Manipulating neurons with light

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Why bother with light, isn’t pharmacology good enough?

Light gives us temporal precision!
Optogenetics
Uncaging
Channelrhodopsin basics

- Blue light (470 nm) activates Channelrhodopsin.
- Channelrhodopsin has all-trans retinal bound to it.
- The activation leads to the transport of Na+ ions from inside the cell to outside the cell.
- eYFP is also present in the cell, likely as a reporter protein.
Advanced opsins

Increased axonal targeting
Increased soma targeting
Increased speed (Cheeta) = better temporal precision
Red shifted opsins (C1V1, Crimson) -> dual color optogenetics
Step function opsins
Optically driven GPCRs

Still incomplete list:
https://web.stanford.edu/group/dlab/optogenetics/sequence_info.html
Not only for neuroscience: optically driven kinases, phosphatases etc.

Detection and manipulation of phosphoinositides
Idevall-Hagren, 2015
Optogenetic inhibitions

Step-Waveform Inhibitory ChannelRhodopsin (SwiChR)
How to target expression: stereotaxic virus injections
Where to inject?
Cell type specific expression: The Cre – loxP system

Cre (Causes recombination)

LoxP (locus of X(cross)-over in P1)
How to control gene expression with Cre-loxP?
Ex vivo optogenetics

You can cut off the cell body, still get responses
-> test inputs from far away brain regions
Comparing inputs from different regions

Lur G. unpublished
In vivo optogenetics - manipulating behavior

SFO neurons control thirst

CaMK2

Vgat

**a**

Trials

- light

+ light

Time (sec)

0

10

20

30

40

licking

+ light

**b**

light

- +

- +

- +

- +

- +

- +

- +

- +

**c**

Trials

1

2

3

4

5

6

7

8

9

10

11

12

**d**

Drinking response (%)

- light

+ light

ChR2

GFP

**e**

Water intake (ml)

water restricted

water satiated

water satiated

+ light

**Slc32a1 (Vgat)-Cre**

Control
Uncaging – basic principles

Goal: spatiotemporally precise neurotransmitter release
Mostly in vitro (but there are exceptions)

Caged compounds:
Glutamate
GABA
IP3
Ca2+
Neuromodulators
Nucleotides like ATP
mRNA & DNA
proteins
Local circuit mapping using one-photon glutamate uncaging
Supra-linear dendritic integration – two-photon glutamate uncaging

Look for work by Jeff Magee and Michael Hausser
Spatial mapping of cellular receptor composition

Lur G. unpublished
Specific control of postsynaptic glutamate receptors

-60 mV

$\Delta Ca^{2+}_{\text{NMDAR}}$

$u\text{EPSC}_{\text{AMPAR}}$

- Control
- $\alpha_1$ agonist
- $\alpha_2$ agonist

5 pA
10 ms

0.2 $\Delta G/G_{\text{sat}}$
100 ms

$u\text{EPSC}_{\text{AMPAR}}$ (pA)

C $\alpha_1$ $\alpha_2$

$\Delta Ca^{2+}_{\text{NMDAR}}$ ($\Delta G/G_{\text{sat}}$)

C $\alpha_1$ $\alpha_2$

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