Novel methodology for the investigation of Dmrt3a interneurons in larval zebrafish

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Background

Understanding the neural circuits that underlie locomotion is key to understanding the causes of motor behaviors and disorders. This project explores the role of **spinal interneurons** (Fig. 1), **linked to the gene Dmrt3a**, in larval zebrafish. Past studies suggest that these interneurons are linked to **speed-shifting and locomotor coordination**.

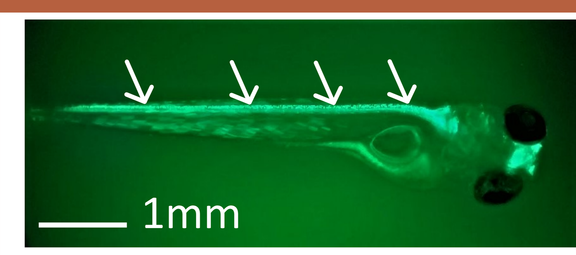

Figure 1. *Dmrt3a-HS:Gal4; Botox-GFP;nacre -/- zebrafish larvae taken under Olympus MVX10 disection scope at 6.3x. White arrows indicate Dmrt3a neurons*

Methods

Control:
8 AB wildtype 6 days post fertilization (dpf) zebrafish

Experimental:
6 Dmrt3a-HS:Gal4;Botox-GFP;nacre -/- 6 dpf zebrafish

Preparation:
Zebrafish larvae were **head-embeded** in 2% low melting point agarose

Video Capture:
Fish were **stimulated to swim using a projected OMR** grating at speeds 10mm/s, 20mm/s, and 30mm/s
Video captured on a custom experimental rig (Fig. 2)

Analysis:
Raw video files were converted from .tiff to uncompressed .AVI
DeepLabCut was used to track points along the bilateral pec fin and tails
Data was processed and analyzed in Python and R

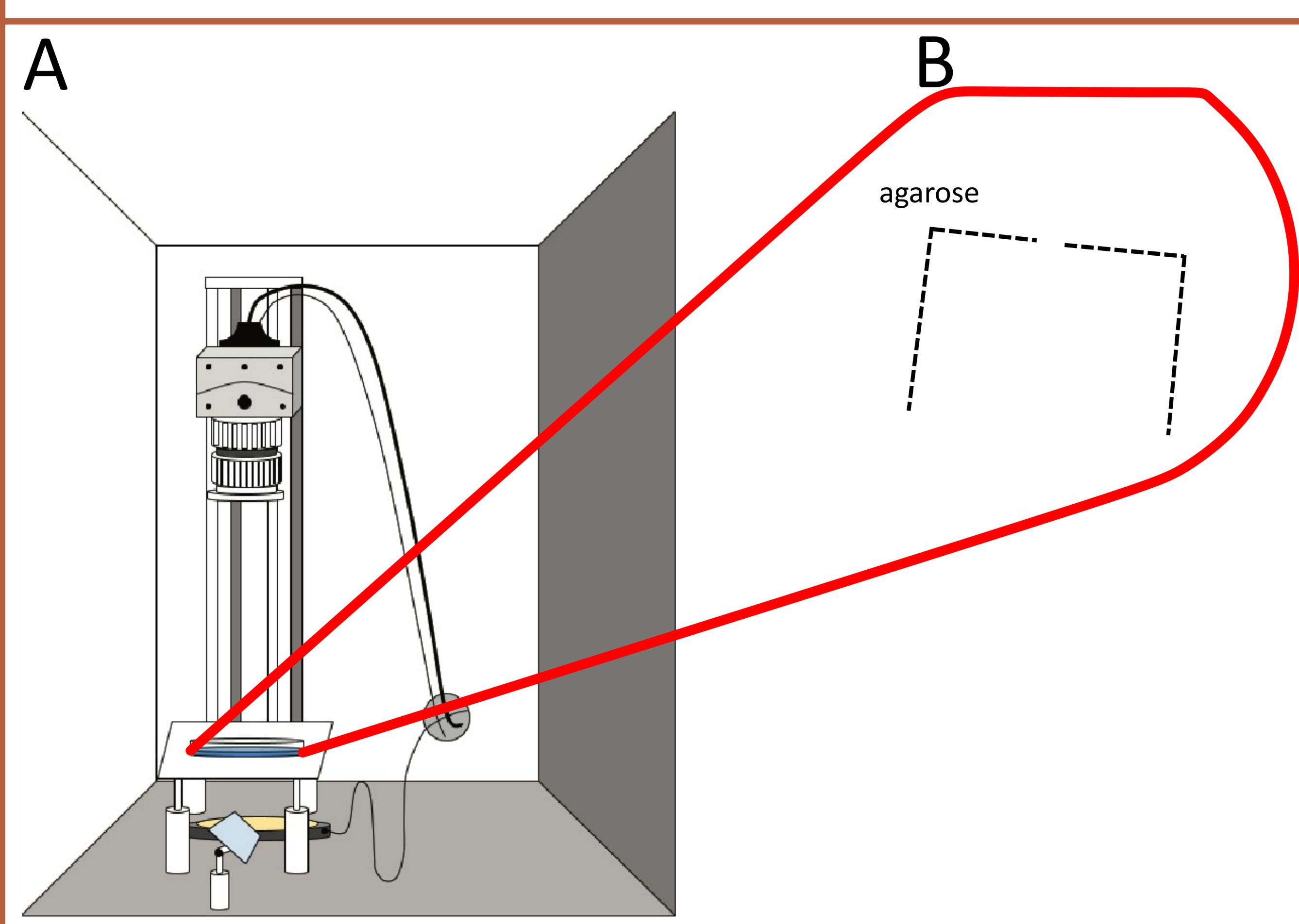

Figure 2. *Behavior rig schematic (A) with insert of head embedded zebrafish (B)*

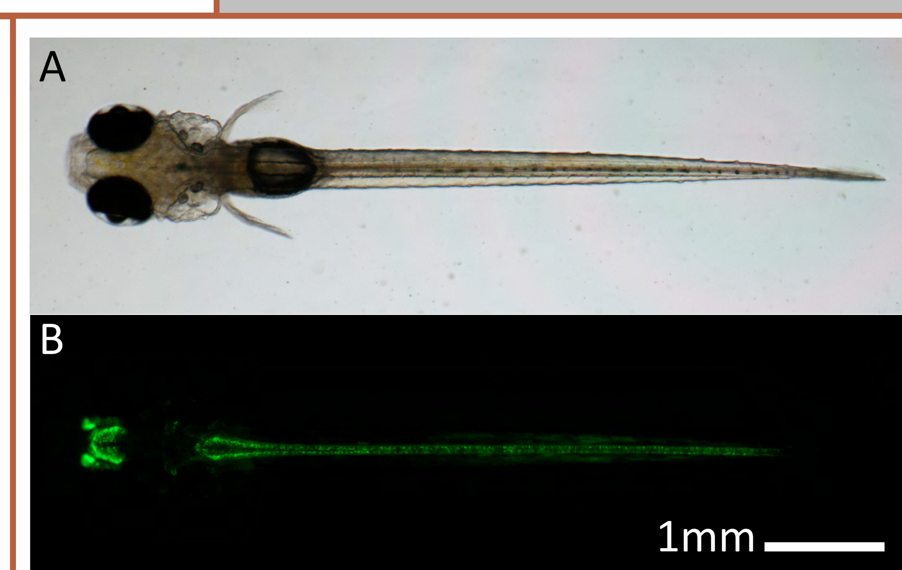

Figure 3. *Dmrt3a-HS:Gal4;Botox-GFP;nacre -/- 6 dpf zebrafish larvae pictured with light (A) and flourescent (B) microscopy. All control and experimental fish were screened using Olympus MVX10 disection scope at 6.3x.*

Preliminary Results

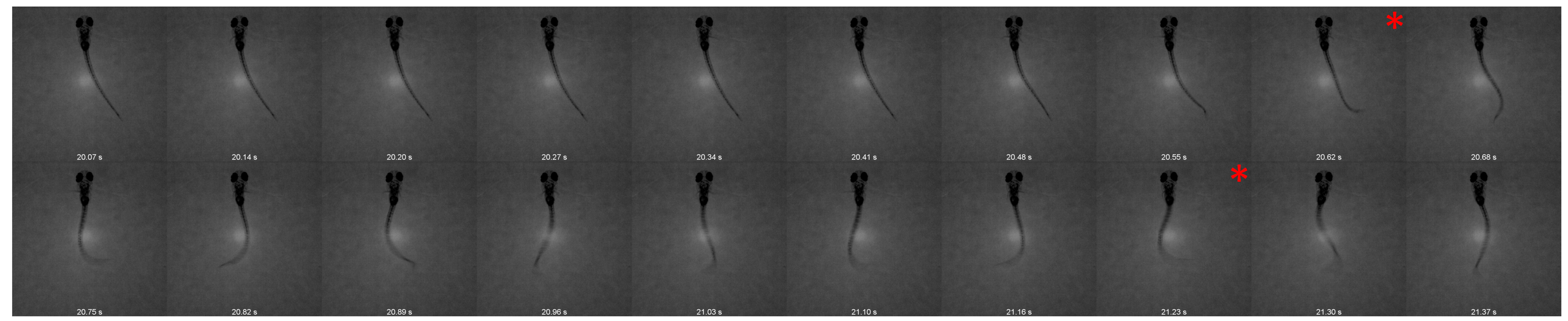

Figure 4. *Example of coordinated motion in AB wildtype fish across gaits. This high speed footage shows both independant and coordinated motion of tail and fins. Of note are possible gait transitions at the marked (*) frames.*

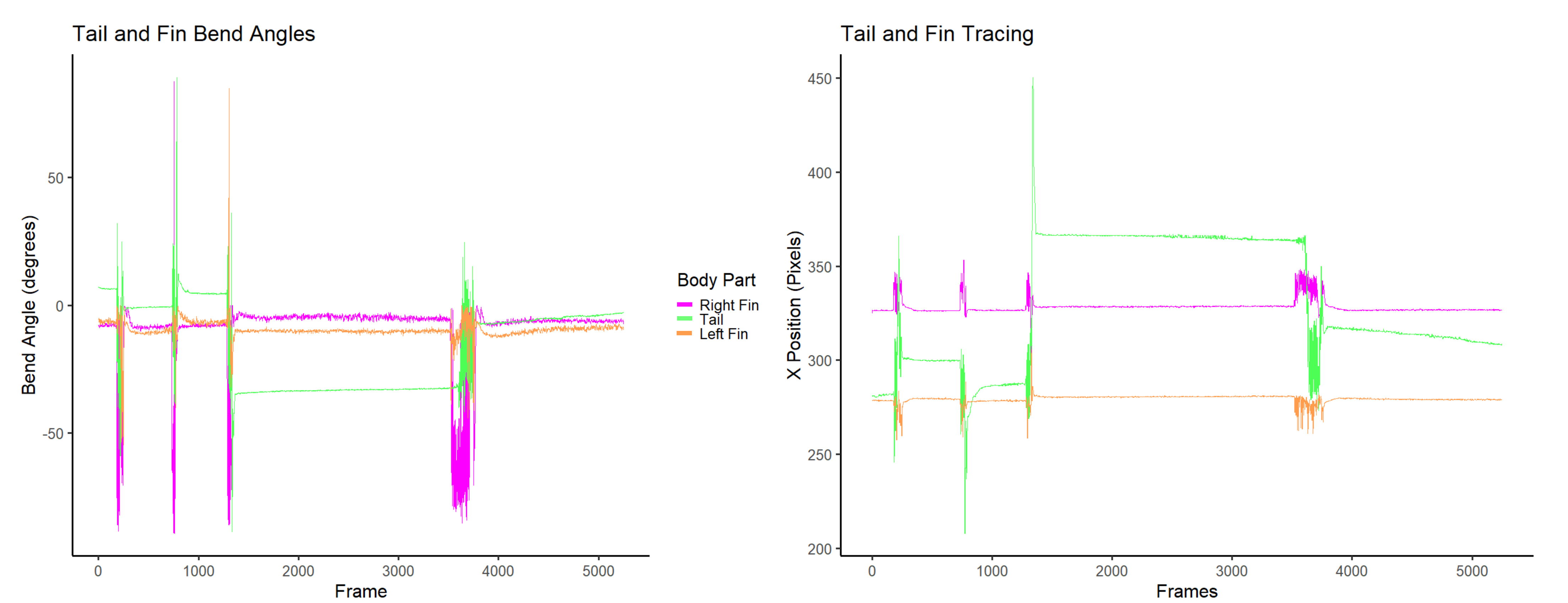

Figure 5. *Comparison of bend angles from one AB wildtype trial at 20 mm/s (A). Comparison of tail and fin tracings from the same fish (B). In this wildtype example, fins and tail are coordinated in "bouts" of motion.*

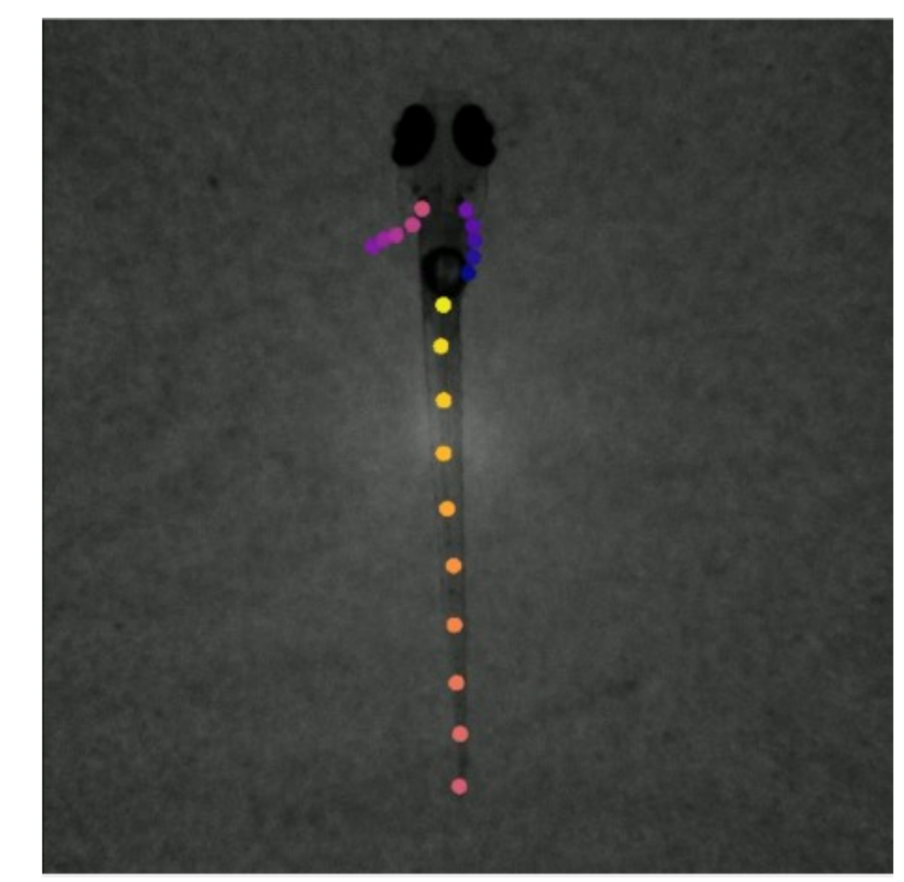

Figure 6. *Preliminary image of tracked points along tail and pec fins*

Discussion & Future Directions

Preliminary data indicates that there may be differences in coordination between AB wildtype fish and Dmrt3a-Botox fish. In order to further explore our hypothesis and confirm the presence of any statistically significant differences several steps are being taken:

1. In order to make meaningful comparisions between fish and trials, **individual bouts of movement must be extracted**. This is currently in progress.
2. **Additional experiments utilizing channelrhodopsin2 (ChR2)** in trangenic fish are planned. These experiments will use a similar head embeded process to allow selective stimulation of Dmrt3a neurons with a fiberoptic light source. This setup will allow for more precise control Dmrt3a activity.
3. Data from free swimming behavioral experiments is currently being analyzed

Use of DLC has allowed for more extensive tracking over more frames and trials then would be practical if working by hand. We are currently exploring how to make use of this broad data set and how to take advantage of machine-learning assisted data collection in future experiments.

Acknowledgements

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