Tools for Mapping the Circuits of Intelligence

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Understanding the brain is a problem of fundamental difficulty, but also of scale
Organized at nanoscale, but spanning centimeters ($10^7$ range)
Computing with millisecond timescale events, but spanning years ($10^{12}$ range)
Principles of neuroengineering design:

Work **backwards** from properties of the brain,

Survey the entire scope of **engineering possibility,**

**Invent** technologies for analyzing and engineering the brain

- **Map**
- **Record**
- **Control**
- **Build**
How does a single neuron compute?

http://www.nature.com/nature/journal/v451/n7174/full/nature06447.html
Whole cell patch clamp: enables simultaneous measurement of electrophysiology, morphology, and gene expression in single cells in living brain.

A robot that can automatically patch clamp neurons in living brain

Commercialized by Neuromatic Devices, Inc. (ESB has no financial affiliation)
Robotic quad patching in living mouse brain
How do neurons work together in entire circuits?
“There are 302 neurons in the nervous system of C. elegans; this number is invariant between animals. Each neuron has a unique combination of properties, such as morphology, connectivity and position, so that every neuron may be given a unique label. Groups of neurons that differ from each other only in position have been assigned to classes. There are 118 classes that have been made using these criteria, the class sizes ranging from 1 to 13. Thus C. elegans has a rich variety of neuron types in spite of having only a small total complement of neurons. This is in marked contrast to structures such as the mammalian cerebellum, which contains more than $10^{10}$ neurons (Braitenberg & Atwood 1958) and yet has only five classes of component neuron (Eccles et al. 1967).”

C. elegans

http://www.wormatlas.org/MoW_built0.92/nervous_system.html
Simultaneous, whole-animal, 3-D microscopy: light-field imaging

Simultaneous, whole-animal, 3-D imaging of neural activity (at 5-50 Hz)

Imaging neural activity throughout organism with known connectome

Imaging zebrafish neural activity in 3-D (at 20 Hz, below)

The world’s smallest mammal: towards whole-organism functional imaging

Michael Brecht, Ian Wickersham, Susan Erdman

Can we understand causally how neurons function in circuits?


Three major optogenetic molecule classes: microbial opsins, seven-transmembrane proteins, binding endogenous all-trans-retinal

Archaerhodopsins and bacteriorhodopsins (e.g., Arch, Mac, BR)

Halorhodopsins (e.g., Halo/NpHR)

Channelrhodopsins (e.g., ChR2)

100% neural silencing in cortical neurons of awake mice mediated by Arch

Halo-expressing neuron in vitro, quieted by yellow light

two different ChR2-expressing neurons in vitro, responding to the same train of blue light pulses

Boyden (2011) Faculty of 1000 Biology Reports 3:11.
Targeting different neurons of the brain in genetic model organisms, and beyond

**Lentiviruses and adeno-associated viruses**

Have intrinsic tropism for certain cell types (e.g., lenti – excitatory neurons of the cortex)

Can tune the promoter: synapsin pan neuronal, CaMKII excitatory, TH/dopamine, GAD, SOM, CCK, ...

AAV serotypes – AAV8, 5, 2, 9, ...

Lots of Cre recombinase expressing mice (e.g., dopamine, serotonin, parvalbumin, etc.)

Administer a floxed and reversed opsin AAV into such a mouse, and the opsin will be flipped around into the correct direction

Takes 2-3 weeks to express after injection; electroporation and other methods may be of use
Rules of thumb for blue/green/yellow light

200 mW/mm² is good **irradiance** to shoot for (higher okay for brief neural activations; lower should be considered for long duration silencings; molecules are sensitive to 0.1-10 mW/mm²)

50 micron **fibers** = can easily go into tetrode drives
100-200 micron fibers = stiff enough to go into brain
400-800 micron fibers = for specialized uses

200 micron fiber, 200 mW/mm² affects ~1 mm³ of tissue

**Close** to fiber tip: light goes forward
Beyond a **scattering** length (~50-100 microns), starts to look spherical (power falls off as $1/r^2$)
Beyond the **absorbance** length (~500-1000 microns), falls off exponentially
Transgenic mice expressing original-N. pharaonis halorhodopsin, tagged with GFP, in hypocretin neurons

Light silences the neurons, resulting in slow-wave sleep

Search locally in genomic space:
ArchT, higher light sensitivity relative of Arch


Search broadly in genomic space:
Mac, blueshifted relative to all other silencers

Noninvasive optogenetic neural silencing: Jaws

Chuong et al. (2014) Nature Neuroscience, accepted.
DAT-Cre + AAV-FLEX-ChR2-tdTomato

Finding circuits in the brain that can mediate reward

Dopamine neurons: implicated in reward and addiction, but largely through pharmacological and electrical means

Is a brief activation of them sufficient to drive reward?

Optogenetic activation of glia can change neural codes

Chronos and Chrimson together

Accessory strategies for a diversity of systems

**Wireless, multisite optogenetics**


**3-D optogenetic control**


**Opto-fMRI**


**Transgenic mice**

Primate optogenetics

Tools distributed to >1000 labs worldwide
- Addgene, DNA
- UNC (Lori Nisi), viruses
- Allen Institute (Hongkui Zeng), floxed-stop transgenics
- Host visitors (1-2x/week) to teach procedures
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Funding
Allen Institute for Brain Science; AT&T; Bahaa Hariri; Benesse Corporation; Jerry and Marge Burnett; DARPA Living Foundries Program HR0011-12-C-0068; DARPA HR0011-11-14-0004; Department of Defense CDMRP PTSD Program; Google; Harvard/MIT Joint Grants Program in Basic Neuroscience; Human Frontiers Science Program; IET A. F. Harvey Prize; Joyce and Jeremy Wertheimer; Lincoln Labs Campus Collaboration Award; MIT Alumni Class Funds; MIT Intelligence Initiative; MIT McGovern Institute and McGovern Institute Neurotechnology (MINT) Program; MIT Media Lab and Media Lab Consortia; MIT Mind-Machine Project; MIT Neurotechnology Fund (& its generous donors); NARSAD; New York Stem Cell Foundation-Robertson Investigator Award; NIH Director’s Pioneer Award 1DP1NS087724 and New Innovator Award 1DP2OD02002, NIH EUREKA Awards 1R01NS087950 and 1R01NS075421, NIH Transformative Awards 1R01MH103910 and 1R01GM104948, NIH Single Cell Grant 1R01EY023173, and NIH Grants 1R01DA029639, 1R43NS070453, 1RC2DE020919, 1RC1MH088182, 2R44NS070453, and 1R01NS067199; NSF INSPIRE Award CBET 1344219, NSF CAREER Award CBET 1053233 and NSF Grants, EFR0835878, DMS0848804, and DMS1042134 (the Cognitive Rhythms Collaborative); Office of the Assistant Secretary of Defense for Research and Engineering; Paul Allen Distinguished Investigator in Neuroscience Award; Skolkovo Institute of Science and Technology; Alfred P. Sloan Foundation; Society for Neuroscience Research Award for Innovation in Neuroscience (RAIN); Stacy and Joel Hock; Synthetic Intelligence Project (& its generous donors); Wallace H. Coulter Foundation. Pre-MIT: Hertz Foundation, Helen Hay Whitney Foundation

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Neural recording: Leaflabs, Konrad Kording, George Church
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In vivo Robotics: Craig Forest
Transgenics: Hongkui Zeng

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