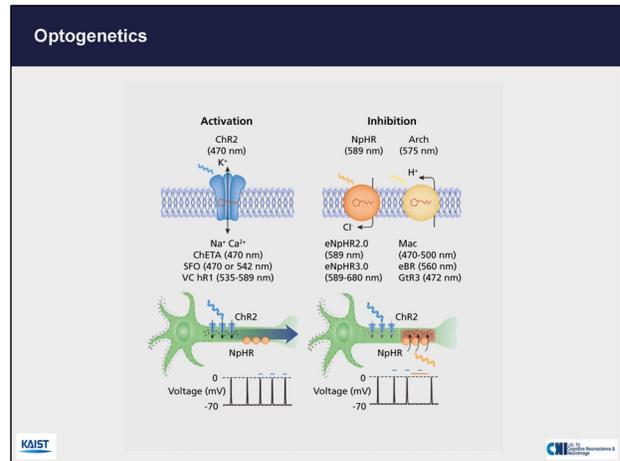
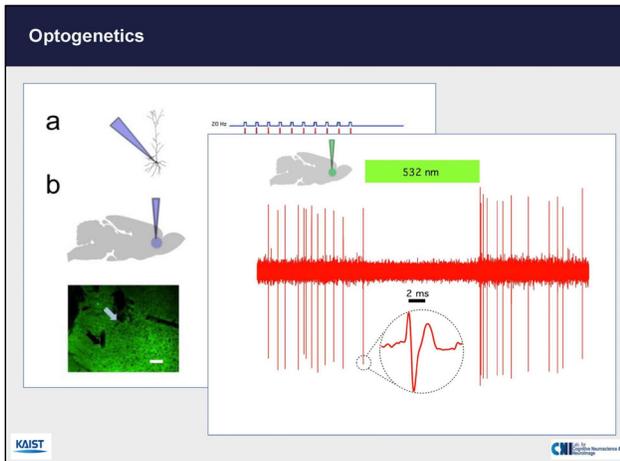


Optogenetics: Control the Brain with Light



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Neuron or Brain area Stimulation/Inhibition

- To see the causality (of certain behavior or cognitive function)
- To modulate (certain brain region or circuit)

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Stimulation methods

- Electrical stimulation : DBS, tCS, TMS
- Optical stimulation
- Chemical stimulation
- Physical, Magnetic, Thermal stimulation : Focused Ultrasound (FUS), magnetogenetics, nanomaterial-based

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Optogenetics의 장점

- Spatial resolution
 - Specific area stimulation
 - Cell specific stimulation
 - Circuit specific stimulation (Circuit dissection)
- High temporal resolution
 - Temporal pattern
- Excitation and Inhibition

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Circuit dissection

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Designing optogenetic experiments to study brain disease

Nature Reviews | Neuroscience

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Designing optogenetic experiments to study brain disease

- **Select the opsin best suited to the experimental goals.** There are trade-offs for different aspects of performance, such as peak photocurrent, kinetics, activation wavelength spectrum and light sensitivity.
- **Select targeting strategy or vector to express opsin in target cells.** Many kinds of viral transduction can restrict cell-body expression of opsin to the injection site, and can be used in wild-type animals or Cre recombinase lines. Some transgenic lines constitutively express opsin.
- **Select light delivery method.** Fiber optics are the most common method of light delivery to deep cell bodies or axon terminals. The numerical aperture, diameter and mode of the fiber will influence the spread of light. There are trade-offs for using acute or chronically implantable fibers, as acute fibers allow for pharmacological manipulations but are delicate and easy to break.
- **Choose appropriate temporal parameters.** Duty cycle, pulse duration, frequency and epoch pattern are the key light-delivery parameters to select. Depending on the behavioral assay, exploring within-session light manipulations can maximize the utility of optogenetic tools.
- **Validate the experimental manipulation.** To verify that the opsin, targeting strategy and illumination parameters are manipulating cells in the intended manner, confirmation using electrophysiology, immunohistochemistry or other measures is crucial for data interpretation.

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Overall Procedure

1. Virus injection
2. Fiber 제작
3. Fiber 삽입 수술
4. Recovery 후 원하는 행동실험

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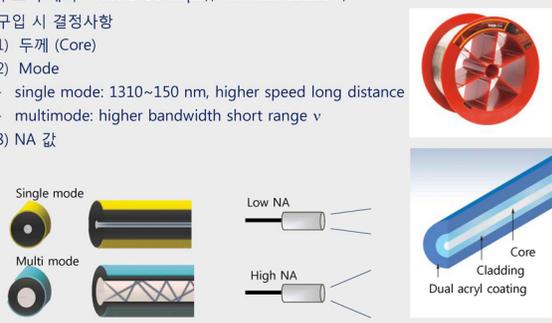
Virus

- 주요 구매처;
 - Penn vector core: <https://www.med.upenn.edu/gtp/vectorcore/>
 - UNC vector core: <https://www.med.unc.edu/genetherapy/vectorcore>
- 직접 제작해서 사용 가능
- 구입 시 결정사항
 - 1) Excitation/Inhibition
 - 2) Virus type
 - 3) Cell type
 - 4) Cre inducible
 - 5) Fluorescent color

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Optical fiber

- 주요 구매처: THORLABS <https://www.thorlabs.com/>
- 구입 시 결정사항
 - 1) 두께 (Core)
 - 2) Mode
 - single mode: 1310~150 nm, higher speed long distance
 - multimode: higher bandwidth short range v
 - 3) NA 값



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Fiber optic ferrule

- 주요 구매처: THORLABS, Doric Lenses, Precision Fiber Products (PFP)
- 구입 시 결정사항
 - 1) Stainless steel/Ceramic
 - 2) Bore size
 - 3) 이후 제작 과정 거칠 것인지/가공된 것을 살 것인지
- Mating sleeve 구매



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Procedure

1. Fiber의 coating 벗기기
 - 다양한 종류의 stripping tool
 - 벗기고자 하는 두께를 확인하고 고르기
2. 다이아몬드 칼로 fiber의 양 끝을 평평하게 자르기
3. Polishing puck과 polishing sheet를 사용하여 더욱 정밀 가공



* 자세한 제작 방법은 jove동영상 참고
<http://www.jove.com/video/50004/fiber-optic-implantation-for-chronic-optogenetic-stimulation-brain>

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Cannula

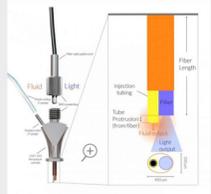
Fiber-optic cannulas



Opto-electric Cannulas



Mono opto-fluid cannulas



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Optical patch cord (cable)

- 구입 시 결정사항
 - 1) 두께 (Core)
 - 2) Mode (single mode/multimode)
 - 3) NA 값
 - 4) 말단의 형태 (FC/PC, SMA, FC/PC to ferrule)
 - 5) Rotary joint 구매 (행동실험)



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Optical patch cord (cable)

- Patch cord 또한 제작 가능
- Patch cord가 많이 필요한 경우라면 제작하는 것이 비용 절감
- 필요한 물품
 - 1) Optical fiber
 - 2) Furcation tubing
 - 3) Polishing puck, polishing sheet
 - 4) FC/PC or SMA end

* 자세한 제작 방법은 jove동영상 참고
<http://www.jove.com/video/50004/fiber-optic-implantation-for-chronic-optogenetic-stimulation-brain>

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Patch cord

Branching Fiber-optic Patch Cords



Dual Fiber-optic Patch Cords



Mono Opto-electric Patch Cords



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Light source

Laser



LED



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Virus Injection

Syringe pump



Syringe



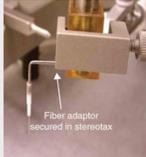
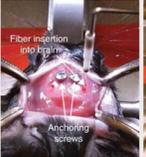



- Stereotaxic frame를 이용해 mouse brain 원하는 부위 타겟
- Virus 양과 속도 결정
- 통합해서 3주 이상 expression 기간 필요

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Optical fiber implantation

- Virus 발현 후 구입하거나 만든 fiber 삽입
- 삽입 후 dental cement로 고정
- 일주일 정도 recovery 후 원하는 행동실험



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Behavior에의 응용

Motor/Sensory behavior



Memory, Locomotion



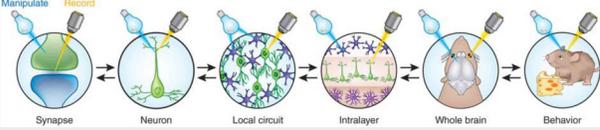
Emotion



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Courses

- Stanford Optgenetic Course
 - <https://web.stanford.edu/group/dlab/optogenetics/oil.html>
 - 3 days or 3 weeks
- Institute Curie
 - <http://enseignement.curie.fr/en/content/optogenetics-course>



Manipulate Record

Synapse Neuron Local circuit Intralayer Whole brain Behavior

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