Probing the Dopaminergic Innervation of a Novel Subtype of HVC Adult-Born Neurons in Male Zebra Finches

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INTRODUCTION

- Zebra finches *(Taeniopygia guttata)* are social animals that communicate via various types of vocalizations, and their song system is an ideal model for studying sensorimotor learning.
- New neurons are added continuously into a telencephalic structure called HVC (proper name), but it is unclear how these new neurons contribute to the stereotyped songs of adult zebra finches. (Walton, et al. 2012)
- Approximately half of these adult-born new HVC neurons (HVC NNs) project to robust nucleus of the arcopallium (RA), but connectivity of the other half remains a mystery. (Scotto-Lomassese, et al., 2007)

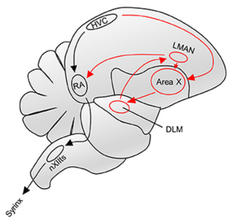


Figure 1: Song system of zebra finches. Black line represents the motor pathway. (Kosubek-Langer, J., Schulze, L., & Scharff, C. (2017). Maturation, Behavioral Activation, and Connectivity of Adult-Born Medium Spiny Neurons in a Striatal Song Nucleus. Frontiers in Neuroscience, 11(323). doi:10.3389/fnins.2017.00323

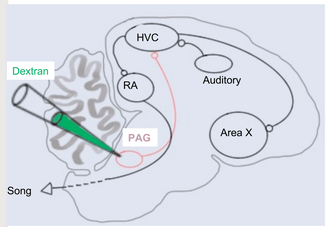
- Previous studies in the Aaron lab showed that about 50% of the adult-born HVC NNs can be labelled with DARPP-32, a phosphoprotein associated with dopamine receptors, and it's exclusive to the 50% *non* HVC-RA neurons. (Aronowitz, et al., 2021, in revision)
- Midbrain structure periaqueductal grey (PAG) is a dedicated supplier of dopamine to HVC. Around 90% of the dopaminergic neurons in PAG send projections to HVC axon terminals (Tanaka, et al., 2018).
- Studies from Duke University suggests a role of the dopaminergic PAG-HVC projections in switching between female-directed courtship songs and undirected songs. (Mor Ben Tov, unpublished data)

OBJECTIVE

We are interested in the function and connections of the non-RA projecting DARPP-32+ HVC NNs. Specifically, we seek to determine whether this subtype of HVC NNs receive dopaminergic input from PAG, and thus has the potential to be the switch of social context-dependent songs.

METHODS

Stereotaxic Surgery



In order to identify any HVC neuron that receives dopaminergic projections from the PAG, the anterograde tracer was stereotaxically injected into the PAG. Birds were placed in a stereotaxic head holder, with the beak in a 40° angle with the horizontal axis. We injected 40 nL of 10% dextran into PAG using the following coordinates: Anterior/Posterior +0.66, Medial-Lateral +0.2 (with the zero-point set at the rostral tip of the cerebellum), Dorsal-Ventral -4.925 (with the zero-point set at brain surface with the pia mater removed).

Tissue Processing and Image Analysis

The brain was immediately removed from the skull after surgery and fixed with 4% PFA for two days, then put in 30% sucrose overnight. Next, the brain was frozen in Tissue-Tek tissue freezing medium and cut coronally into 50 μm sections using the cryostat. Sections were also stained with Hoechst nuclear stain.

RESULTS

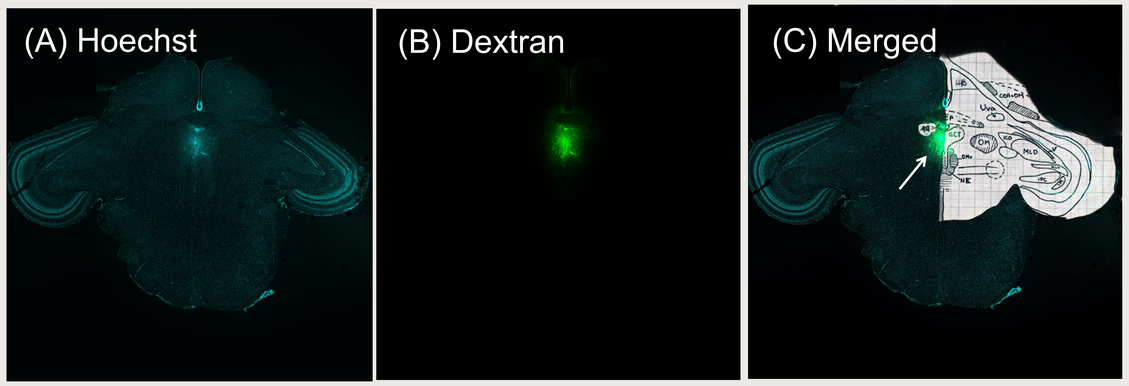


Figure 2: (A) Hoechst 33258 labels all cell nuclei and fluoresces blue in the UV channel. **(B)** The anterograde tracer Dextran Fluorescein (MW=10 kDa) fluoresces green in FITC channel. **(C)** The two channels are merged, along with adapted picture of transverse plate 31 from the zebra finch brain atlas, which confirms that the injection site is at where we expected. The white arrows points to the PAG.

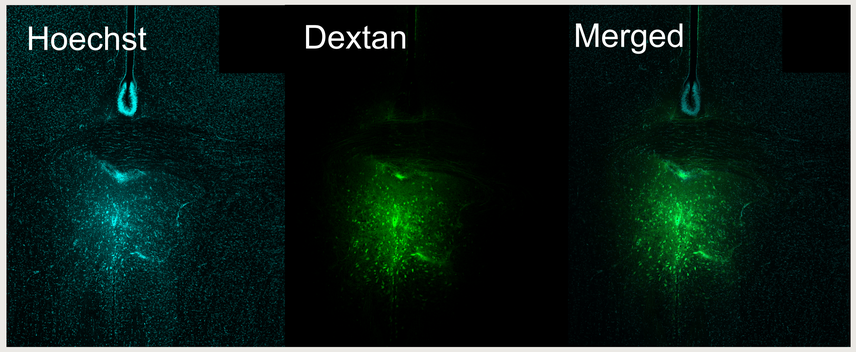
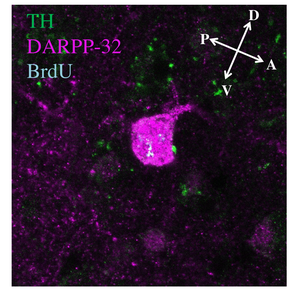


Figure 3: a higher magnification image of Figure 2. Injected Dextran (green fluorescence, FITC channel is generally constricted in the PAG area and didn't diffuse far off to other areas of the brain.

FUTURE DIRECTIONS

- Over the summer, we have successfully identified the stereotaxic coordinates for the PAG, using the zebra finch brain atlas and other studies as guidance. This was a challenging process, as this surgery has not been performed extensively in the field, and the brain atlas used a stereotaxic apparatus different than ours.
- We plan to modify our coordinates to keep the surgeries survivable and allow sufficient time for Dextran to be transported into the HVC, then perform triple-label immunohistochemistry with Tyrosine Hydroxylase, DARPP-32 and BrdU. In the future, if we observe that DARPP-32+ NNs have Dextran+ axon terminals, then we can tentatively conclude that DARPP-32+ NNs receive dopamine from the PAG.



We expect to see "triple-labeling" similar to in this picture, except in addition to tyrosine hydroxylase (TH, green), fibers containing Dextran will also make contacts onto the neuron. (Aronowitz, et al., 2021, in revision)

- Relating this expected finding to the unpublished results from Duke University, DARPP-32+ NNs' functionality in social context of the song can be further explored with functional analysis by selectively increasing and decreasing the activity of these neurons.

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REFERENCES

1. Walton, C., Pariser, E., & Nottebohm, F. (2012). The Zebra Finch Paradox: Song Is Little Changed, But Number of Neurons Doubles. The Journal of Neuroscience, 32(3), 761-774. doi:10.1523/jneurosci.3434-11.2012

2. Scotto-Lomassese, S., Rochefort, C., Nshdejan, A., & Scharff, C. (2007). HVC interneurons are not renewed in adult male zebra finches. European Journal of Neuroscience, 25(6), 1663-1668. doi:https://doi.org/10.1111/j.1460-9568.2007.05418.x

3. Aronowitz, J. V., Kirn, J. R., Pytte, C. L., & Aaron, G. B. (2021, , in revision at J Comp Neuology). A Subtype of Adult-Born Neuron in Male Zebra Finch HVC Expresses DARPP-32 and Does Not Project to RA as part of the Vocal Motor Pathway. bioRxiv, 2020.2008.2031.271783. doi:10.1101/2020.08.31.271783

4. Kosubek-Langer, J., Schulze, L., & Scharff, C. (2017). Maturation, Behavioral Activation, and Connectivity of Adult-Born Medium Spiny Neurons in a Striatal Song Nucleus. Frontiers in Neuroscience, 11(323). doi:10.3389/fnins.2017.00323

5. Tanaka, M., Sun, F., Li, Y., & Mooney, R. (2018). A mesocortical dopamine circuit enables the cultural transmission of vocal behaviour. Nature, 563(7729), 117-120. doi:10.1038/s41586-018-0636-7