

The motor programs underlying navigation in *Drosophila* larva

based on *PLoS ONE*, 6:e23180 (2011), with K. Shen, M. Klein, A. Tang,
E. Kane, M. Gershow, P. Garrity, and A.D.T. Samuel

Subhaneil Lahiri

Harvard University

Dec. 13, 2011

Motor programs in *Drosophila* larvae

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We will look at the motor behaviour of the *Drosophila* larva during navigational motion, paying attention to which segments are used, in which order, etc.

We want to get some insight into the circuits that control this behaviour and the role of sensory feedback by quantifying the motor output at high resolution.

Introduction

We will look at the motor behaviour of the *Drosophila* larva during navigational motion, paying attention to which segments are used, in which order, etc.

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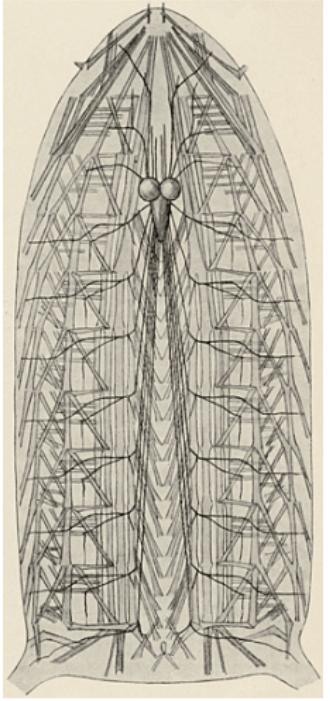
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└ Introduction

1. Ultimately: trace out full pathway sensory *to* decision *to* motor
2. future: interfere, now: just look at normal behaviour

Drosophila larva



[Hertweck (1931)]

$\sim 10^4$ neurons.

Has CNS, spiking neurons,...

Many genetic tools.

Transparent \Rightarrow optogenetics.

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└ Drosophila larva

1. factor of 10 < adult
2. unlike *c. elegans*
3. sequenced genome, GAL4/UAS system - target cell types

Drosophila larva



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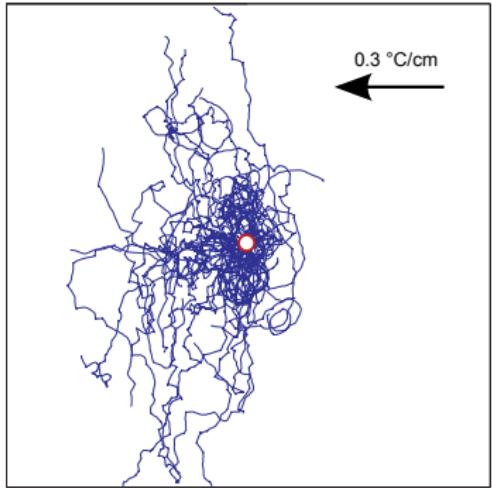
└ Outline

- ① Navigation and locomotion
- ② Imaging and analysis of fluorescent muscles
- ③ Results
- ④ Conclusions and future directions

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Biased random walks



Alternating runs and reorientations.

Effectively point-like sensor.

Similar to *E. coli* and *C. elegans*.

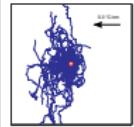
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└ Navigation and locomotion

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Biased random walks



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Head-sweeps

Moves head from side-to-side to sample environment and pick a direction to travel.

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└ Navigation and locomotion

└ Head-sweeps

Moves head from side-to-side to sample environment and pick a direction to travel.

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1. accepted
2. rejected

Navigation strategy

For thermo-/chemo-/photo-taxis, larva modulates:

- head-sweep frequency
- head-sweep size
- head-sweep acceptance probability

Depending on whether conditions are improving/worsening.

[Luo et al. (2010)]

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└ Navigation and locomotion

└ Navigation strategy

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Questions

Different types of head-sweep:

- Different circuits?
- When is decision made? With what info?
- Mechano-sensory feedback?

Look for differences in mechanics of different types of head-sweep.

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└ Navigation and locomotion

└ Questions

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- Different types of head-sweep:
• Different circuits?
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Look for differences in mechanics of different types of head-sweep.

Locomotion and sensory feedback

Crawl using peristaltic waves from posterior to anterior that lift and push the body forwards.

Several types of Multidendritic (md) sensory neurons.

Repeated in each segment. Possibly used for proprioception.

[Bodmer and Jan (1987), Grueter et al. (2002)]

md neurons are used for locomotion:

- Turn off all types → no locomotion [Song et al. (2007)]
- Turn off certain subsets → disrupt pattern (toothpasting) [Hughes and Thomas (2007)]

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Navigation and locomotion

Locomotion and sensory feedback

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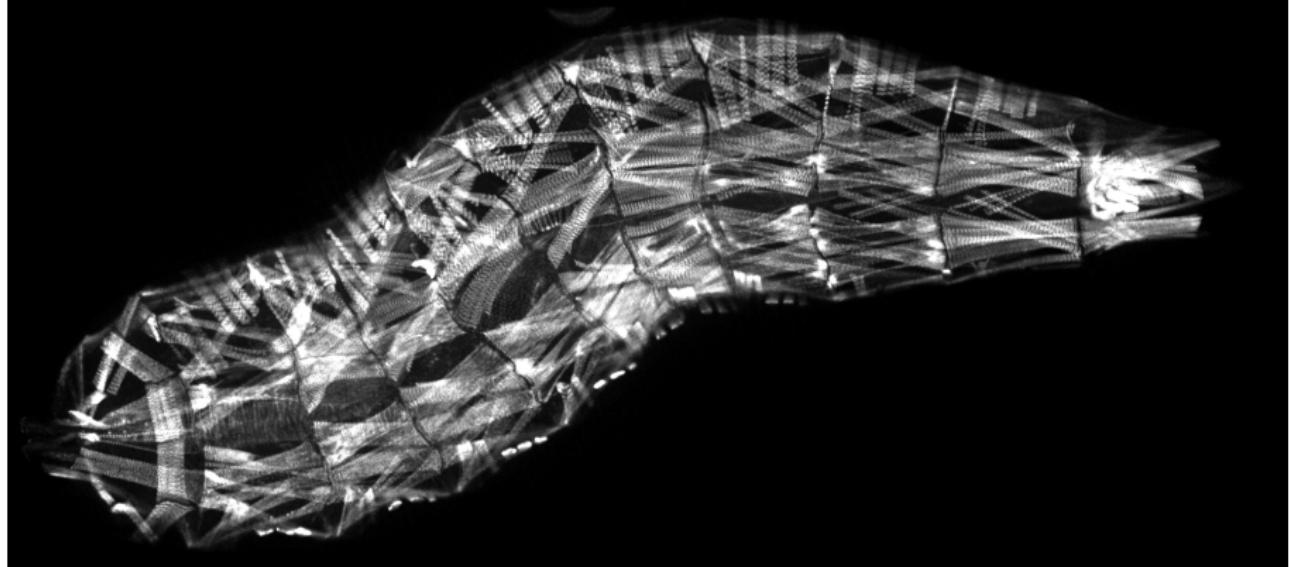
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Fluorescent muscles

Mutant: $w^-; \frac{mhc-GFP^{0110}}{CyO}$

[Hughes and Thomas (2007)]



Can see segment boundaries → measure length → which segment contracts.

Motor programs in Drosophila larvae

└ Imaging and analysis of fluorescent muscles

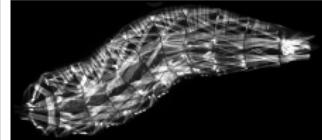
└ Fluorescent muscles

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Fluorescent muscles

Mutant: $w^-; \frac{mhc-GFP^{0110}}{CyO}$

[Hughes and Thomas (2007)]



Can see segment boundaries → measure length → which segment contracts.

1. we see 11 segments, some people talk about A9 (terminal, too small), mouth segment (involute during early development)
2. can't automate this yet.

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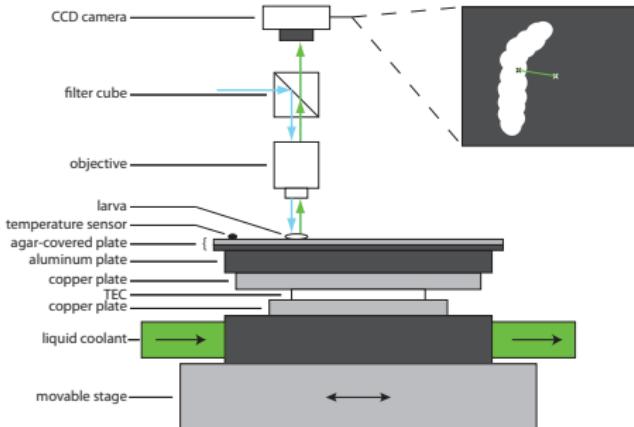
└ Intensity pattern

Muscles contract → same GFP in smaller volume → increase concentration → increase brightness.

1. another measure of contraction. less noisy

Muscles contract → same GFP in smaller volume → increase concentration → increase brightness.

Apparatus



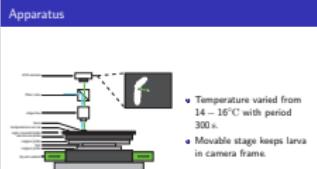
- Temperature varied from 14 – 16°C with period 300 s.
- Movable stage keeps larva in camera frame.

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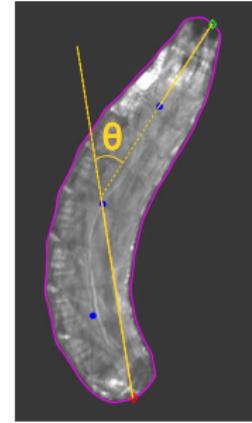
Motor programs in Drosophila larvae

- Imaging and analysis of fluorescent muscles

Apparatus



1. triggers many head-sweeps
2. allows comparison of head-sweepin warming/cooling



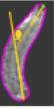
Find boundary, head, tail and bend angle automatically

Motor programs in Drosophila larvae

- Imaging and analysis of fluorescent muscles

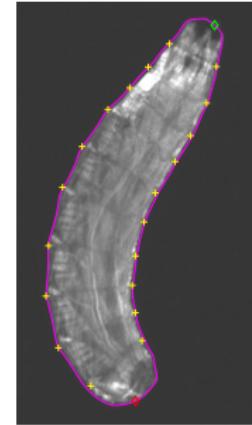
Image analysis

1. allows us to flag interesting bits
2. slowest part
3. automatic again. look for asymmetry
4. less noisy



Find boundary, head, tail and bend angle automatically

Image analysis

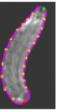


User clicks on segment boundaries

Motor programs in Drosophila larvae
└ Imaging and analysis of fluorescent muscles

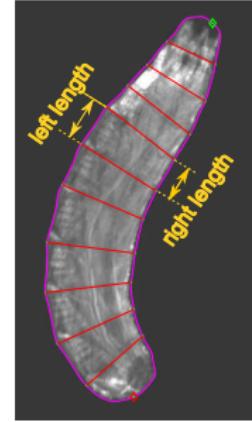
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Map to boundary. Find segment lengths.

Motor programs in Drosophila larvae

- Imaging and analysis of fluorescent muscles

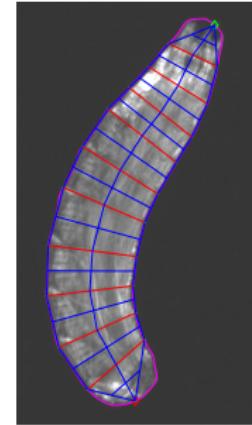
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Map to boundary. Find segment lengths.



Split segment into quadrants. Mean pixel value → intensity.

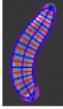
Motor programs in Drosophila larvae

- Imaging and analysis of fluorescent muscles

Image analysis

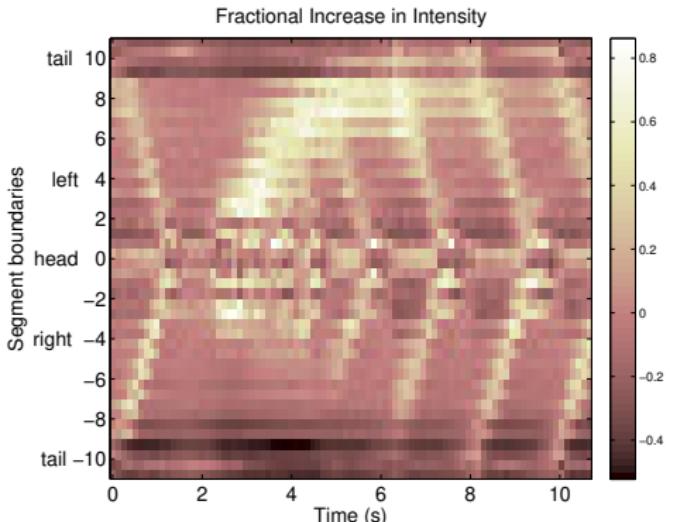
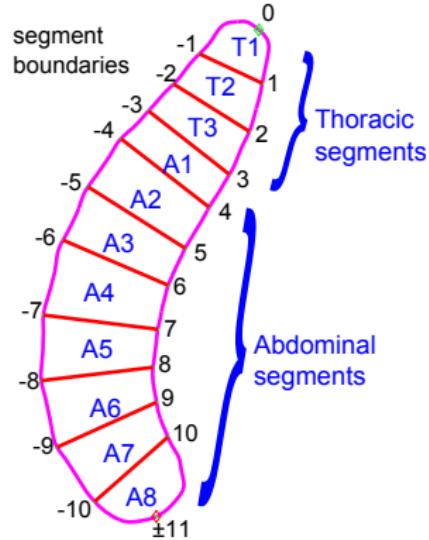
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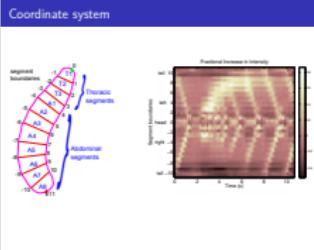
Coordinate system



Motor programs in Drosophila larvae
└ Imaging and analysis of fluorescent muscles

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└ Coordinate system

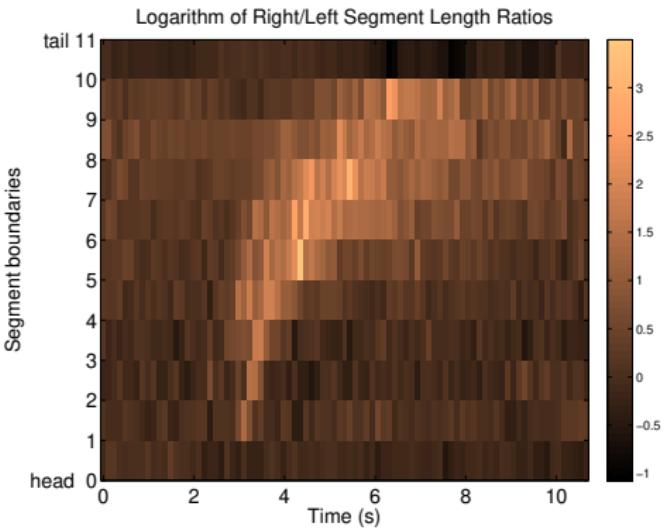
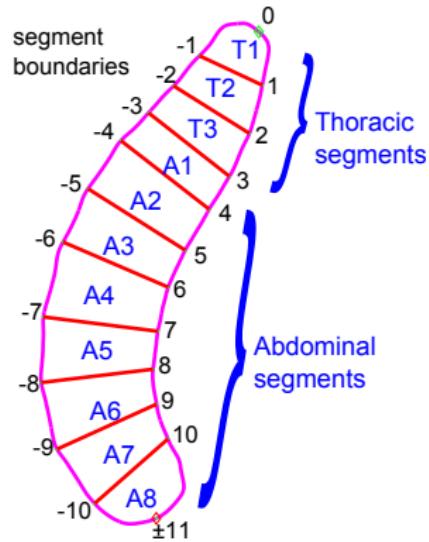


this slide just to explain how to read graphs. Interpret later.

thorax -3 to 3, rest abdomen

1. Head in middle, left above, right below.
Bright spots: contraction. See peristalsis go from tail to head
2. Head at bottom, tail at top. Remove peristalsis, just see bend.
Bright: left bend, dark: right bend.

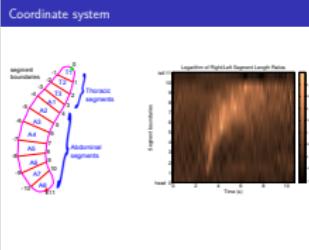
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Motor programs in Drosophila larvae
└ Imaging and analysis of fluorescent muscles

└ Coordinate system

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Results

Forward motion

Pulse travels from tail to head. New pulse starts after previous reaches head.

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1. Mouth hooks drown out all else (ratio) in T1,T2.
2. If we interfere with sensory feedback, could use this to measure effects.

Pulse travels from tail to head. New pulse starts after previous reaches head.

Small accepted head-sweep

Motor programs in Drosophila larvae

Results

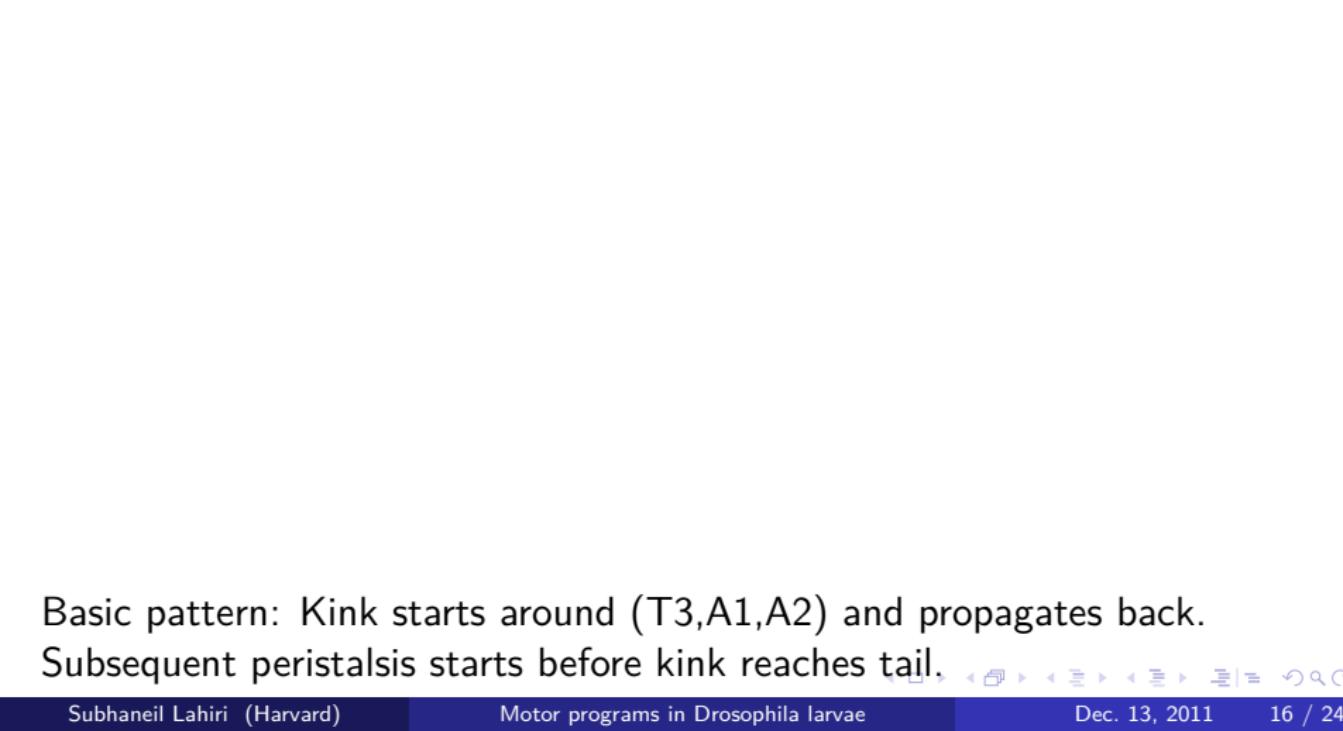
Small accepted head-sweep

Basic pattern: Kink starts around (T3,A1,A2) and propagates back.
Subsequent peristalsis starts before kink reaches tail.

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Small accepted head-sweep

Small accepted head-sweep



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└ Large accepted head-sweep

Basic pattern: Kink starts around (T3,A1,A2) and propagates back.
Subsequent peristalsis starts from kink, **not** tail.

1. same as small, statistics later

Basic pattern: Kink starts around (T3,A1,A2) and propagates back.
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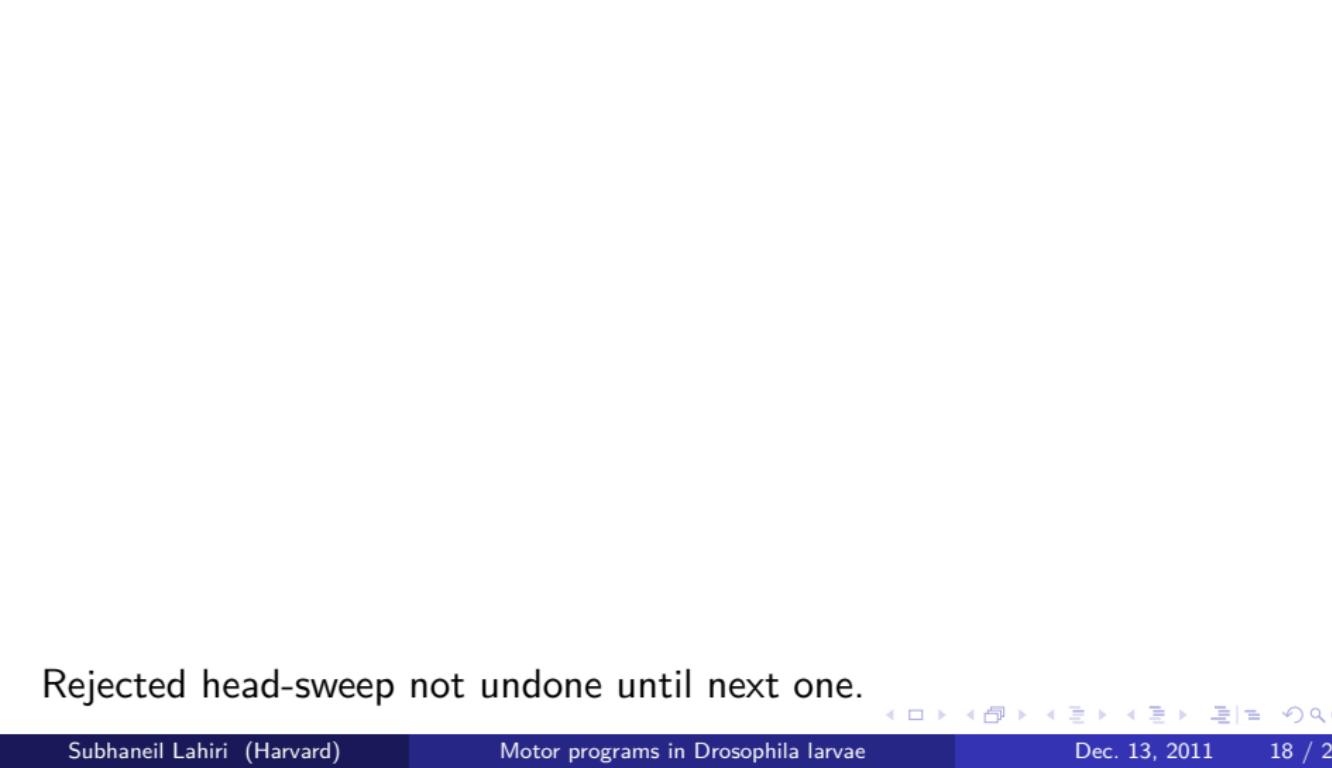
Rejected head-sweep

Motor programs in Drosophila larvae

Results

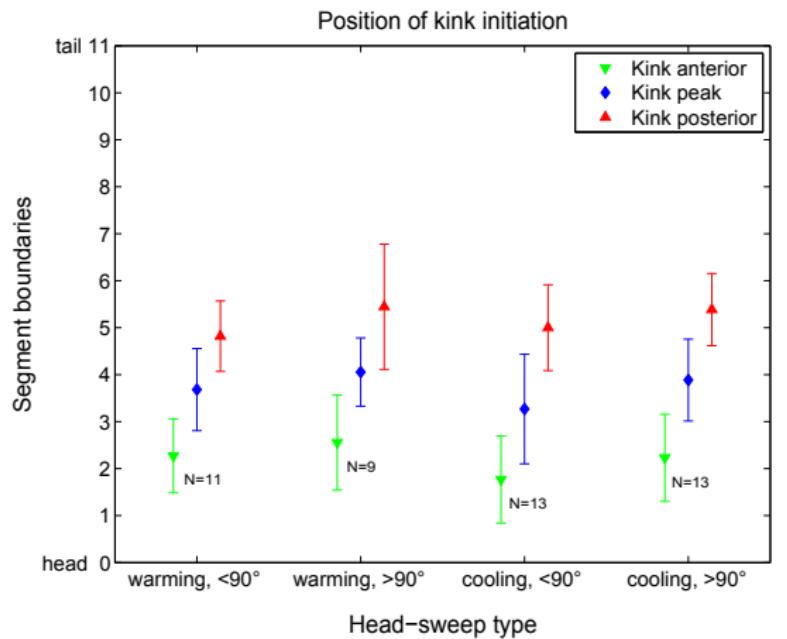
Rejected head-sweep

Rejected head-sweep not undone until next one.



Rejected head-sweep not undone until next one.

Position of initial bend



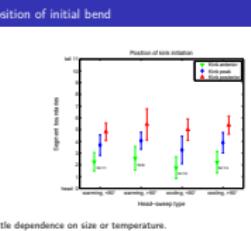
Little dependence on size or temperature.

Motor programs in Drosophila larvae

Results

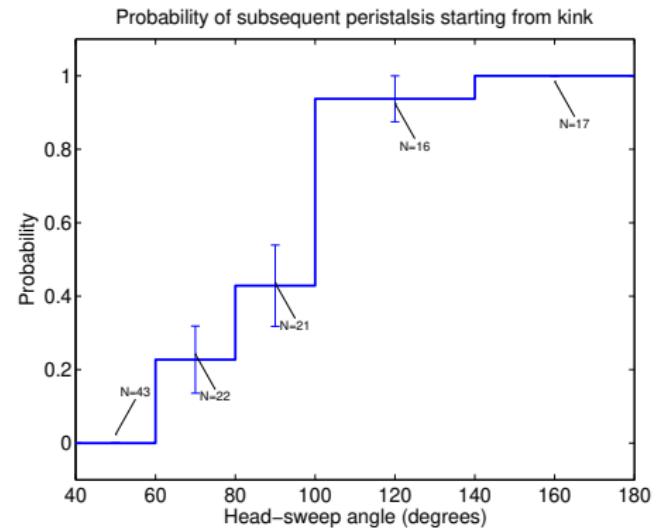
Position of initial bend

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1. error bars ar std dev, not std err.

Position of start of peristalsis



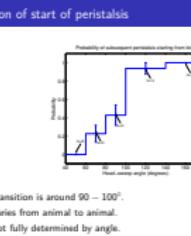
- Transition is around $90 - 100^\circ$.
- Varies from animal to animal.
- Not fully determined by angle.

Motor programs in Drosophila larvae

Results

Position of start of peristalsis

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Possible explanations

- Mechanical reason?

> 90° tail would move wrong way.

< 90° starting from kink would be slower.

- Neural circuit?

Stretch-sensors involved in locomotion pattern. If one side is already contracted, segment just anterior to kink might think peristaltic pulse has already reached it

- Central pattern generator?

Body re-coupling in mid-cycle – dependence on head-sweep size?

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Conclusions

All head-sweeps start at the same segments. Same circuits? Decision on size of head-sweep made later?

Navigation results from combining two basic motor programs: peristalsis and asymmetric contraction. Pathway from sensory input → motor output simpler than previously thought.

No “unbending” motor program.

Large head-sweeps: subsequent peristalsis starts at kink. Shows that peristalsis can start anywhere. Implications for circuits that control forward motion.

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Conclusions and future directions

Conclusions

1. need to interfere with sensory input during head-sweep optogenetically.
2. only need to decide when to switch programs
3. can only reject by going other way.
4. peristalsis initiator not localised

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Future directions

Interfere with motor patterns (optogenetically).

Fully automate image analysis.

Other stimuli.

Reverse crawling, hunching, and rolling.

Motor programs in Drosophila larvae

Conclusions and future directions

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Acknowledgements

Thanks to:

- Konlin Shen
- Anji Tang
- Mason Klein
- Liz Kane
- Ashley Carter
- Aravi Samuel
- Garrity lab

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└ Conclusions and future directions

└ Acknowledgements

1. Last slide!

Thanks to:
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• Mason Klein
• Liz Kane
• Ashley Carter
• Aravi Samuel
• Garrity lab

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References

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References I



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References

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Development, 129:2867–2878, (Jun, 2002) , PubMed:12050135.
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└ Toothpasting

back