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

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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.

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.

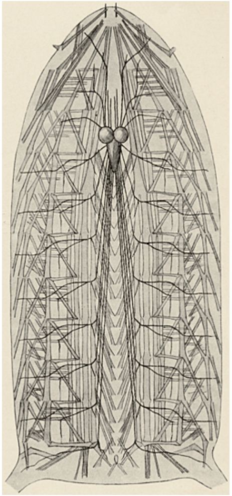
Introduction

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.

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.

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

Drosophila larva



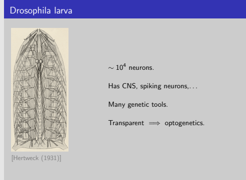
[Hertweck (1931)]

$\sim 10^4$ neurons.

Has CNS, spiking neurons,. . .

Many genetic tools.

Transparent $\implies$ optogenetics.



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

Outline

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

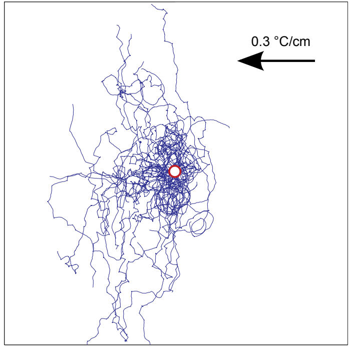
1. review how D.larvae navigate, what's known about locomotion circuits
2. how larvae with fluorescent muscles will help us, how we use them
3. results of this analysis
4. conclusions and future directions

Section 1

Navigation and locomotion

Section 1
Navigation and locomotion

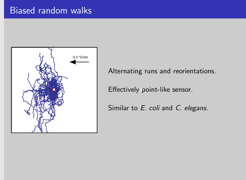
Biased random walks



Alternating runs and reorientations.

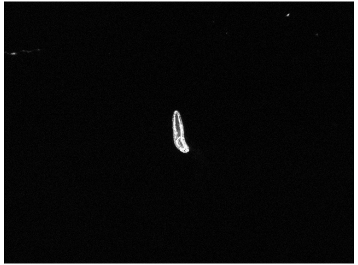
Effectively point-like sensor.

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

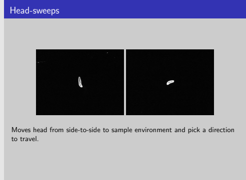


1. longer runs in good directions
2. has to move sensor to measure gradients
3. can do more
4. the thing that allows D.larvae to do more...

Head-sweeps

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



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)]

Navigation strategy
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- head-sweep frequency
- head-sweep size
- head-sweep acceptance probability
Depending on whether conditions are improving/worsening.
[Luo et al. (2010)]

1. turns more when things are getting worse
2. larger turns when things are getting worse
3. more likely to accept when better

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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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.

1. difference in initiation $\rightarrow$ makes decision before

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), Grueber 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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1. We'll see lots of videos of this later.
2. each segments waits for posterior segment to contract.
3. in future: interfere

Section 2

Imaging and analysis of fluorescent muscles

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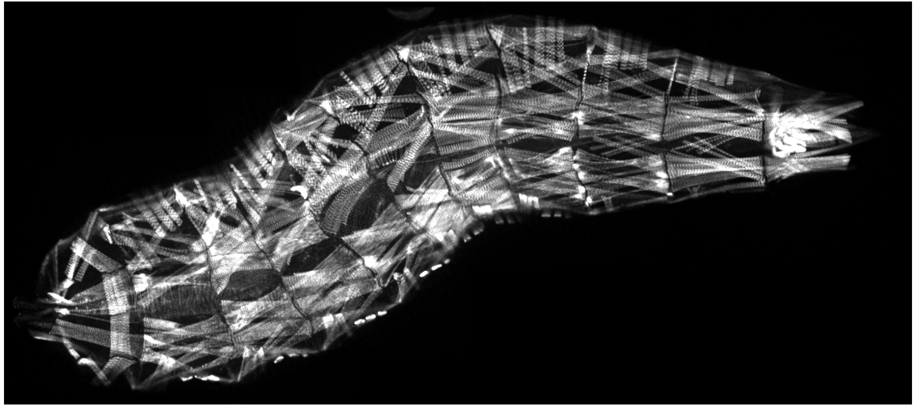
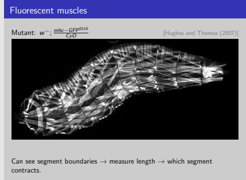
Fluorescent muscles

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.

Intensity pattern

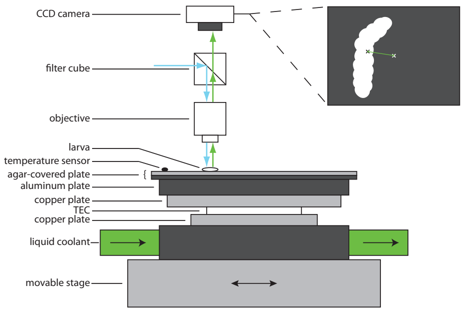
Muscles contract $\rightarrow$ same GFP in smaller volume $\rightarrow$ increase concentration $\rightarrow$ increase brightness.

Intensity pattern

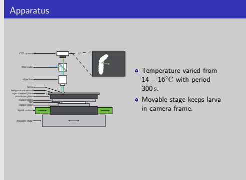
Muscles contract → same GFP in smaller volume → increase concentration → increase brightness.

1. another measure of contraction. less noisy

Apparatus

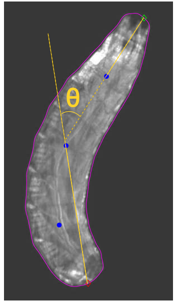


- Temperature varied from $14 - 16^{\circ}\mathrm{C}$ with period $300\,\mathrm{s}$.
- Movable stage keeps larva in camera frame.

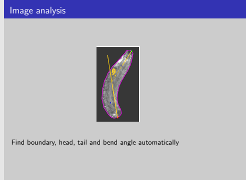


1. triggers many head-sweeps
2. allows comparison of head-sweepin warming/cooling

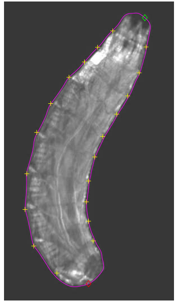
Image analysis



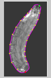
Find boundary, head, tail and bend angle automatically



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



User clicks on segment boundaries



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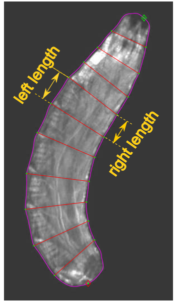

Map to boundary. Find segment lengths.

Image analysis

Map to boundary. Find segment lengths.

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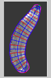
Image analysis

Split segment into quadrants. Mean pixel value $\rightarrow$ intensity.

Image analysis



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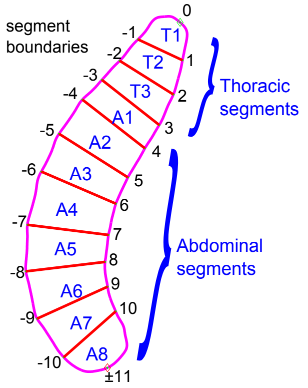
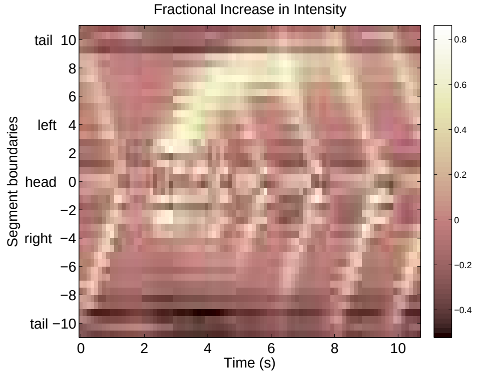
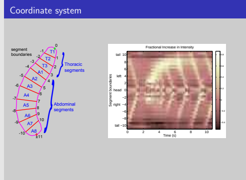
Coordinate system





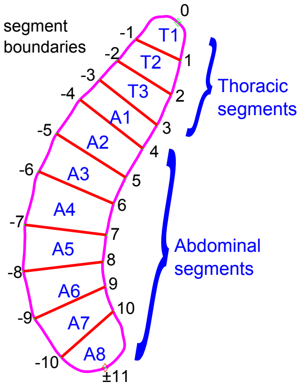


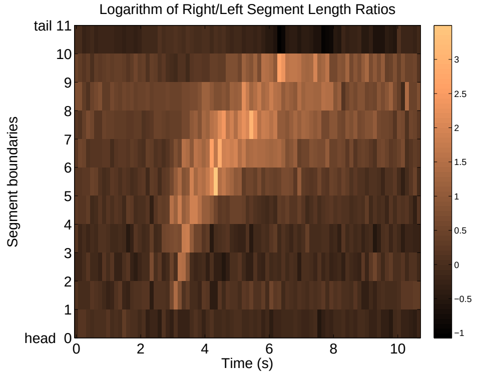
this slide just to explain how to read graphs. Interpret later.

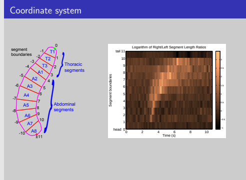
thorax -3 to 3, rest abdomen

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.

Coordinate system







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Section 3

Results

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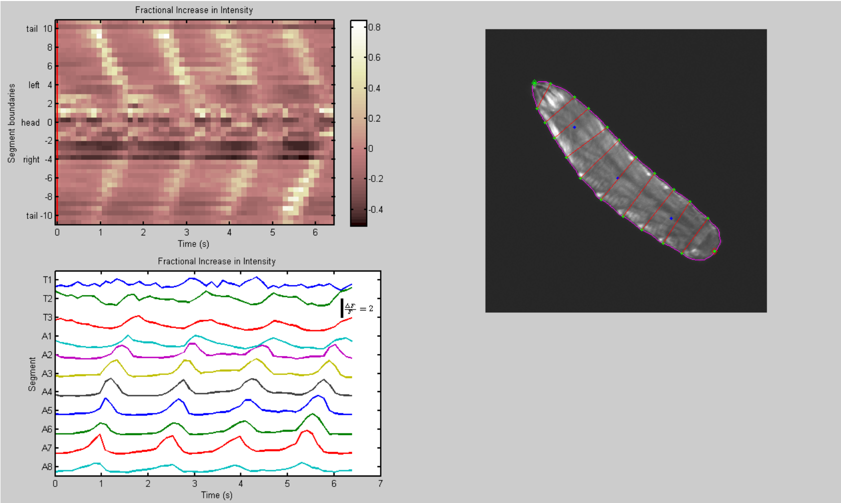
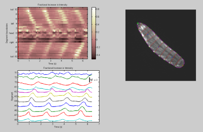
Forward motion



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

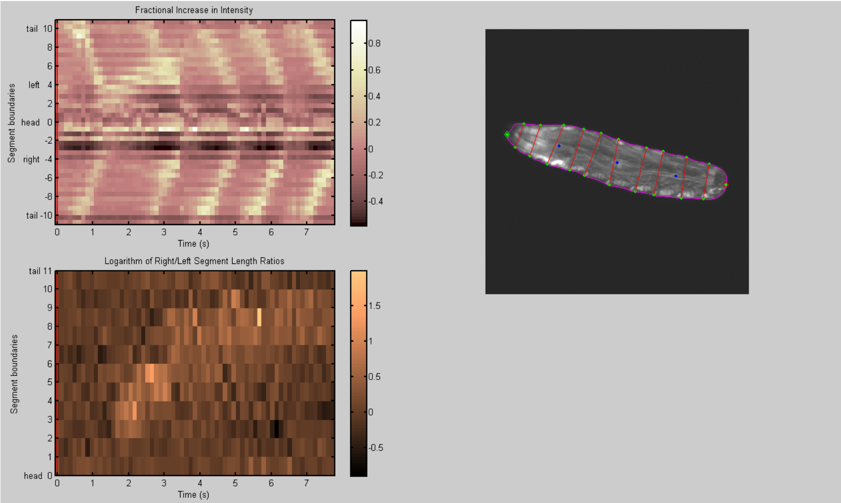
Forward motion



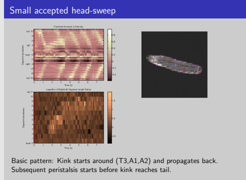
Pulse travels from tail to head. New pulse starts after previous reaches head.

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

Small accepted head-sweep

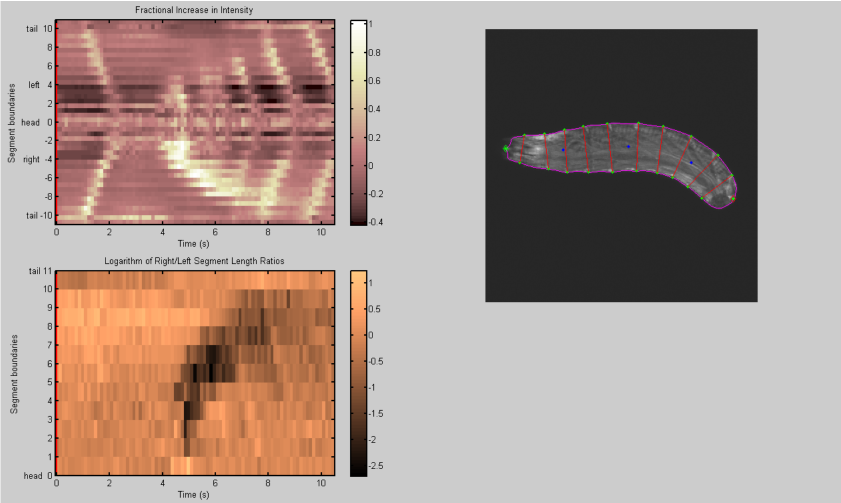


Basic pattern: Kink starts around (T3,A1,A2) and propagates back.
Subsequent peristalsis starts before kink reaches tail.



1. Completes head-sweep with peristalsis, not unbending.
2. non-overlapping

Large accepted head-sweep



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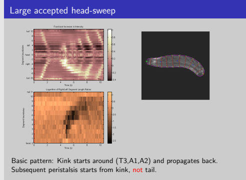
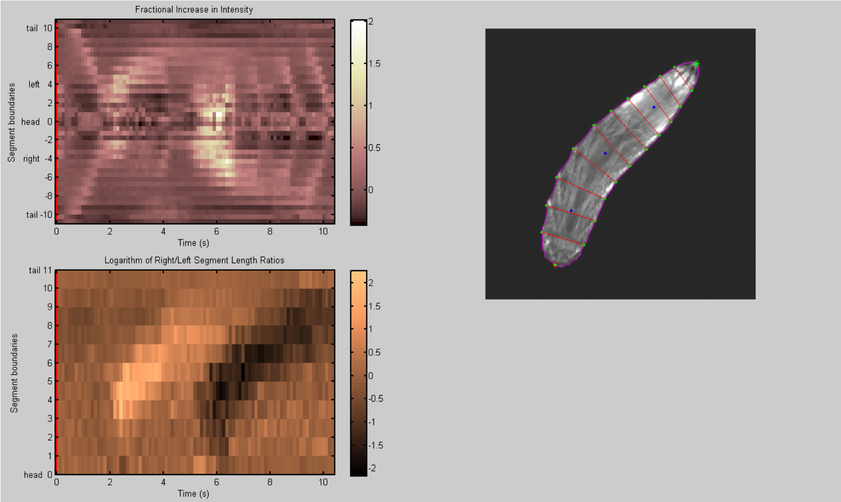
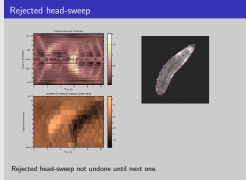


1. same as small, statistics later

Rejected head-sweep

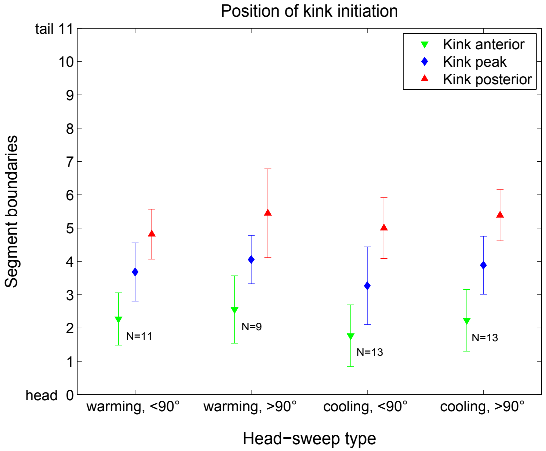


Rejected head-sweep not undone until next one.

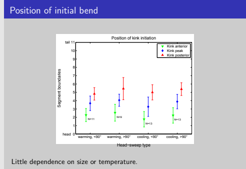


1. no unbending program

Position of initial bend

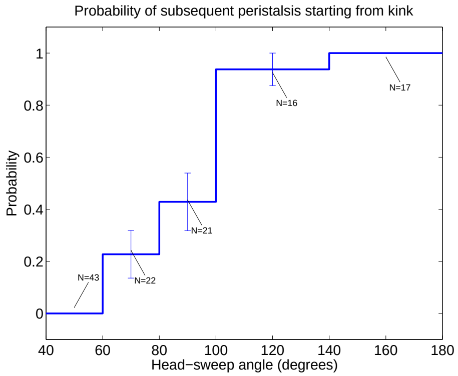


Little dependence on size or temperature.

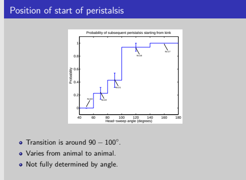


1. error bars ar std dev, not std err.

Position of start of peristalsis



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



Possible explanations

- Mechanical reason?
  $> 90^\circ$ tail would move wrong way.
  $< 90^\circ$ 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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Section 4

Conclusions and future directions

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

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

Future directions

Interfere with motor patterns (optogenetically).

Fully automate image analysis.

Other stimuli.

Reverse crawling, hunching, and rolling.

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Interfere with motor patterns (optogenetically).

Fully automate image analysis.

Other stimuli.

Reverse crawling, hunching, and rolling.

1. requires next point
2. machine learning - training data
3. we did temperature, could do odour. light difficult. Unlikely to be any difference.
4. nociceptive and rapid avoidance responses

Acknowledgements

Thanks to:

- Konlin Shen
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- Ashley Carter
- Aravi Samuel
- Garrity lab

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1. Last slide!

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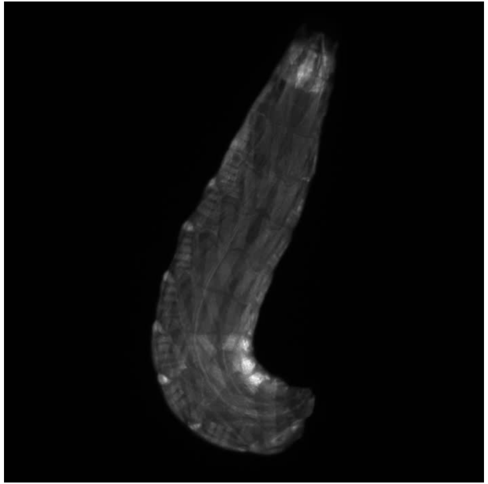
Toothpasting



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