What needs to be done for 6 pages in Nature

[Asymmetric neurotransmitter release enables rapid odour lateralization in *Drosophila*. (2013)
Q Gaudry, EJ Hong, J Kain, BL de Bivort & RI Wilson]

Tea talk #VI
January 22, 2013

Step I: Intriguing question

- How to sense direction with bilateral neurons?

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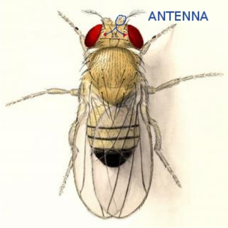

- Olfactory receptors send info bilaterally
- Downstream neurons receive info from both antennas

Step I: Intriguing question

How to de-entangle information from the two antennas?

- Other neurons responsible?
- One side Faster?
- One side Stronger?
- Any mechanism? (pre-, post-synaptic?)

Step II: Record behaviour

- Fly on a treadmill. Stabilised with wax.
- Movement recorded with an optical mouse.

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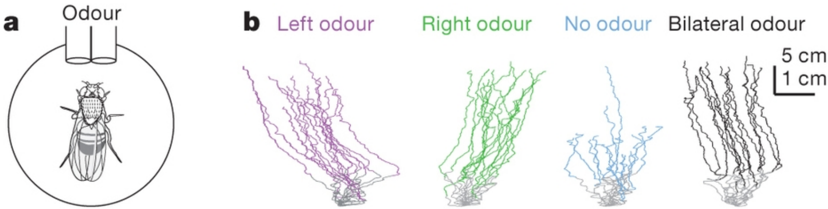
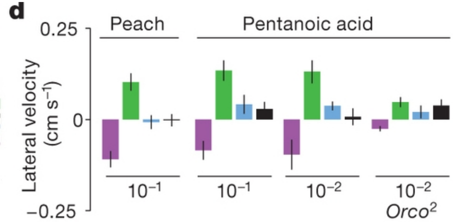


- Unilateral odour stimulation induces directed movement.

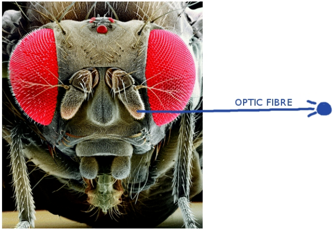
Step III: Behaviour+Genetics.



- Choose simple odour that evokes similar behaviour: pentanoic acid, specific to one receptor, with bilateral axons.
- Genetic silencing of this receptor decreases turning.
- Unilateral ORNs are intact.
- Bilateral communication plays role in directed movement.

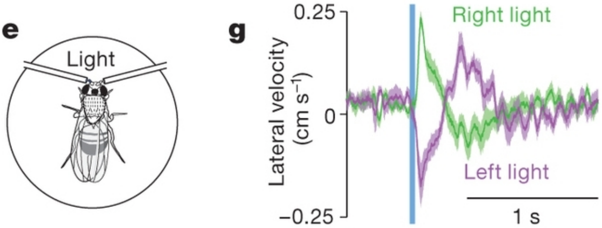
Step IV: Behaviour+Optogenetics.

- Replace odour with light.



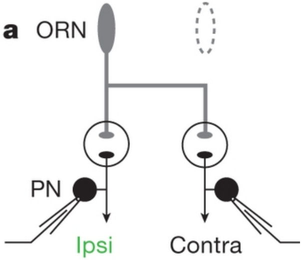
- Modify channelrhodopsin-2 in a bilateral receptor (Or42b).
- 50 ms light stimulation $\Rightarrow$ 3–4 spike burst.
- Light off $\Rightarrow$ decrease under baseline.

Step IV: Behaviour+Optogenetics.



- Light on — turn (70 ms latency).
- Light off — compensatory turn.
- Rapid (70 ms) extraction of lateral information.

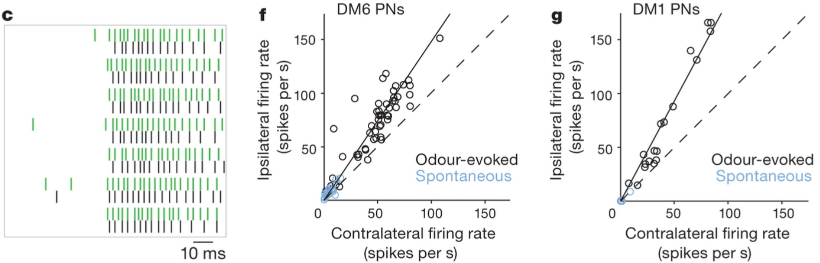
Step V: Electrophysiology. [How?]



- Simultaneous recording from sister neurons (GFP-targeted).
- One antenna removed.

Step V: Electrophysiology. Cell attached.

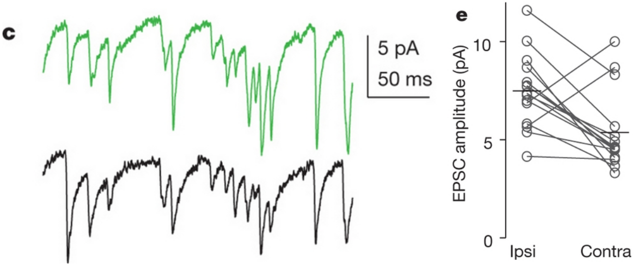
- Odour-evoked



- Ipsilateral spikes
  - Faster (but only 1–2 ms).
  - Stronger (50% higher firing rates).
- Inhibition (GABA) doesn't play a role.

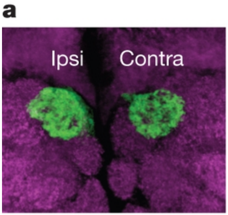
Step V: Electrophysiology. Whole-cell.

- Spontaneous (no odour)



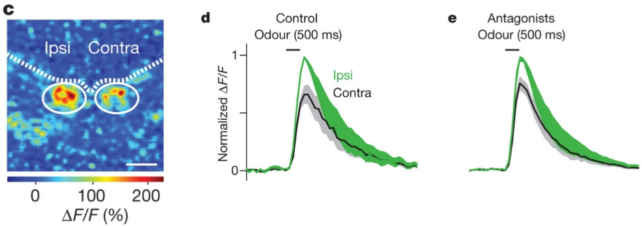
- Paired spontaneous events in sister neurons.
- Ipsilateral EPSCs
  - somewhat faster (0.806±0.51 ms).
  - stronger (40%).

Step VI: Structural imaging (GFP). [Where?]



- Synaptobrevin-GFP fluorescence was 40% higher on the ipsilateral side
- Assymetric number or size of neurotransmitter release sites

Step VII: Functional imaging (Ca^{++}, 2-photon).



- GCaMP3.0 in receptor neurons, 2-photon microscopy in principal neurons (DM6)
- Presynaptic calcium fluorescence was 40% higher on the ipsilateral side.
- No feedback required (tested acetlocholine, $GABA_A$ and $GABA_B$ influence).

Step VIII: Functional imaging (LFP).

- Bilateral field recordings — confirm presynaptic currents are larger ipsilaterally.
- Asymmetry in EPSC amplitudes has a presynaptic origin.
- Possibly: ipsilateral arbor is 40% larger than the contralateral arbor.

Wrap-up

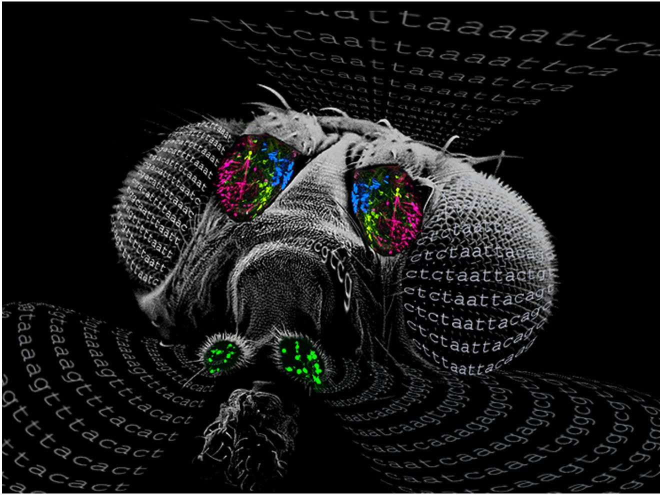
- How to sense direction with bilateral neurons?

- Other neurons responsible? Not only [Behaviour/genetics]
- Ipsilateral Faster? Yes, a little
- Ipsilateral Stronger? Yes, a lot
  [Optogenetics/Electrophysiology (GFP targeted, paired)]
- Any mechanism? Yes, presynaptic
  [Structural and functional imaging (GFP, Ca^{++}, LFP)]

Take-home message

- Some (experimentalists) have to work really hard for their 6 pages (in Nature)...

Thank you!



[Molecular zip codes for odor receptor gene choice in *Drosophila*.
Design: Anandasankar Ray and Woodstock Tom; SEM: Jennifer Perry]