Molecular Origins of the Pair1 and Moonwalker Descending Neuron's Neural Circuitry in *Drosophila*

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Background

- Moonwalker Descending Neurons (MDNs) and Pair1 Neurons (Pair1s) function in a well-characterized neural circuit and are essential for initiating backwards movement in *Drosophila* (fruit flies).[1]
- Neurons communicate in circuits to elicit behaviors including movements as seen in the MDN-Pair1 circuitry.
- MDNs initiate backwards movement and activate the Pair1s which generate a pause in both adults and larvae (Fig. 1).
- The MDNs and Pair1s are formed from neuroblasts (also known as stem cells in mammals).
- Before the embryos hatch into larvae, the neuroblasts (NB) divide into progeny neurons, in distinct windows marked by temporal transcription factor (tTF) expression. tTFs are proteins that influence the expression of specific genes.
- Hunchback (Hb) is expressed first followed by Kruppel (Kr), then Castor (Cas), and Grainyhead (Grh) (Fig. 2).

Figure 1

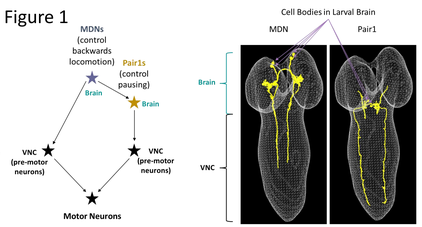
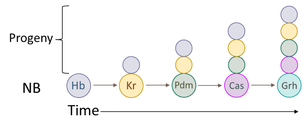


Figure 2



Big Objective: Determine which tTF the MDN and Pair1 neurons derive from and examine how morphology and behavior is affected in larvae.

Methods

tTF Identification

Figure 3

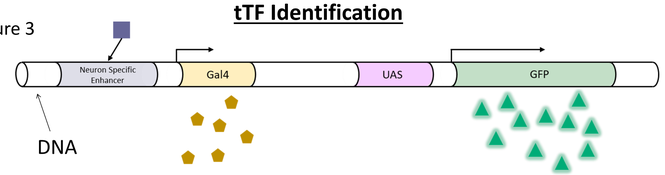


Fig 3. The Gal4/UAS system is used to express membrane-bound GFP in only the MDNs or Pair1s in fruit fly larvae. The temporal TFs can then be labeled with an antibody to observe overlap with these neurons.

Hb Manipulations

Figure 4

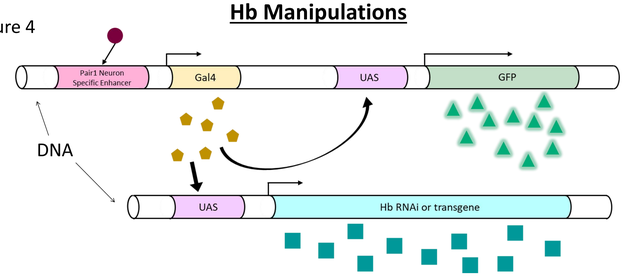


Fig 4. Hb can be knocked down (Hb RNAi) and overexpressed (Hb transgene) in the Pair1s through the Gal4/UAS system.[2]

Figure 5

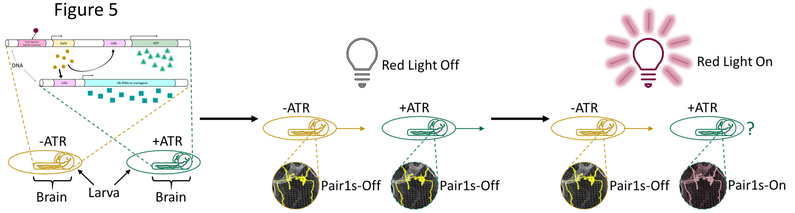


Fig 5. The behavior associated with the Hb manipulations in Pair1s can be analyzed using the Gal4/UAS system with optogenetics. By treating the larvae with retinol (+ATR), the Pair1s can be activated using red light and then compared to the pausing behavior exhibited by larvae not treated with retinol (-ATR).

The MDN-Pair1 circuit is born in the Hb temporal window which is important for circuit formation and behavior.

Results

tTF Identification of MDN

Figure 6

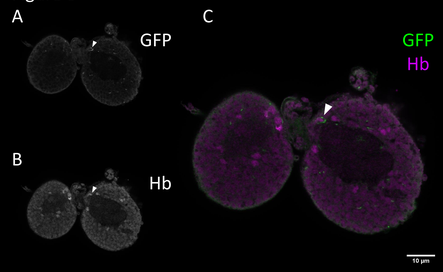


Fig 6. (A-B) MDNs (GFP) show a significant overlap with the tTF Hb in fruit fly larvae. (E) Percentage of TF overlap with MDN was calculated by dividing the number of MDNs with tTF by the total number of MDNs observed. No overlap was seen with Kr, Cas, or Grh.

tTF Identification of Pair1

Figure 7

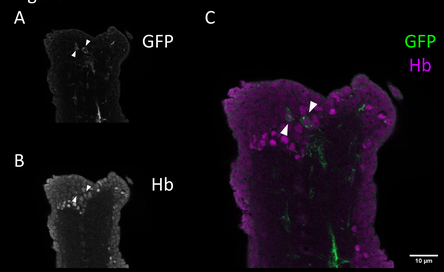


Fig 7. (A-D) Pair1s (GFP) show a significant overlap with the tTF Hb in fruit fly larvae. (E) Percentage of TF overlap with Pair1 was calculated by dividing the number of Pair1s with tTF by the total number of Pair1s observed. No overlap was seen with Kr, Cas, or Grh.

Hb Manipulations

Figure 8

Fig 8. (A) Control larvae shows two Pair1 neurons (GFP) with fully descending axons down the side of the neuropil. NC82 is labeling the neuropil (synapse rich areas).

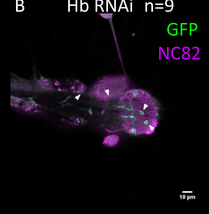


Fig 8. (B) When Hb is knocked down in the larvae's Pair1 neurons (GFP), the axons no longer descend. NC82 is labeling the neuropil (synapse rich areas).

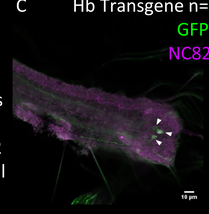


Fig 8. (C) When Hb is overexpressed in the larvae's Pair1 neurons (GFP), a third Pair1 neuron develops. NC82 is labeling the neuropil (synapse rich areas).

Figure 9

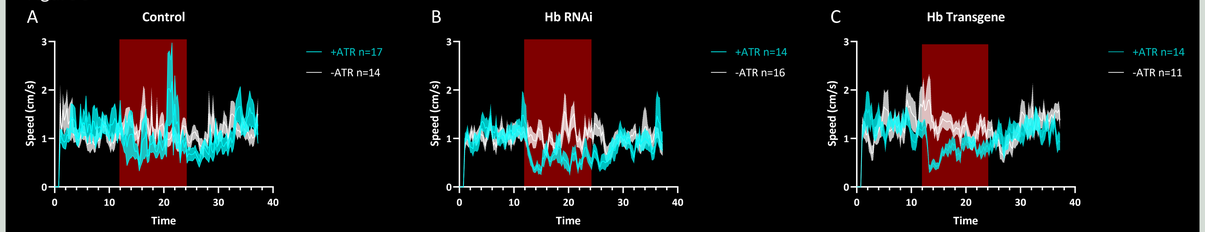


Fig 9. (A) When the light turns on, the +ATR larvae slow down. The +ATR larvae then speed up as light turns off. These changes in speed appear immediate with the light. The –ATR larvae keep their speed through the light.

Fig 9. (B) When the light turns on, the +ATR larvae slow down, but take longer to speed up after the light turns off. These changes in speed appear delayed to the light. The –ATR larvae keep their speed through the light.

Fig 9. (C) When the light turns on, the +ATR larvae slow down, but gradually speed up immediately after without regards to the light turning off. The –ATR larvae keep their speed through the light.

Conclusions

- MDN and Pair1 neurons are derived from the same tTF, which could indicate that neurons from similar developmental origins function together in neural circuits.
- Changing the amount of hunchback in Pair1 neurons affects morphology and behavior.
- New morphologies and behaviors suggests that normal Pair1 circuitry formation is altered by changing molecular origins.

Future Directions

- Determine morphological and behavioral changes with Hb knock down or overexpression in MDNs.
- Determine morphological and behavioral changes with Hb knock down or overexpression in Pair1s and MDNs in adult fruit flies.
- Preform neurite mapping of the Pair1s when Hb is knocked down or over expressed.

References

1 Carreira-Rosario, A., Zarin, A. A., Clark, M. Q., Manning, L., Fetter, R. D., Cardona, A., & Doe, C. Q. (2018). MDN brain descending neurons coordinately activate backward and inhibit forward locomotion. ELife, 7. doi:10.7554/elife.38554

2 Hirono, K., Kohwi, M., Clark, M. Q., Heckscher, E. S., & Doe, C. Q. (2017). The Hunchback temporal transcription factor establishes, but is not required to maintain, early-born neuronal identity. Neural Development, 12(1). doi:10.1186/s13064-017-0078-1

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