

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

January 7, 2013

2013-01-07

Motor programs in *Drosophila* larvae

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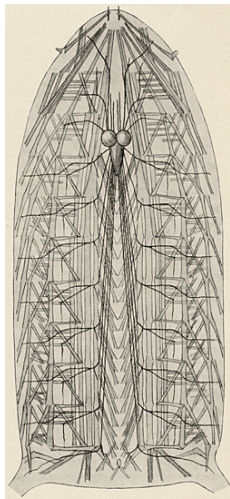
January 7, 2013

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



[Hertweck (1931)]

$\sim 10^4$ neurons.

Has CNS, spiking neurons,...

Many genetic tools.

Transparent \implies optogenetics.

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└ Drosophila larva



$\sim 10^4$ neurons.
Has CNS, spiking neurons...
Many genetic tools.
Transparent \implies optogenetics.

[Hertweck (1931)]

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

Outline

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

- 1 Navigation and locomotion
- 2 Imaging and analysis of fluorescent muscles
- 3 Results
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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

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Motor programs in Drosophila larvae
└─ Navigation and locomotion

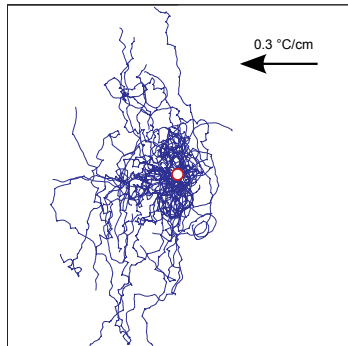
Section 1

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*.

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Motor programs in *Drosophila* larvae

Navigation and locomotion

Biased random walks



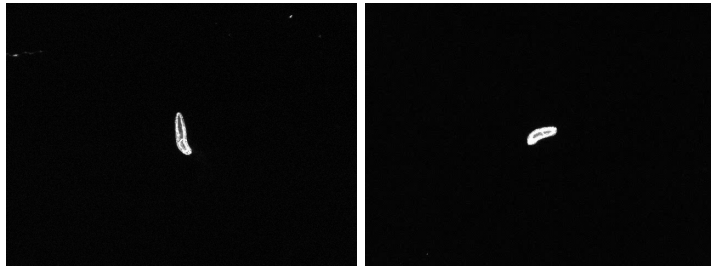
Alternating runs and reorientations.

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.

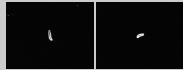
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Motor programs in *Drosophila* larvae

└ Navigation and locomotion

└ Head-sweeps

Head-sweeps



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

1. accepted
2. rejected

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

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

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

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 → makes decision before

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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Motor programs in Drosophila larvae

└ Navigation and locomotion

└ Locomotion and sensory feedback

Locomotion and sensory feedback

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

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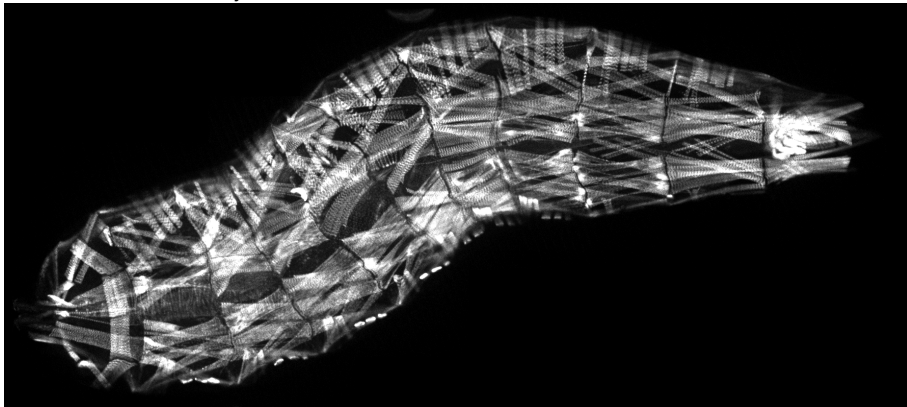
1. We'll see lots of videos of this later.
2. each segments waits for posterior segment to contract.
3. in future: interfere

Imaging and analysis of fluorescent muscles

Fluorescent muscles

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

[Hughes and Thomas (2007)]



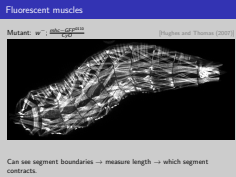
Can see segment boundaries → measure length → which segment contracts.

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Motor programs in Drosophila larvae

└ Imaging and analysis of fluorescent muscles

└ Fluorescent muscles



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.

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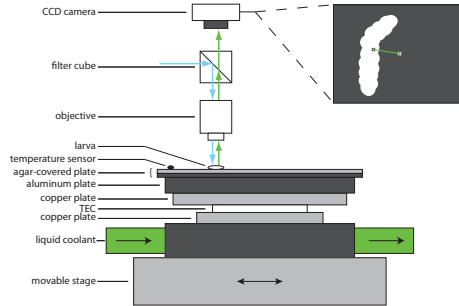
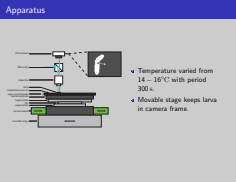
Motor programs in *Drosophila* larvae
└ Imaging and analysis of fluorescent muscles
└ Intensity pattern

Intensity pattern



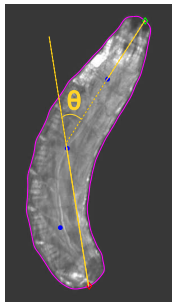
Muscles contract \rightarrow same GFP in smaller volume \rightarrow increase concentration \rightarrow increase brightness.

1. another measure of contraction. less noisy



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

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



Find boundary, head, tail and bend angle automatically

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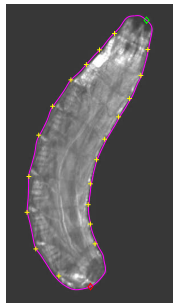
Motor programs in *Drosophila* larvae
└ Imaging and analysis of fluorescent muscles
└ Image analysis

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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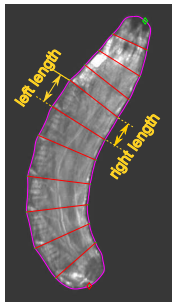
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└ Imaging and analysis of fluorescent muscles
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Image analysis



User clicks on segment boundaries



Map to boundary. Find segment lengths.

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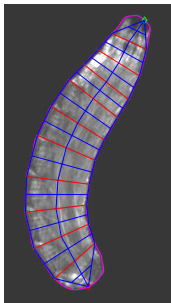
- Motor programs in Drosophila larvae
 - Imaging and analysis of fluorescent muscles
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Image analysis



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

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

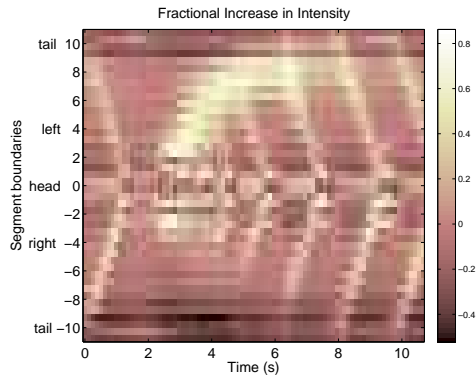
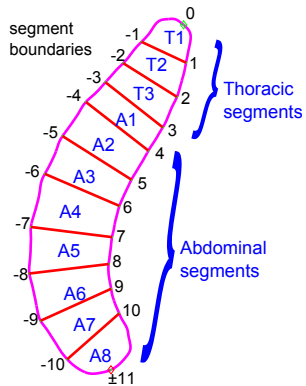
Image analysis



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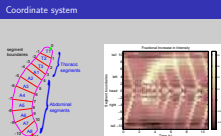
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Coordinate system



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- Motor programs in Drosophila larvae
 - Imaging and analysis of fluorescent muscles
 - Coordinate system

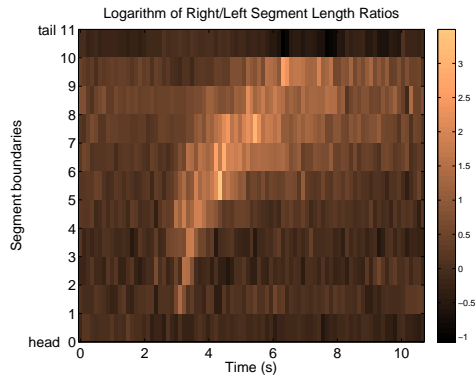
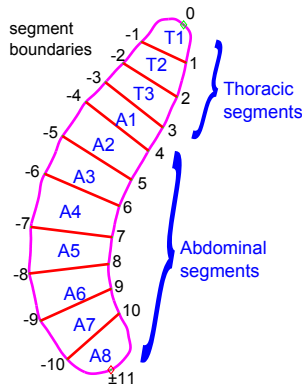


this slide just to explain how to read graphs. Interpret later.

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.

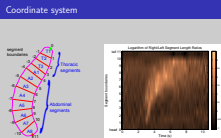
Coordinate system



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Motor programs in Drosophila larvae

- Imaging and analysis of fluorescent muscles
- Coordinate system



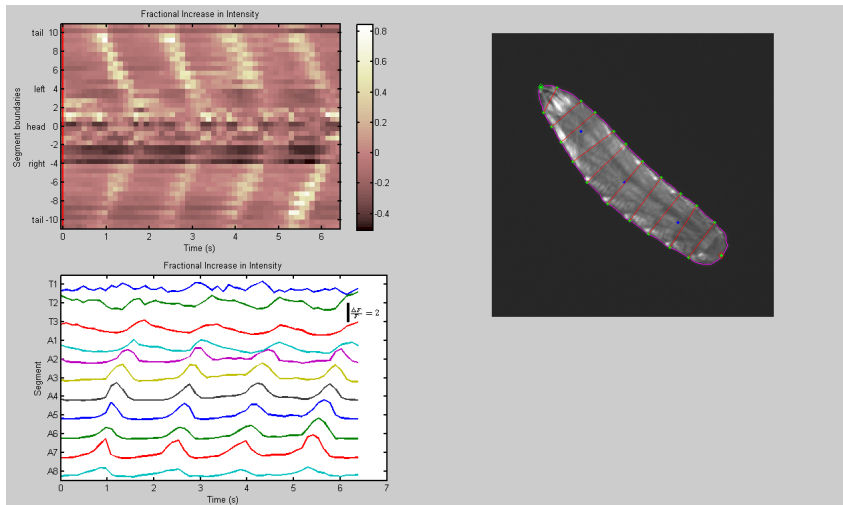
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Results

Forward motion



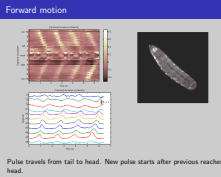
Pulse travels from tail to head. New pulse starts after previous reaches head.

Motor programs in Drosophila larvae

Results

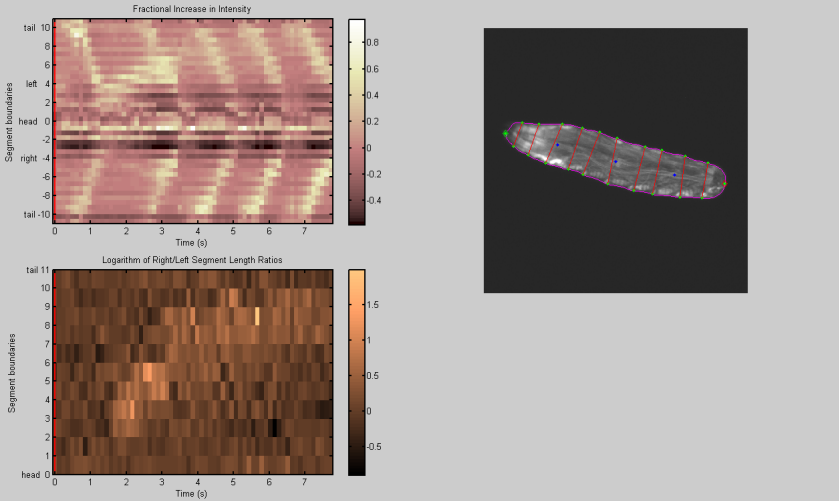
Forward motion

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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.

Small accepted head-sweep



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

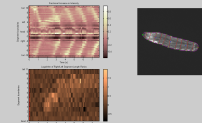
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Motor programs in Drosophila larvae

Results

Small accepted head-sweep

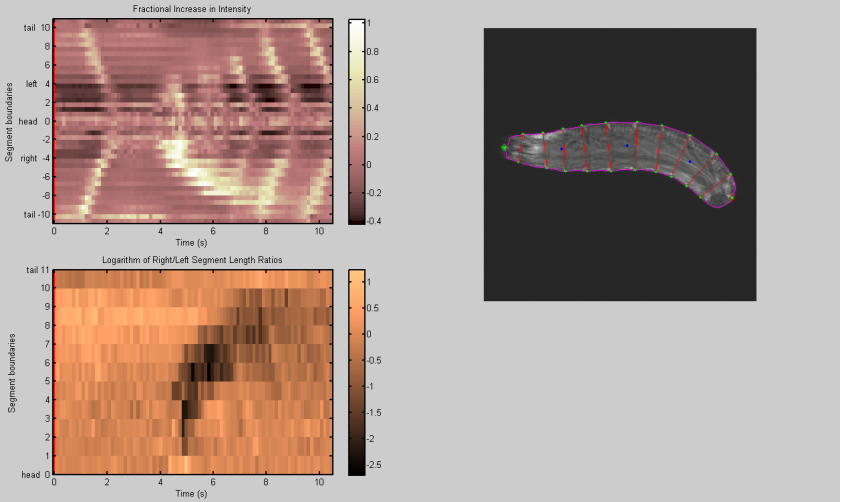
Small accepted head-sweep



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.
Subsequent peristalsis starts from kink, **not** tail.

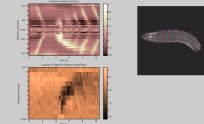
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Motor programs in Drosophila larvae

Results

Large accepted head-sweep

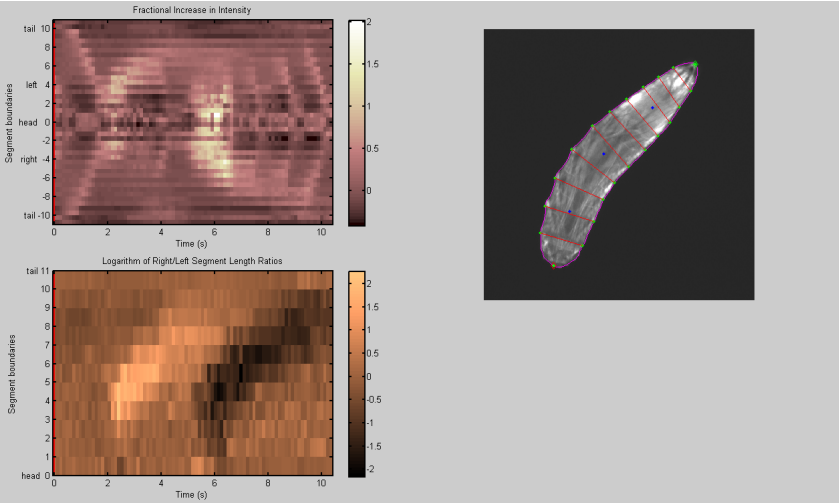
Large accepted head-sweep



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



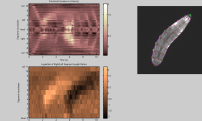
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Motor programs in Drosophila larvae

Results

Rejected head-sweep

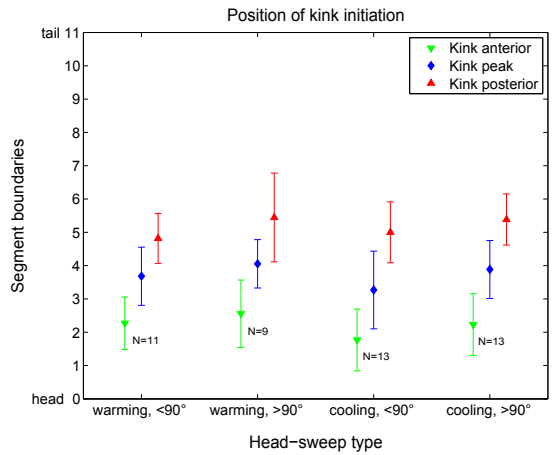
Rejected head-sweep



1. no unbending program

Rejected head-sweep not undone until next one.

Position of initial bend



Little dependence on size or temperature.

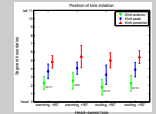
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Motor programs in Drosophila larvae

Results

Position of initial bend

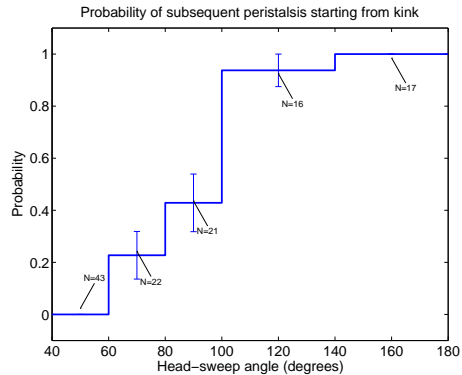
Position of initial bend



Little dependence on size or temperature.

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

Position of start of peristalsis



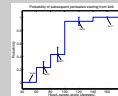
- Transition is around $90 - 100^\circ$.
- Varies from animal to animal.
- Not fully determined by angle.

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Motor programs in *Drosophila* larvae

Results

Position of start of peristalsis



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

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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Motor programs in Drosophila larvae

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Motor programs in *Drosophila* larvae
└ Conclusions and future directions

Section 4

Conclusions and future directions

Section 4

Conclusions and future directions

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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Motor programs in *Drosophila* larvae

└─ Conclusions and future directions

└─ Conclusions

Conclusions

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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.

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

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- Motor programs in Drosophila larvae
 - Conclusions and future directions
 - Future directions

Future directions

Interfere with motor patterns (optogenetically).

Fully automate image analysis.

Other stimuli.

Reverse crawling, hunching, and rolling.

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

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Motor programs in Drosophila larvae
└─ Conclusions and future directions
 └─ 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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“Two alternating motor programs drive navigation in *Drosophila* larva”.

PLoS ONE, 6:e23180, 2011, PubMed:21858019.



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“Navigational decision making in *Drosophila* thermotaxis”.

J. Neurosci., 30:4261–4272, Mar 2010, PubMed:20335462.

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- Motor programs in *Drosophila* larvae
 - Conclusions and future directions
 - References

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Development Genes and Evolution, 196:69–77, 1987.

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11



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Development, 129:2867–2878, Jun 2002, PubMed:12050135.

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Motor programs in *Drosophila* larvae

└ Conclusions and future directions

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C. L. Hughes and J. B. Thomas.

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11

13

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Motor programs in *Drosophila* larvae

Conclusions and future directions

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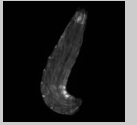
Toothpasting



2013-01-07

- Motor programs in Drosophila larvae
 - Conclusions and future directions
 - Toothpasting

Toothpasting



back