

Singing Related Activation of DARPP-32+ Adult Born Neurons in Zebra Finch HVC

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Introduction

Background: HVC DARPP-32+ NNs are distinct from HVC-RA NNs

Conclusions

Neurogenesis is the process by which neurons are born and eventually are integrated into pre-existing networks in the brain. About 50% of HVC adult-born neurons (HVC NNs) project to RA (HVC-RA NNs), while there is no positive identification of the remaining 50%. About 50% of HVC NNs also express DARPP-32, a marker that labels dopaminergic neurons.

Interestingly, the HVC NNs that express DARPP-32 (DARPP-32+ NNs) cannot be backfilled from RA, suggesting that they may be the so far unidentified neurons that do not project to RA.

DARPP-32+ NNs do not project to Area X or Nucleus Accumbens, the two other projection targets of HVC, and they are not inhibitory interneurons, leading us to believe they may be excitatory interneurons and have a different function than the HVC-RA NNs.

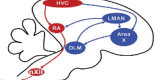
Directed song occurs when a female is present and the male sings directly at a potential mate. Undirected song is sung in the absence of a female.

We also believe dopamine plays a key role in regulating the social aspects of song. A study showed that when a female is present, but dopamine is blocked from binding in HVC, directed song from the male is eliminated but undirected song, which remains functional.

Given the proposed differential dopaminergic innervation of HVC-RA NNs and HVC DARPP-32+ NNs, we want to investigate whether these two groups are differentially activated in socially directed versus undirected song. We believe that DARPP-32+ NNs could be related to the switch between directed and undirected song production.

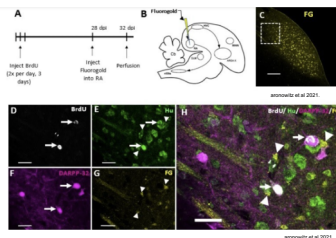
Given that dopamine in HVC is required for directed song and that HVC DARPP-32+ NNs putatively express dopamine receptors, we hypothesize that there is a higher proportion of DARPP-32+ NNs activated in directed song compared to HVC-RA NNs.

We can test this hypothesis by using ZENK, an immediate early gene that can be used as a measure of neuronal activity. Previous studies have shown the HVC-RA NNs express ZENK immediately after a singing bout. We will repeat and expand on these studies by comparing ZENK activity levels after both directed and undirected song and compare HVC-RA NNs to HVC DARPP-32+ NNs.



A visual of the avian song system. We are looking mainly at the connections between RA and HVC in the vocal motor pathway shown in red.

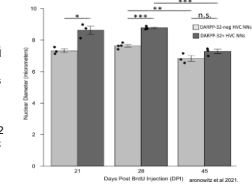
Wood et al., 2013.



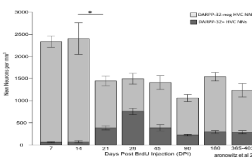
Of the 46.4% of HVC NNs that do project to RA 99.4% do not express DARPP-32, providing evidence for the difference between HVC-RA NNs and DARPP-32+ NNs

To test if DARPP-32+ HVC NNs (non-RA projecting) project to RA, we birth-coated NNs with BrdU and injected Fluorogold into RA. Birds were killed at 32 dpi, and it was found that 46.4% of HVC NNs were RA-projecting. The RA-projecting NNs examined were also almost exclusively DARPP-32 neg. All but one DARPP-32+ HVC NN was FG-, showing that DARPP-32+ NNs are not backfilled from RA and therefore may be a different sub-type of HVC NN.

The proportion of DARPP-32+ NNs changes over a year. After 14 days dpi the number of DARPP-32+ NNs increases and at 28 dpi there is stability in the number of overall HVC NNs showing that DARPP-32 expression may be decreased after maturation is reached. It also shows a culling period from 14 dpi to 21 dpi where neurons that did not form synaptic connections will die as the overall number of neurons decreases



DARPP-32+ NNs are significantly larger than DARPP-32 neg NNs until 45 dpi where they are no longer statistically significant. At 45 dpi both neuron types seem to converge to a common size.



Methods

HVC DARPP-32+ NNs receive TH terminals and are active during singing

Future Directions

- Multiple lines of evidence suggest DARPP-32 NNs are a unique subtype of new neuron in HVC

- DARPP-32+ HVC NNs express ZENK during song production

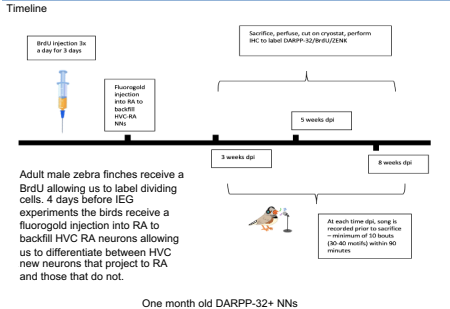
- We will determine whether DARPP-32+ adult-born respond differently to directed or undirected song

- We will observe if dopamine in HVC plays a key role in the social aspects of directed versus undirected song

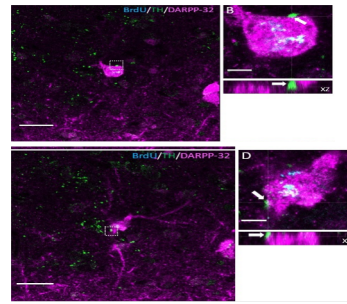
- We will probe the effect of social context on the activation of DARPP-32+ HVC NNs within three age groups (3, 5, and 8 week old neurons) during both directed and undirected song

Acknowledgements

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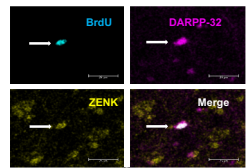
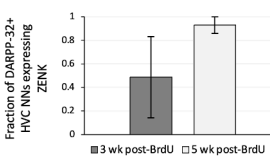


TH+ & DARPP-32+ NN Connections

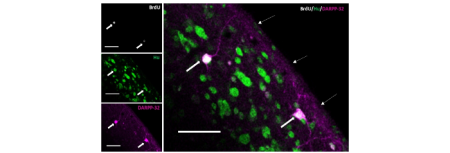


The green indicates tyrosine hydroxylase positive synapse connections. We see TH+ synapses interacting with DARPP-32+ NNs, meaning they receive either dopamine, epinephrine or norepinephrine. Scale bars: 10 μm: (B, D), 25 μm (A, C).

DARPP-32+ HVC NNs are active in song production



DARPP-32+ ZENK neuron, showing activation after song production. Scale bars: 25 μm



One month old DARPP-32+ NNs