Optogenetics

n. genetics  A science that combines optics and genetics to probe neural circuits  
(Wiktionary)
Early origins of optogenetics

Selective Photostimulation of Genetically ChARGed Neurons

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Neurotechnique

Expression of fly arrestin in vertebrate neurons

Remote Control of Behavior through Genetically Targeted Photostimulation of Neurons

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Purinoreceptor (must inject caged ATP)
Really early origins of optogenetics

Hypothesized evolution in the the Volvocales

- Chlamydomonas Unicellular
- Gonium Colonial multicellular
- Eudorina ECM expansion multicellular
- Pleodorina Cell type specification multicellular
- Volvox Differentiated tissues Germ/Soma multicellular

~200 Ma

Brad Olsen
Suddenly, neuroscientists are interested in algae

Chlamydomonas reinhardtii

Thank you, green C. reinhardtii

Nagel, Szellas, Huhn, Kateriya, Adesshvill, Berthold, Ollig, Hegemann, Bamberg (2003) PNAS
(ChR2 is directly light-gated, depolarizes cells when expressed in cultured cells)

(ChR2 can be expressed in vertebrate neurons and can control activity on ms time scales)

(ChR2 can be used to remotely activate neurons in freely behaving nematodes)
What do you need to do basic optogenetic experiments in flies?

Transgenic flies (freely available)  
Inexpensive blue light source

Hornstein et al., 2009, *J. Vis. Exp.*

***Expensive***
Implementation in teaching labs

Prepare LEDs, larvae with ChR2 in defined populations of neurons

Students activate neuronal populations with blue light

Observe, quantify behavioral responses, compare to controls

Students activate neuronal populations with blue light

Observe, quantify responses in semi-intact preparations

Students activate neuronal populations with blue light

Measure neural basis of ChR2 behavior responses (synaptic potentials at the neuromuscular junction)
What will students see when all motor neurons are stimulated at once?
Light-evoked synaptic potentials at the larval neuromuscular junction

***Nuts and bolts: before poking anything, check for visible contractions in response to light light
Pickpocket-GAL4 expresses in larval nociceptive neurons
PPK neurons are necessary and sufficient for evoking a larval ‘nocifensive’ behavior

Movies in

Hwang et al., 2007, *Curr. Biol.*
Stimulation of sensory neurons in teaching labs

ChR2 expressed in ‘nociceptive’ neurons
Student-initiated analysis of behavior

<table>
<thead>
<tr>
<th></th>
<th>UAS control</th>
<th>All sensory</th>
<th>Nociceptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Ambient light</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Blue light</td>
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</tbody>
</table>

# forward peristaltic waves / s

- Ambient light
- Blue light

* One way ANOVA, Tukey-Kramer post-hoc test

- * indicates significant difference
- n.s. indicates no significant difference
Student-initiated analysis of behavior

All genotypes under white light & wild type under blue light

All sensory under blue light

Nociceptive under blue light

*Size of boxes represents proportion of time spent in given behavior. Thickness of arrows indicates relative probabilities of transitions from one behavior to another. Analysis by S. Albers and Z. Lorsch.
Activation of sensory neurons triggers EJPs at NMJ

***Specific mission: compare delay to first EJP in motor neuron vs sensory***
Rhythmic bursts of EJPs with constant stimulation

![Diagram of a brain with segmental nerves and blue light illumination, indicating activation of nociceptive neurons and measurement of voltage in muscle m6.]

<table>
<thead>
<tr>
<th>Condition</th>
<th>cycle period [s]</th>
<th>inst burstfreq [Hz]</th>
<th>burst duration [s]</th>
<th>dutycycle</th>
<th>interburst interval [s]</th>
<th>#spikes in burst</th>
<th>spikefreq in burst [Hz]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient light</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60 sec blue light</td>
<td>1.7 ± 0.5</td>
<td>0.58 ± 0.1</td>
<td>1.12 ± 0.4</td>
<td>0.49 ± 0.1</td>
<td>1.08 ± 0.2</td>
<td>39.4 ± 15.4</td>
<td>35.1 ± 4.7</td>
</tr>
</tbody>
</table>
Students: Are these exercises actually effective?

[Bar charts showing student responses to exercises.]

- This exercise helped me understand how sensory input can reconfigure the output of central pattern generators.
- This exercise did NOT increase my interest in studying neurophysiology and animal behavior.
- I was motivated by the fact that the techniques used here have not been extensively used in research articles.

- It was helpful to start the lab by looking at behavioral responses in Channelrhodopsin-2 expressing animals.
- I was able to see clear, reproducible behavior responses when all peripheral sensory cells were stimulated.

[Charts illustrating student responses with percentages.]

- Percent responding:
  - Strongly agree
  - Agree
  - Neutral
  - Disagree
  - Strongly disagree
Educators: Are these exercises actually effective?
DISCUSSION POINTS

• What is it about this technology that is so helpful?

• When isn’t it helpful? What are the limitations?

• Who made this discovery?

• Why do we have this new technology?