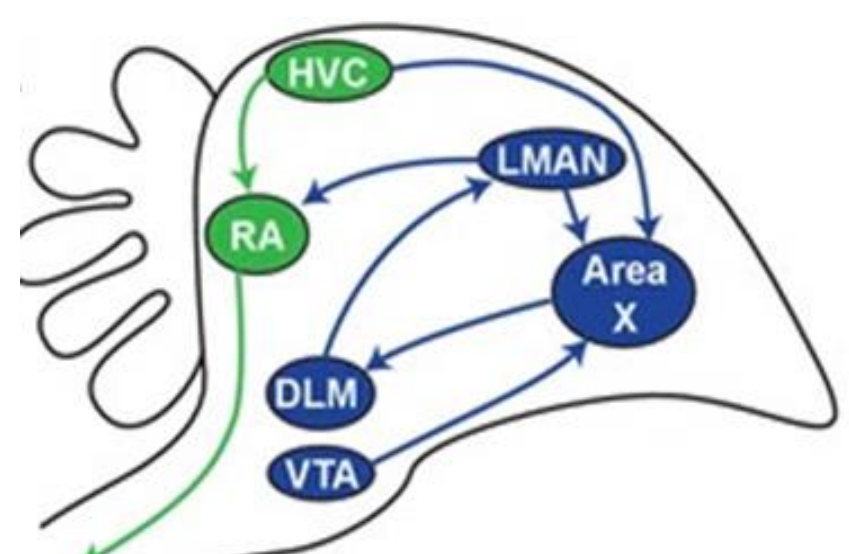
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## Background

### Zebra Finch Songbirds:

- Young humans and zebra finch songbirds both learn speech and language incrementally by imitating adults.
- The comparable learning process makes zebra finches a suitable model for simulating the language learning process of humans.



**Figure 1:** The song nuclei of the zebra finch songbird including the motor pathway (green) and the anterior forebrain pathway (blue)



**Figure 2:** An adult female (left) and male (right) zebra finch

### Selection of Research Genes:

- Mutations to both Forkhead box G1 (FOXP1) and methyl CpG binding protein 2 (MeCP2) have been implicated in impeding speech and language development

### FOXP1:

- Acts as a transcription factor that regulates numerous developmental genes
- Mutations are known to cause FOXP1 syndrome characterized by physical and cognitive difficulties in tandem with language/speech impairment

### MeCP2:

- Acts as a transcriptional repressor that responds to epigenetic biomarkers
- Mutations are known to cause Rett (RTT) syndrome which causes a loss of motor skills and language impairment during childhood development

## Research Aims

1. To determine where in the bird brain the genes MeCP2 and FOXP1 have heightened expression
2. To determine if these genes are developmentally regulated

## Methods

### 1. Brain Harvesting:

Birds were sacrificed and perfused at specific age points including:

- 30 days
- 45 days
- 60 days
- >100 days (adult)

Brains were then sliced using a cryostat and stored for future use



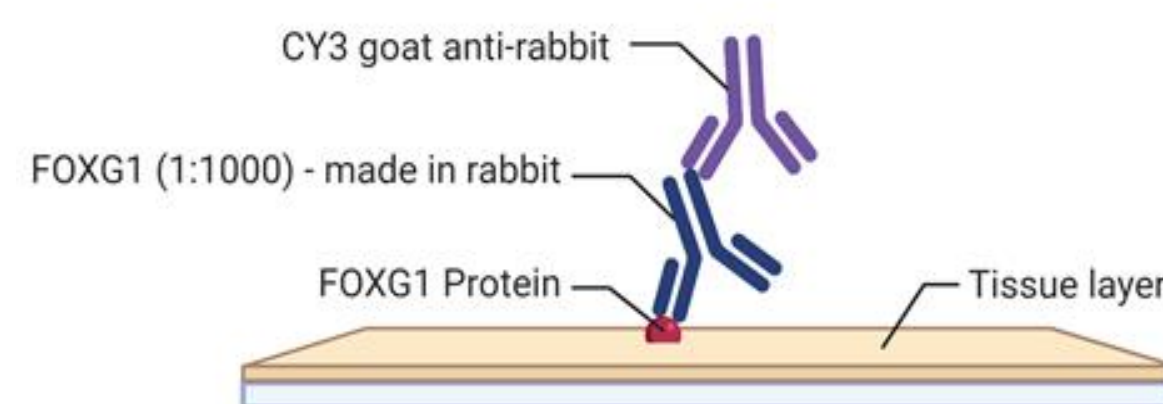
**Figure 3:** The developmental timeline of the zebra finch



**Figure 4:** Basic overview of the brain collection process

### 2. Immunohistochemistry (IHC):

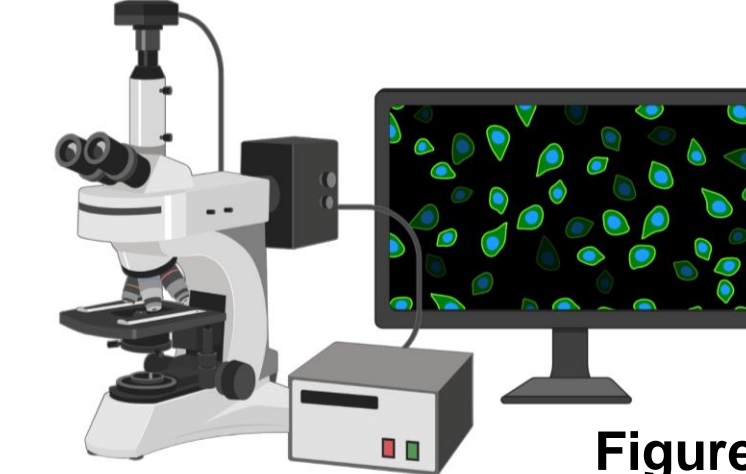
Sliced brain sections were washed and treated with specific antibodies targeted to either FOXP1 or MeCP2



**Figure 5:** IHC Overview

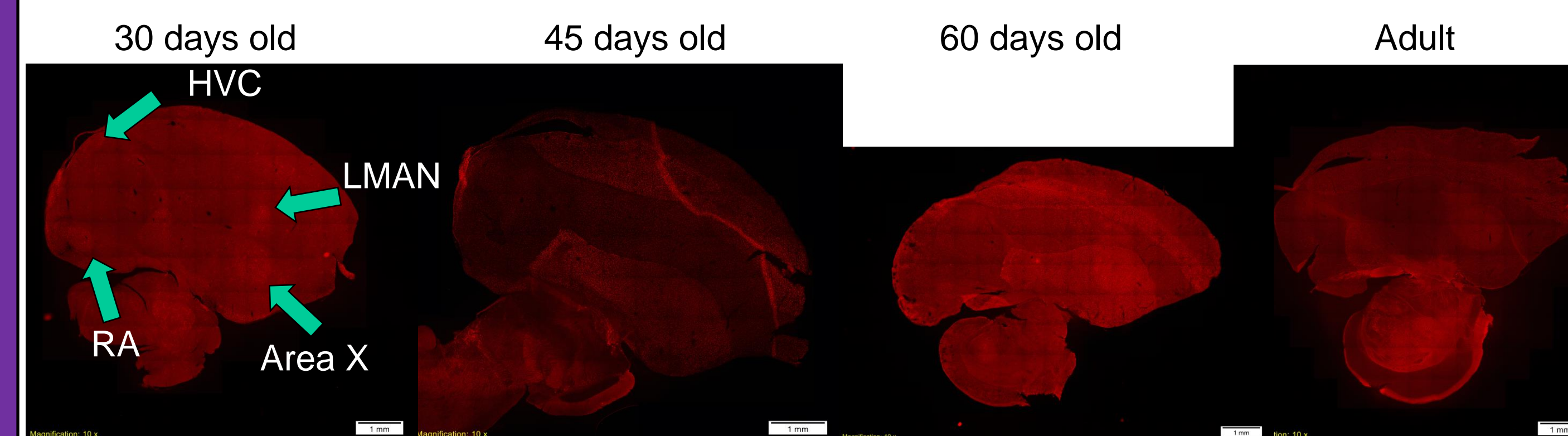
### 3. Imaging:

Sections that have been mounted and gone through IHC treatment were visualized using confocal fluorescent microscopy



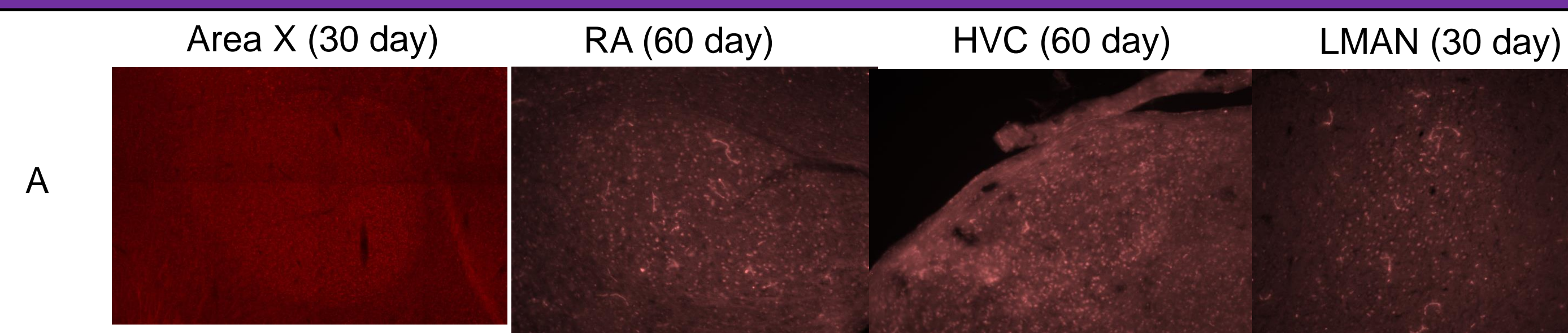
**Figure 6:** Diagram of fluorescent microscopy

## FOXP1 Expression in Song Circuit is Developmentally Regulated

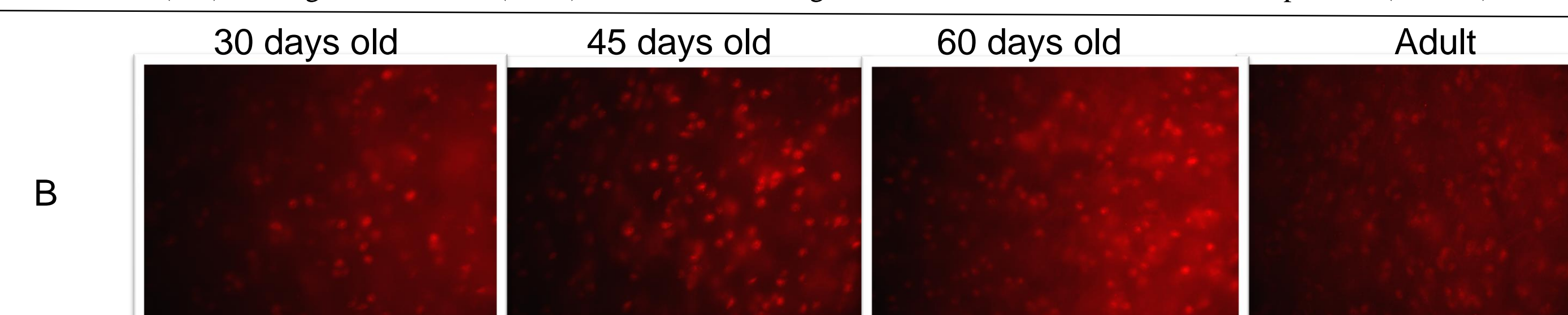


**Figure 7:** Full brain montages of birds Green 4L (30 days old), Yellow 126R (45 days old), Spearmint 60R (60 days old), and Blue 111L (adult) using the CY3 to show FOXP1 expression

## FOXP1 Expression in Song Nuclei

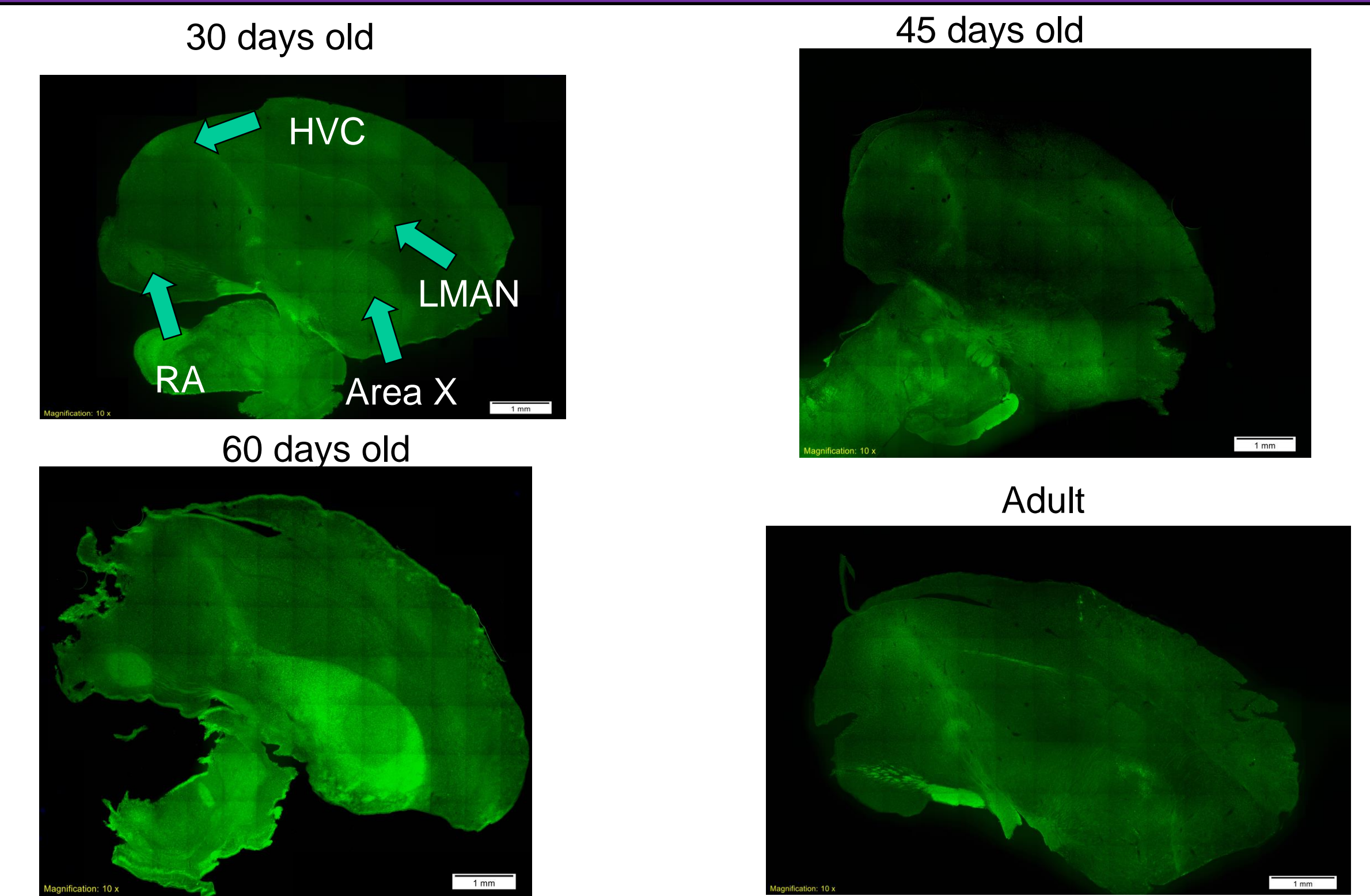


**Figure 8A:** 10x Magnification images of all notable song nuclei including the robust nucleus of the arcopallium (RA), the high vocal center (HVC), and the lateral magnocellular nucleus of the anterior nidopallium (LMAN)



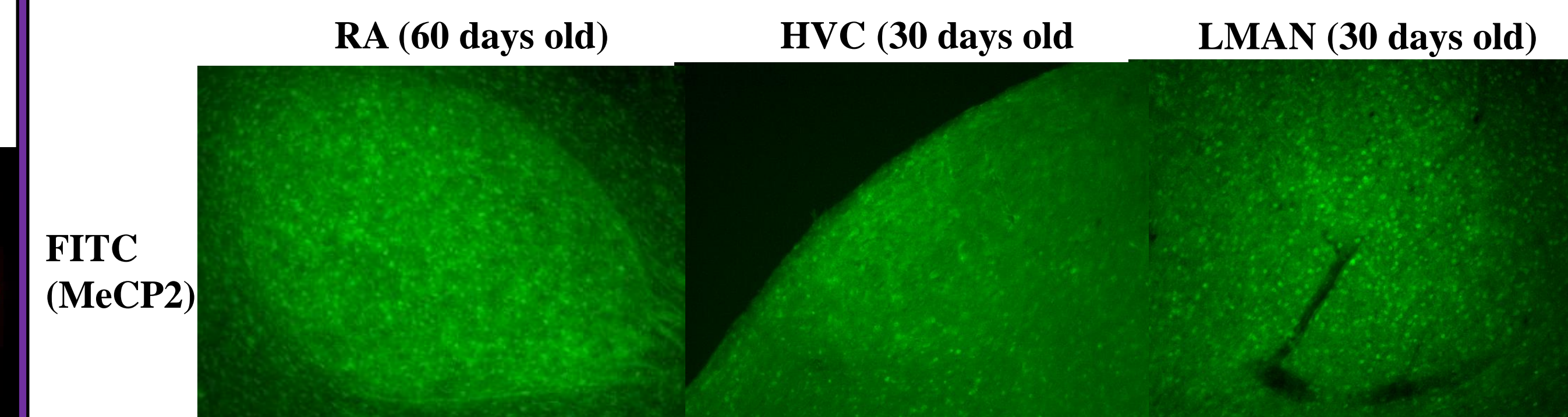
**Figure 8B:** 40x Magnification images within Area X across all developmental stages

## MeCP2 Expression in Song Circuit is Developmentally Regulated



**Figure 9:** Full brain montages of birds Green 4L (30 days old), Yellow 126R (45 days old), Spearmint 60R (60 days old), and Blue 111L (adult) using the FITC and DAPI channels to show MeCP2 expression

## MeCP2 Expression in Song Nuclei



**Figure 10:** Individual images of other notable song nuclei including the robust nucleus of the arcopallium (RA), the high vocal center (HVC), and the lateral magnocellular nucleus of the anterior nidopallium (LMAN) taken at 10x magnification

## Conclusion

- FOXP1 and MeCP2 expression is enriched in the song circuit
- FOXP1 and MeCP2 appears to be developmentally regulated