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

December 10, 2011

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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.

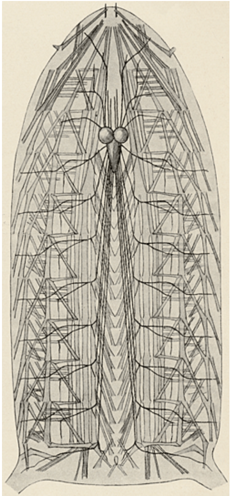
Introduction

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. future: interfere, now: just look at normal behaviour

Drosophila larva



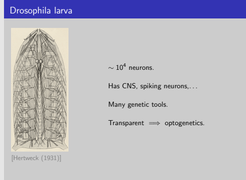
[Hertweck (1931)]

$\sim 10^4$ neurons.

Has CNS, spiking neurons,. . .

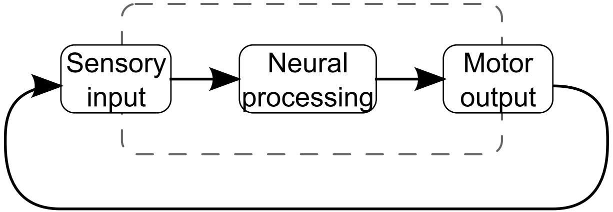
Many genetic tools.

Transparent $\implies$ optogenetics.



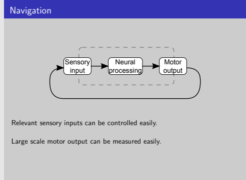
1. factor of 10 ¡ adult
2. unlike c. elegans
3. sequenced genome, GAL4/UAS system - target cell types

Navigation



Relevant sensory inputs can be controlled easily.

Large scale motor output can be measured easily.



1. Temperature, odour, light
2. path travelled, turning decisions

Outline

1 Navigation and locomotion

2 Imaging and analysis of fluorescent muscles

3 Results

4 Conclusions and future directions

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

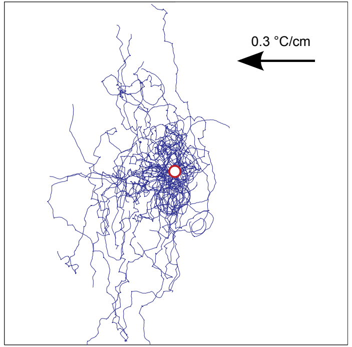
1. review how D.larvae navigate, what's known about locomotion circuits
2. how larvae with fluorescent muscles will help us, how we use them
3. results of this analysis
4. conclusions and future directions

Section 1

Navigation and locomotion

Section 1
Navigation and locomotion

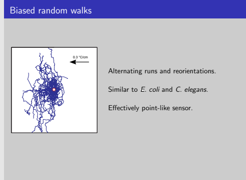
Biased random walks



Alternating runs and reorientations.

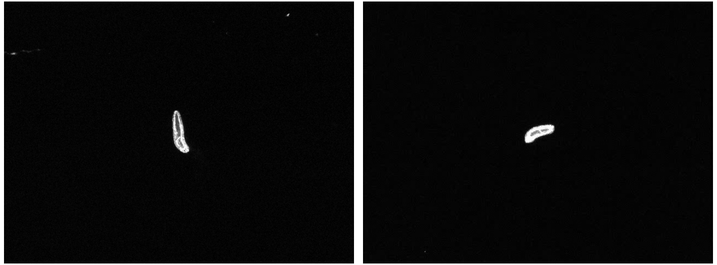
Similar to *E. coli* and *C. elegans*.

Effectively point-like sensor.

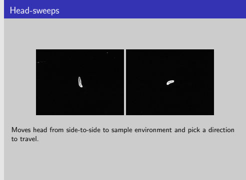


1. longer runs in good directions
2. can do more
3. has to move sensor to measure gradients

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
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)]

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

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)]

For finer analysis: quantify patterns of muscle use.

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1. in future: interfere

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 circuits?
- When is decision made? With what info?
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Look for differences in mechanics of different types of head-sweep.

1. difference in initiation → makes decision before

Section 2

Imaging and analysis of fluorescent muscles

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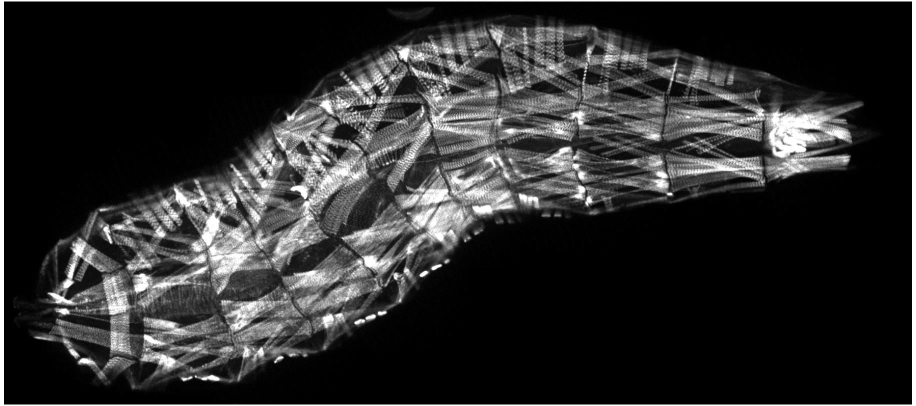
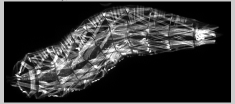
Fluorescent muscles

Mutant: $w^{-}; \frac{mhc{-}GFP^{0110}}{CyO}$ [Hughes and Thomas (2007)]



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

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

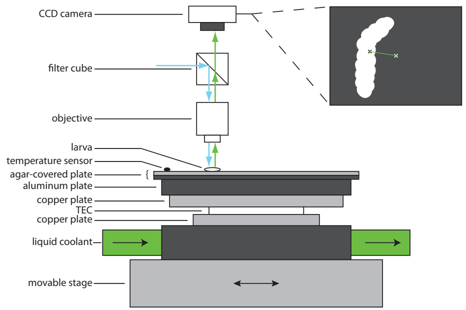
Muscles contract → same GFP in smaller volume → increase concentration → increase brightness.

Intensity pattern

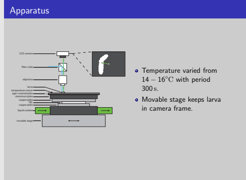
Muscles contract → same GFP in smaller volume → increase concentration → increase brightness.

1. another measure of contraction. less noisy

Apparatus

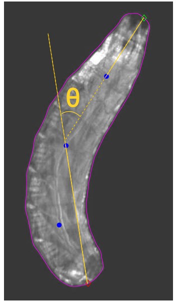


- Temperature varied from $14 - 16^{\circ}\mathrm{C}$ with period $300\,\mathrm{s}$.
- Movable stage keeps larva in camera frame.

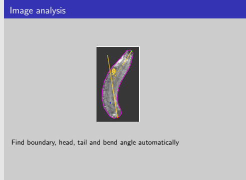


1. triggers many head-sweeps
2. allows comparison of head-sweepin warming/cooling

Image analysis

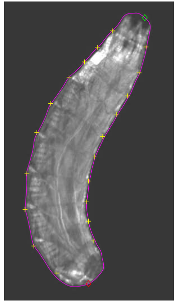


Find boundary, head, tail and bend angle automatically

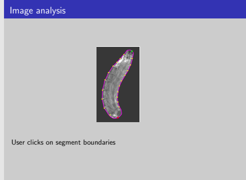


1. allows us to flag interesting bits
2. slowest part
3. automatic again
4. less noisy

Image analysis



User clicks on segment boundaries



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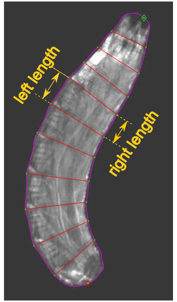

Map to boundary. Find segment lengths.

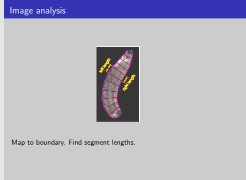

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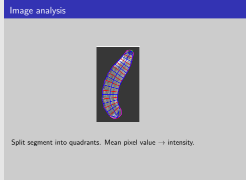
Image analysis

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



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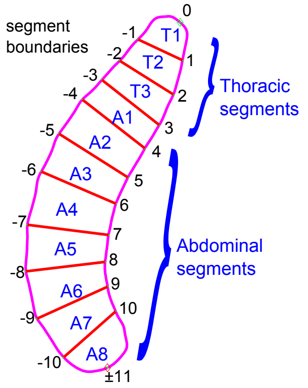
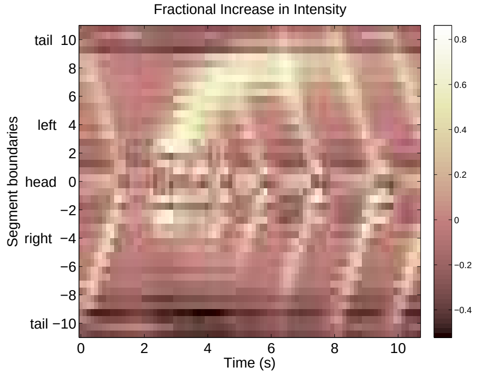
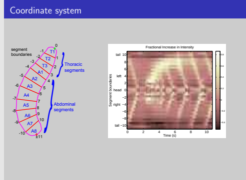
Coordinate system





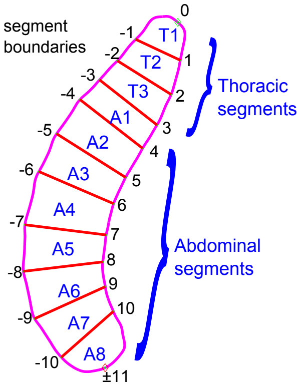


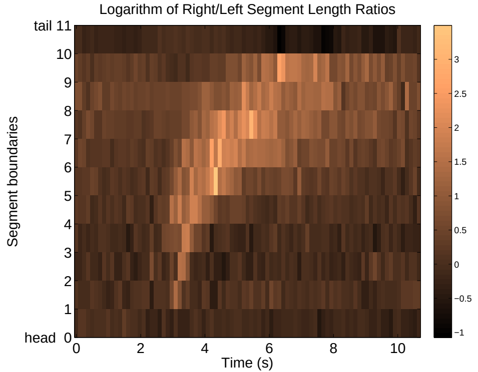
this slide just to explain how to read graphs. Interpret later.

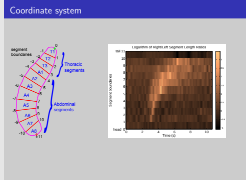
thorax -3 to 3, rest abdomen

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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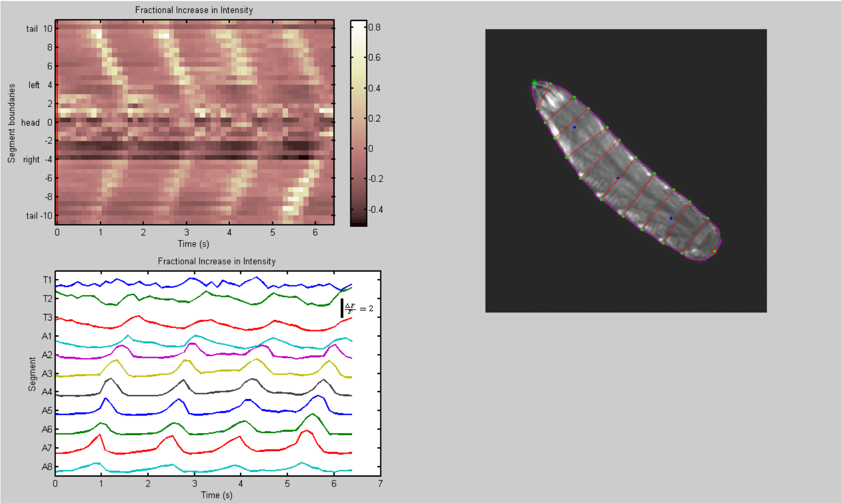
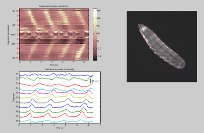
Forward motion



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

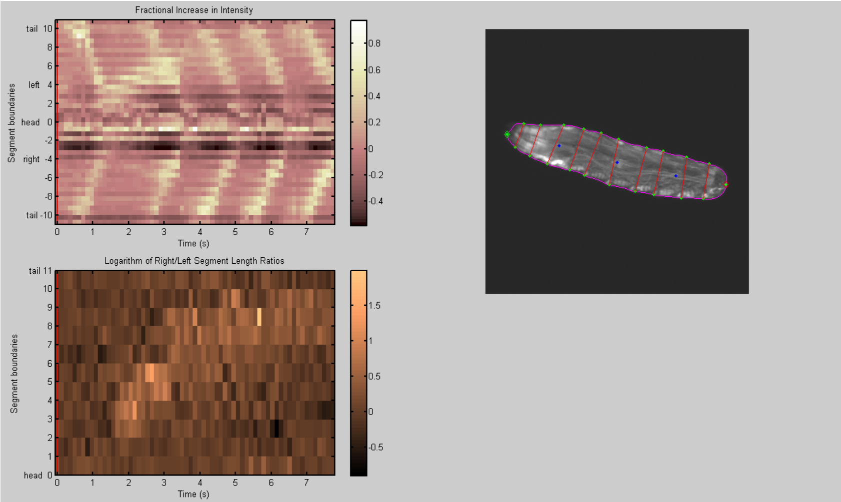
Forward motion



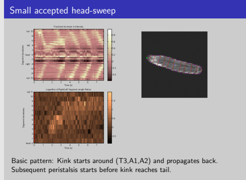
Pulse travels from tail to head. New pulse starts after previous reaches head.

1. Mouth hooks drown out all else (ratio) in T1,T2.

Small accepted head-sweep

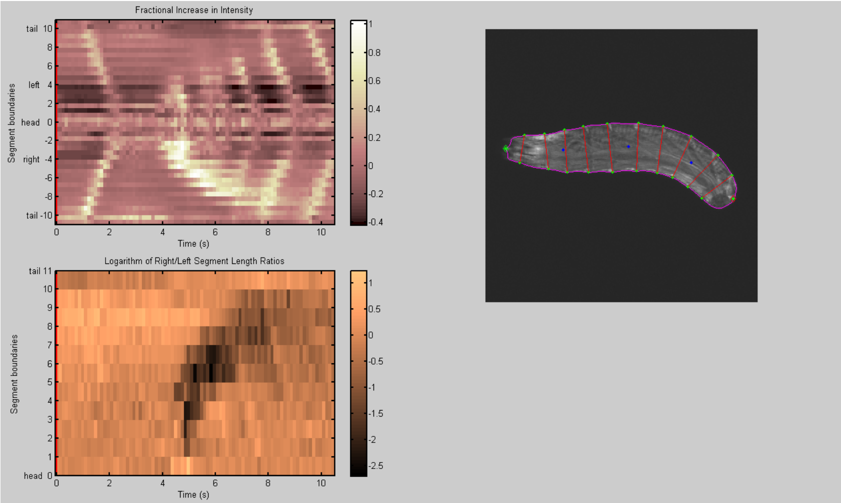


Basic pattern: Kink starts around (T3,A1,A2) and propagates back. Subsequent peristalsis starts before kink reaches tail.

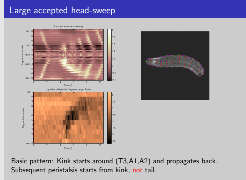


1. Completes head-sweepwith peristalsis, not unbending.

Large accepted head-sweep

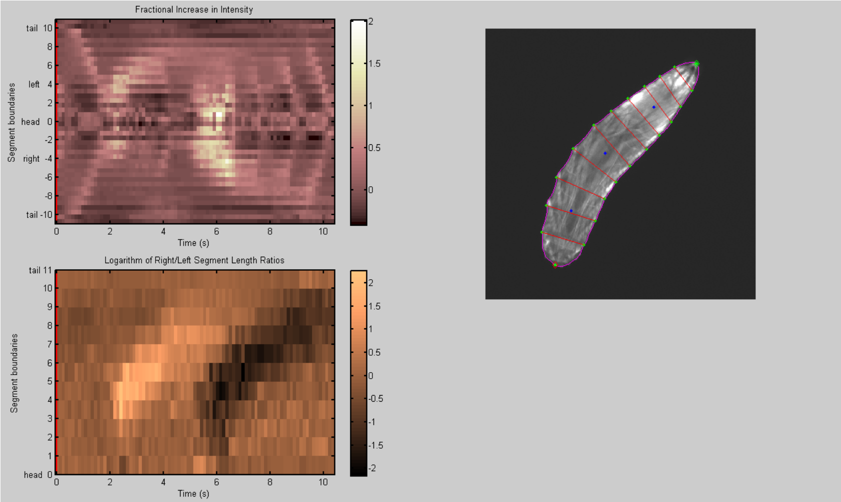


Basic pattern: Kink starts around (T3,A1,A2) and propagates back.
Subsequent peristalsis starts from kink, not tail.

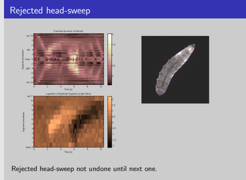


1. same as small, statistics later

Rejected head-sweep

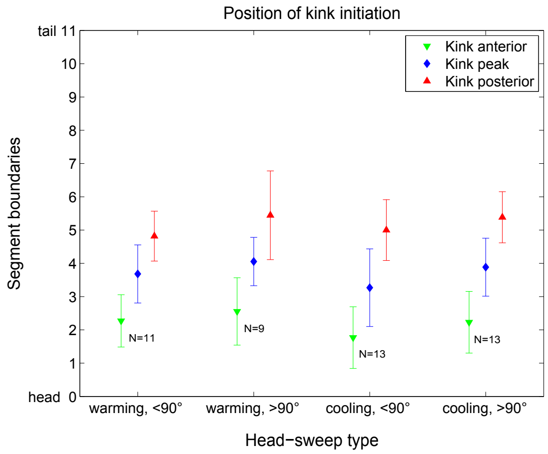


Rejected head-sweep not undone until next one.

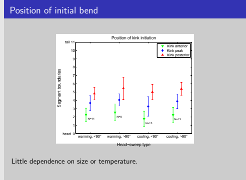


1. no unbending program

Position of initial bend

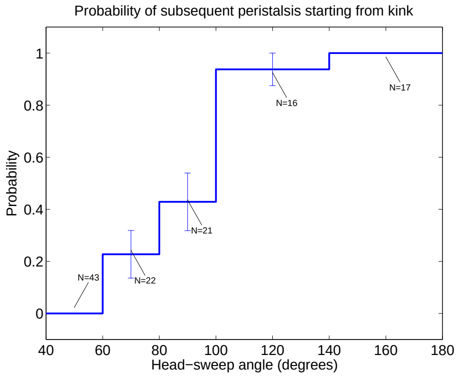


Little dependence on size or temperature.

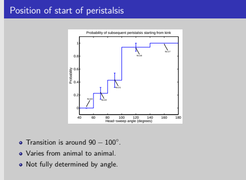


1. error bars ar std dev, not std err.

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-sweepsize?

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Section 4

Conclusions and future directions

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

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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.

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Future directions

Interfere with motor patterns (optogenetically).

Fully automate image analysis.

Other stimuli.

Reverse crawling, hunching, and rolling.

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

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1. Last slide!

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"Navigational decision making in Drosophila thermotaxis".
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2011-12-10

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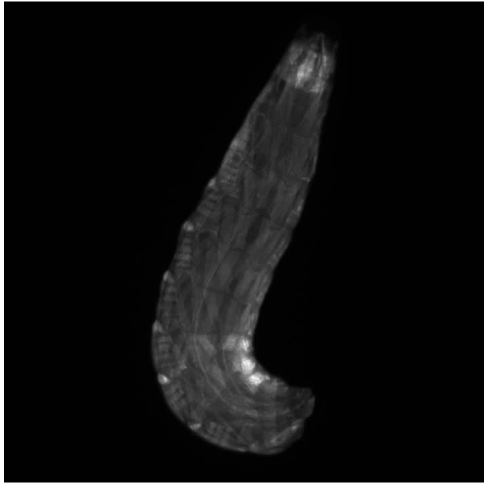
Toothpasting



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