

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

February 7, 2013

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Motor programs in *Drosophila* larvae

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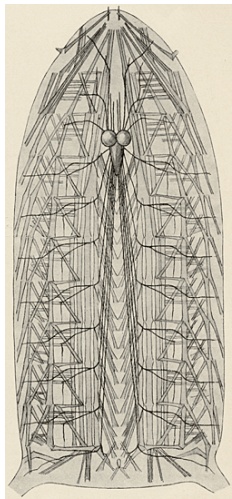
February 7, 2013

└ 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



[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

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

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Motor programs in Drosophila larvae

Outline

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

- Navigation and locomotion
- Imaging and analysis of fluorescent muscles
- Results
- Conclusions and future directions

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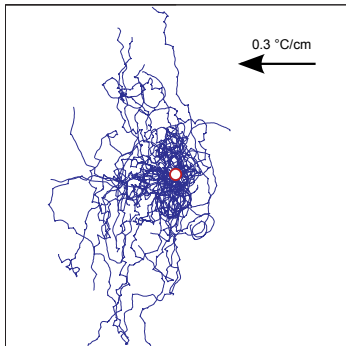
Motor programs in Drosophila larvae
└ Navigation and locomotion

Section 1

Navigation and locomotion

Section 1

Navigation and locomotion



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

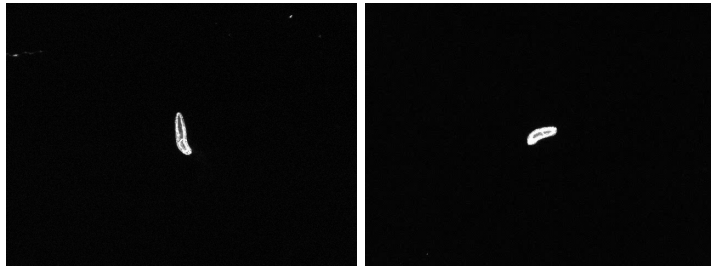
└ Biased random walks



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 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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Motor programs in Drosophila larvae

└ Navigation and locomotion

└ 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.

1. difference in initiation → 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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Motor programs in *Drosophila* larvae

- Navigation and locomotion

- └ Locomotion and sensory feedback

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

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

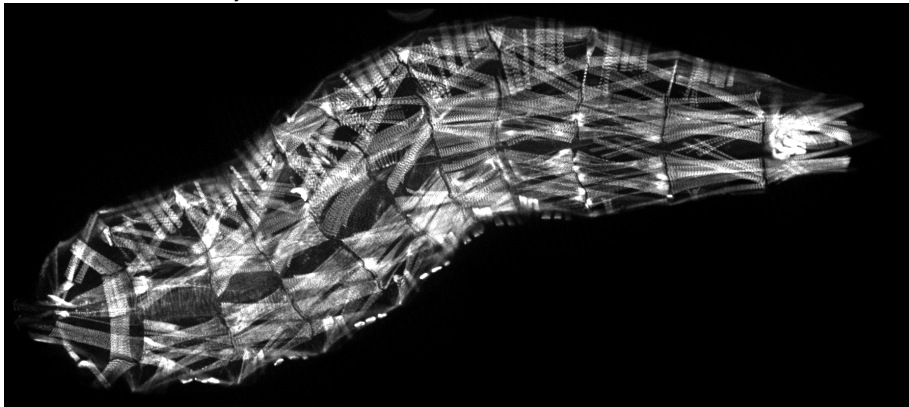
Imaging and analysis of fluorescent muscles

Imaging and analysis of fluorescent muscles

Fluorescent muscles

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

[Hughes and Thomas (2007)]



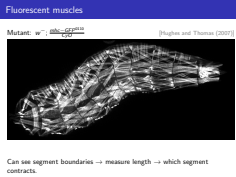
Can see segment boundaries \rightarrow measure length \rightarrow which segment contracts.

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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

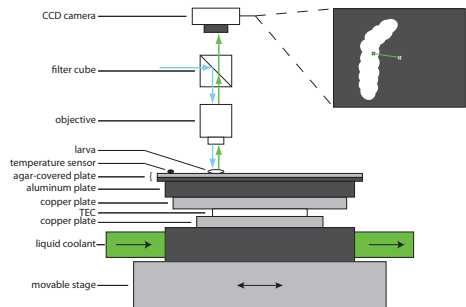
- Intensity pattern

Intensity pattern



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

1. another measure of contraction. less noisy



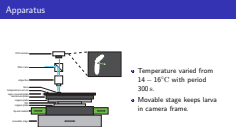
- Temperature varied from 14 – 16°C with period 300 s.
- Movable stage keeps larva in camera frame.

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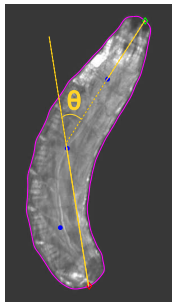
Motor programs in Drosophila larvae

└ Imaging and analysis of fluorescent muscles

└ Apparatus



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



Find boundary, head, tail and bend angle automatically

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Motor programs in Drosophila larvae

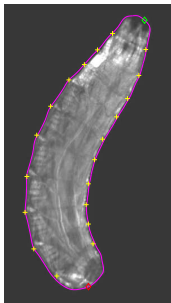
└ Imaging and analysis of fluorescent muscles

└ Image analysis



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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Motor programs in Drosophila larvae

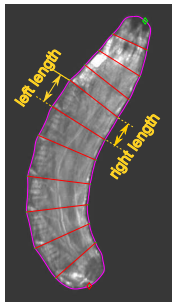
└ Imaging and analysis of fluorescent muscles

└ Image analysis

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User clicks on segment boundaries



Map to boundary. Find segment lengths.

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Motor programs in Drosophila larvae

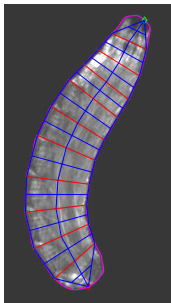
└ Imaging and analysis of fluorescent muscles

└ 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

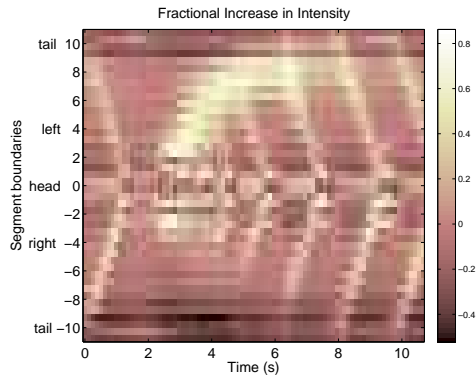
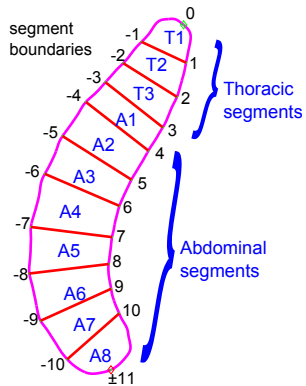
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Coordinate system

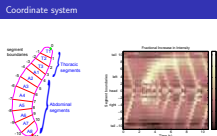


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Motor programs in Drosophila larvae

Imaging and analysis of fluorescent muscles

Coordinate system

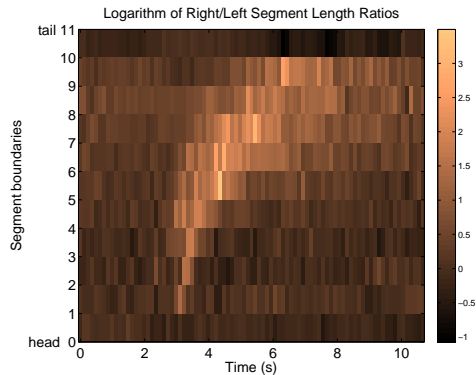
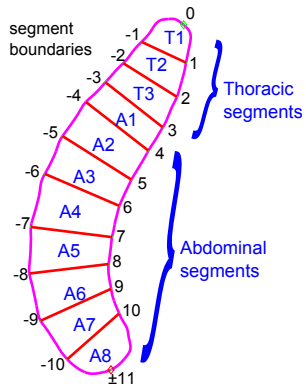


this slide just to explain how to read graphs. Interpret later.

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

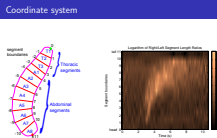


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Motor programs in Drosophila larvae

Imaging and analysis of fluorescent muscles

Coordinate system

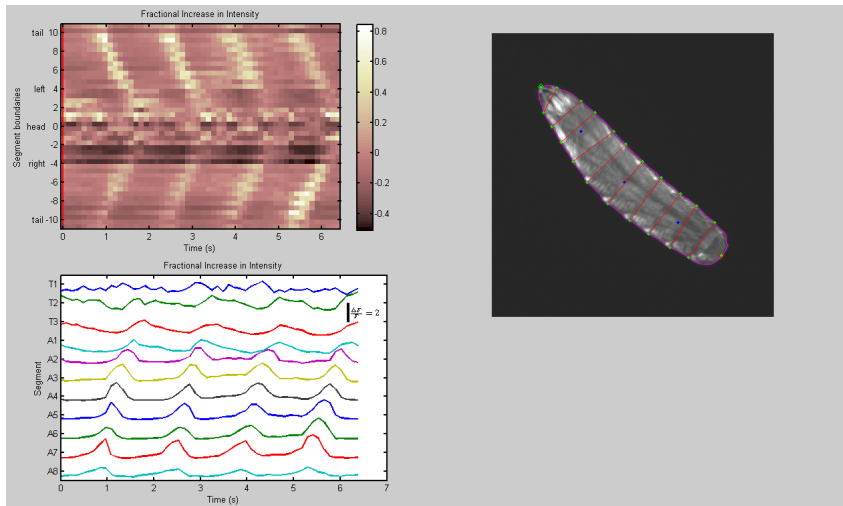


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Forward motion



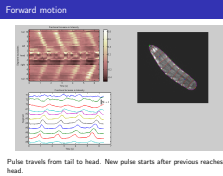
Pulse travels from tail to head. New pulse starts after previous reaches head.

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Motor programs in Drosophila larvae

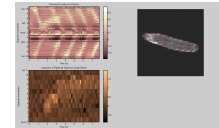
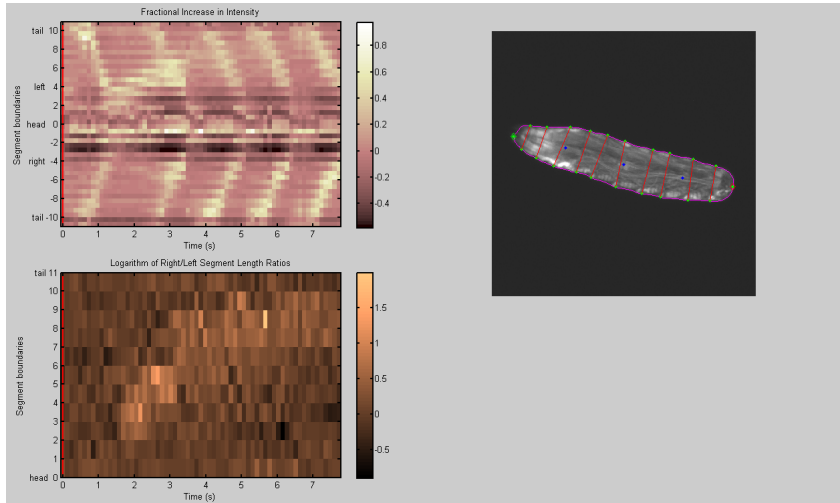
Results

Forward motion



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

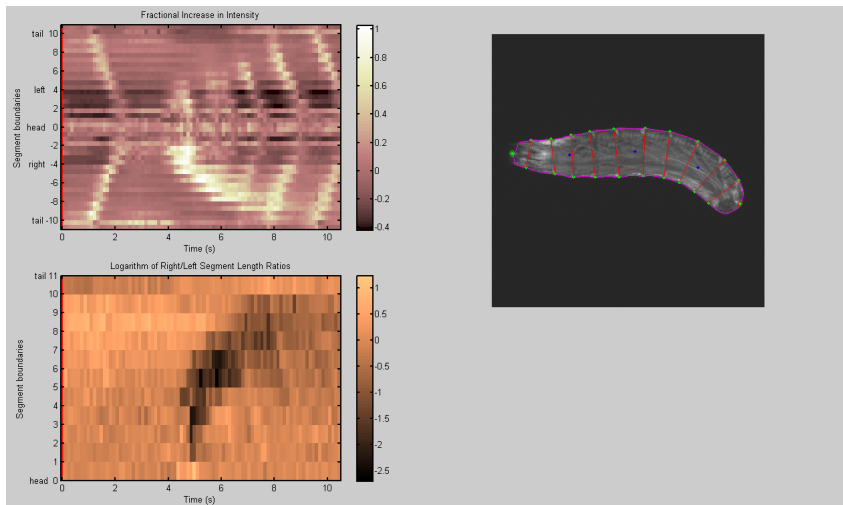
Results

Small accepted head-sweep

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

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

Large accepted head-sweep

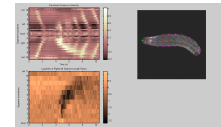


Basic pattern: Kink starts around (T3,A1,A2) and propagates back.
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Motor programs in Drosophila larvae
└ Results
└ Large accepted head-sweep

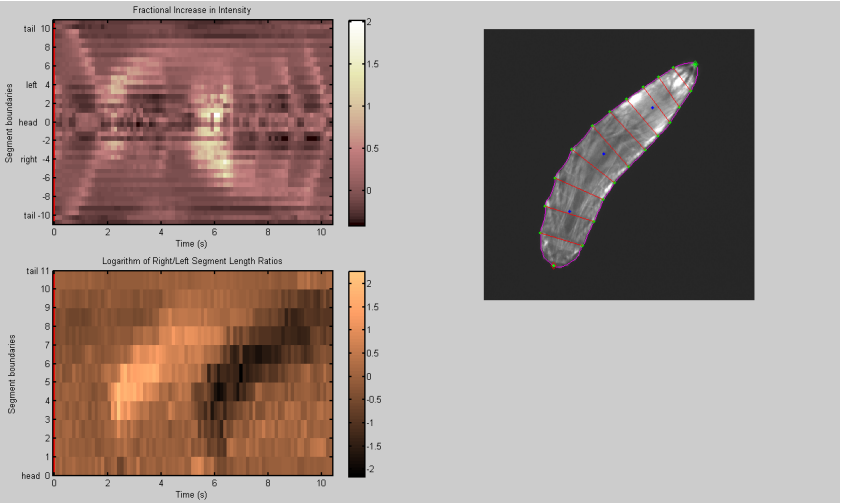
Large accepted head-sweep



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



Rejected head-sweep not undone until next one.

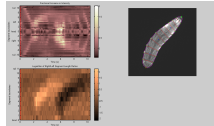
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Motor programs in Drosophila larvae

└ Results

└ Rejected head-sweep

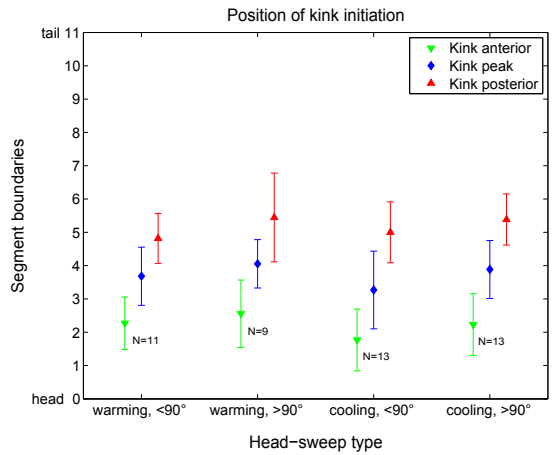
Rejected head-sweep



Rejected head-sweep not undone until next one.

1. no unbending program

Position of initial bend



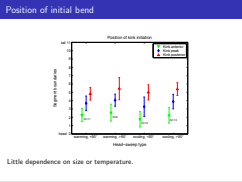
Little dependence on size or temperature.

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Motor programs in Drosophila larvae

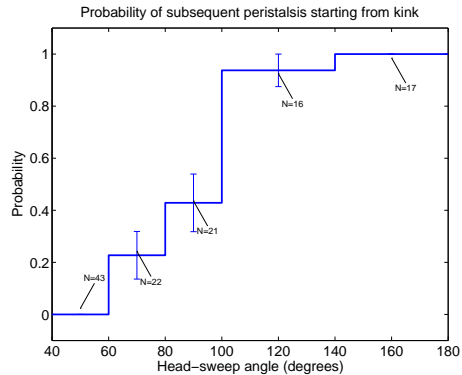
Results

Position of initial bend



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.

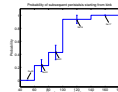
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Motor programs in Drosophila larvae

Results

Position of start of peristalsis

Position of start of peristalsis



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- 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

Results

Possible explanations

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

Conclusions and future directions

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

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

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

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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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Motor programs in Drosophila larvae

└─ Conclusions and future directions

└─ Future directions

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

Future directions

Interfere with motor patterns (optogenetically).

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Other stimuli.

Reverse crawling, hunching, and rolling.

Thanks to:

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

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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

1. Last slide!



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L. Luo, M. Gershow, M. Rosenzweig, K. Kang, C. Fang-Yen, P. A. Garrity, and A. D. Samuel.

“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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11



W. B. Grueber, L. Y. Jan, and Y. N. Jan.

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

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- Motor programs in *Drosophila* larvae
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11

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Motor programs in *Drosophila* larvae

Conclusions and future directions

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Toothpasting



back

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Motor programs in Drosophila larvae

└ Conclusions and future directions

└ Toothpasting

Toothpasting

