

Prerequisites to Tackling Disorders via Modulating Peripheral Nervous System

1. wiring diagrams(at target organs)
2. genetic makeup of innervated cell types
3. functional maps for nerves
4. interventional strategies informed by 1-3

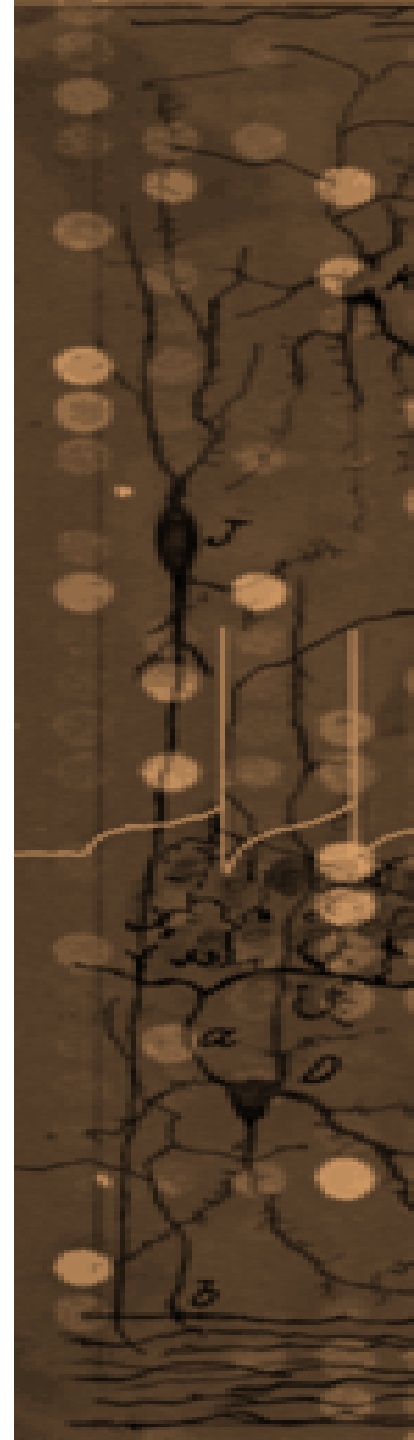
neural circuits correlates
of **function** and **dysfunction**

enabling technologies:

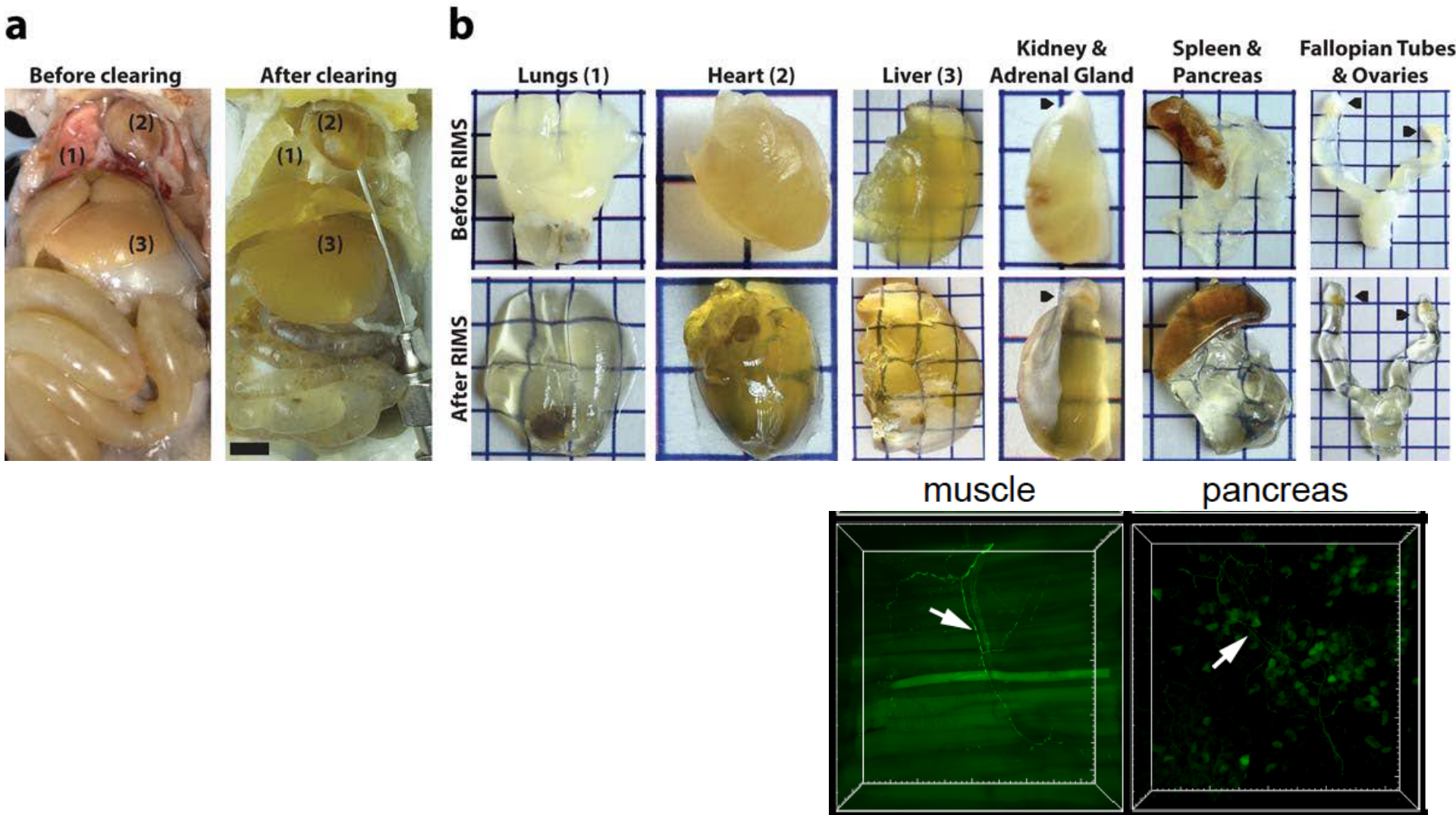
Tissue Clearing for anatomical / phenotypic mapping

Optogenetics for functional mapping

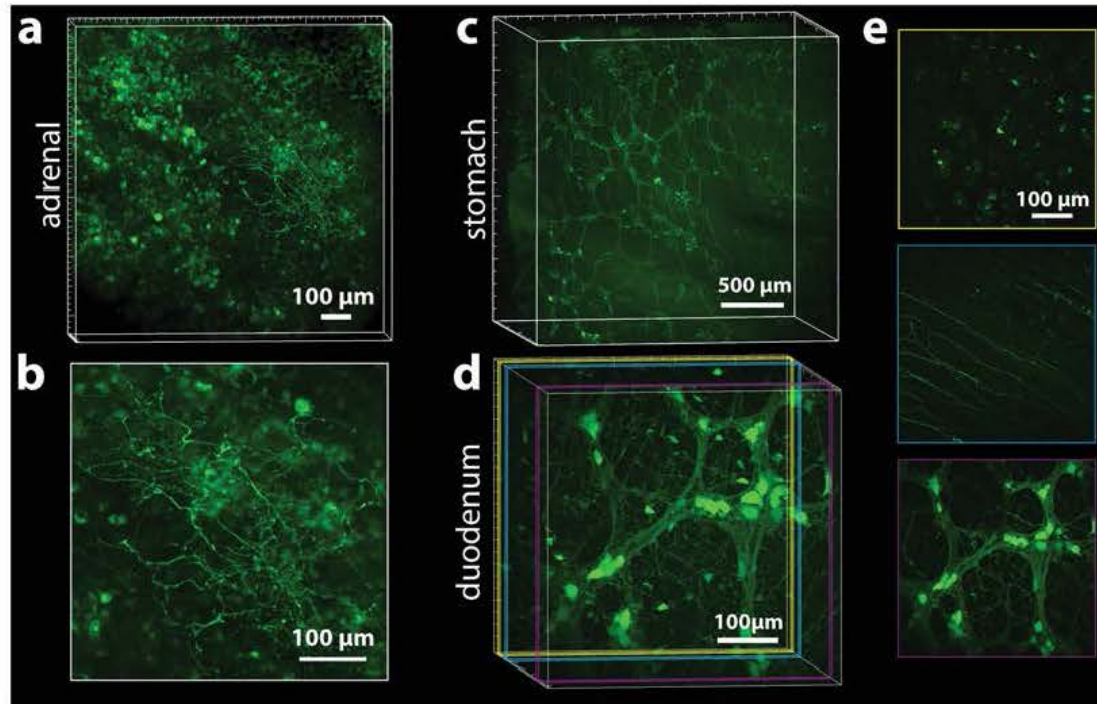
Viral vectors for nerve-type specific targeting



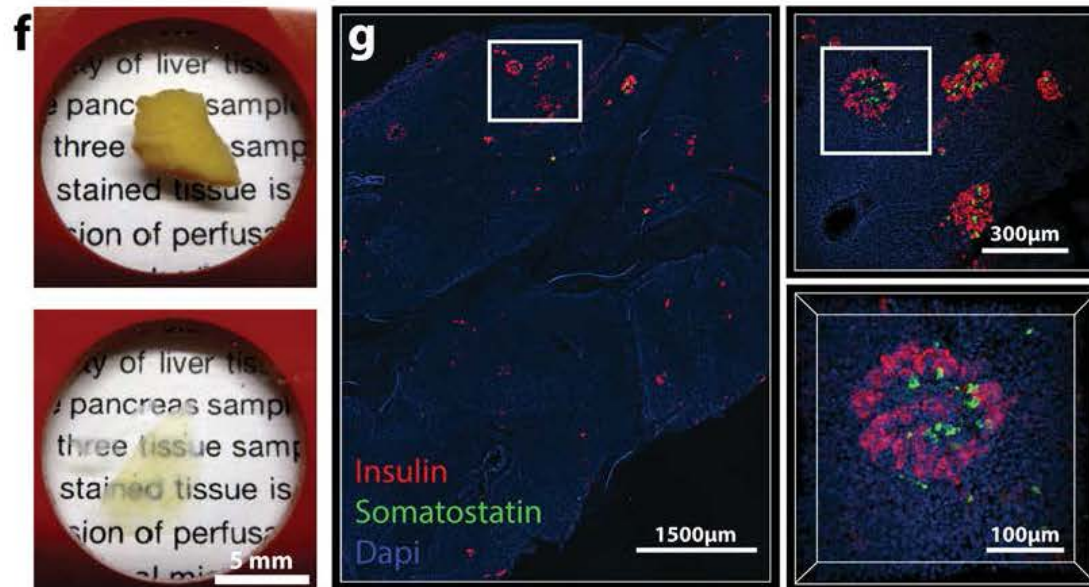
Phenotypic Mapping of Nerves and Organs with Cleared Tissues



PARS cleared mouse tissues - endogenous GFP fluorescence



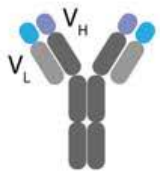
PACT cleared human pancreas - immunostaining



Need: smaller immunolabels for thick tissues

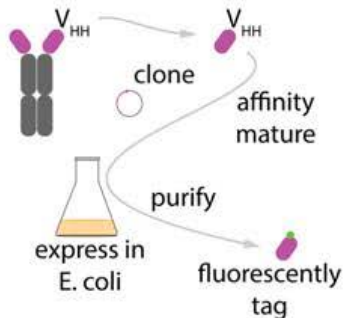
a

conventional antibody



camelid

nanobody



Pros

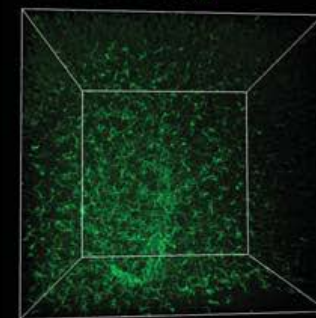
- penetrates well
- produce large quantities cheaply
- highly stable

Cons

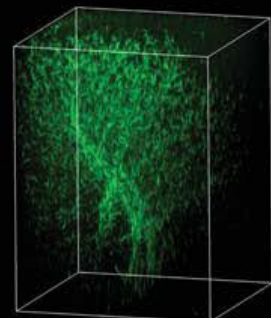
- current selection limited
- expensive to develop

b

GFAP nanobody



top view

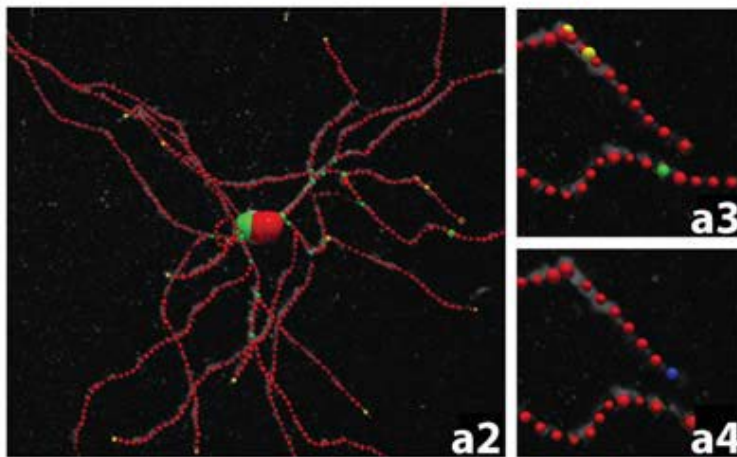
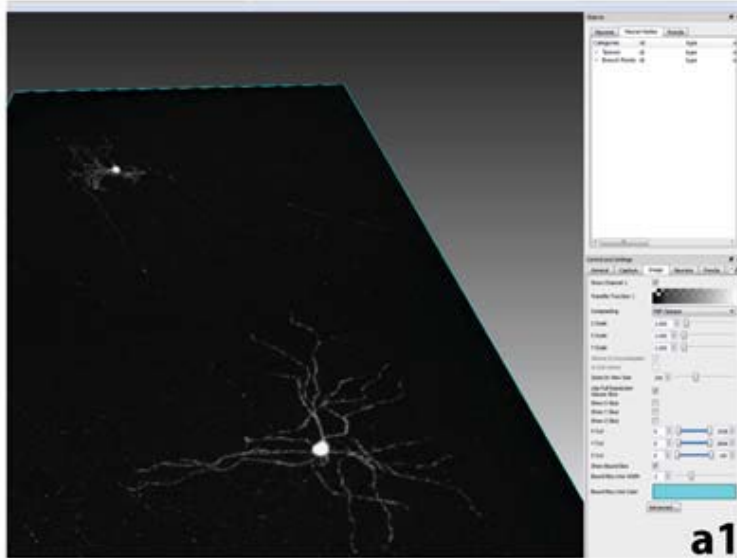


side view
850 micron depth

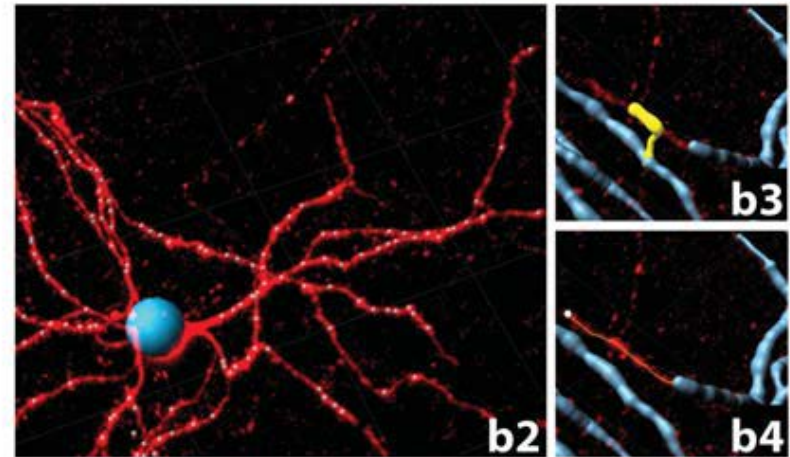
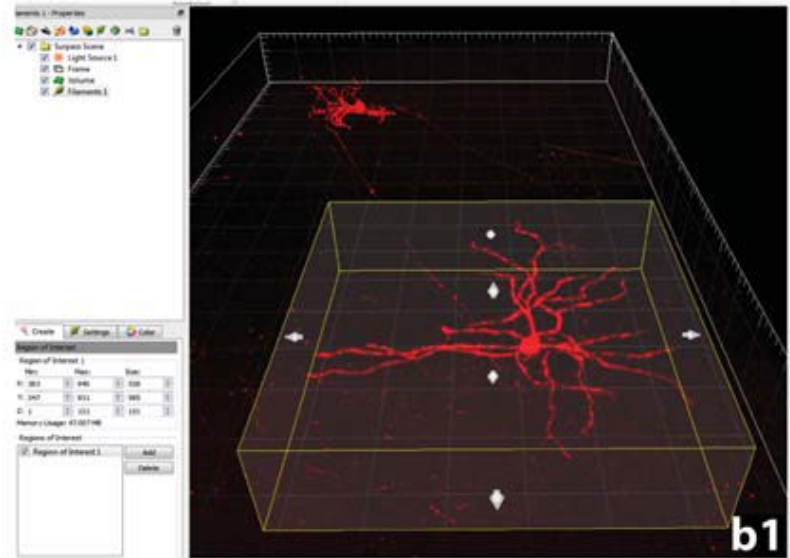
200 μm

Need: Tract Tracing / Data Analysis

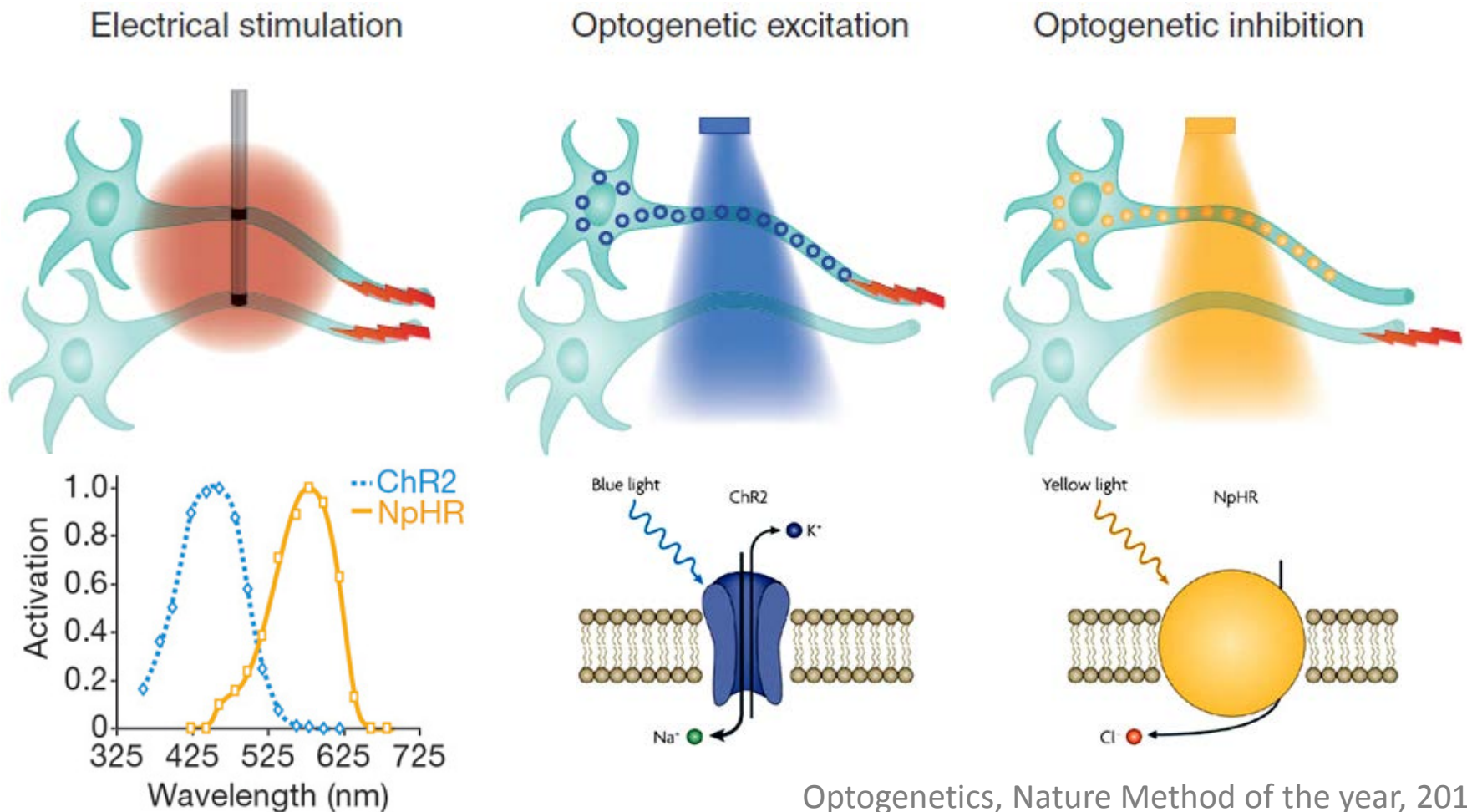
a



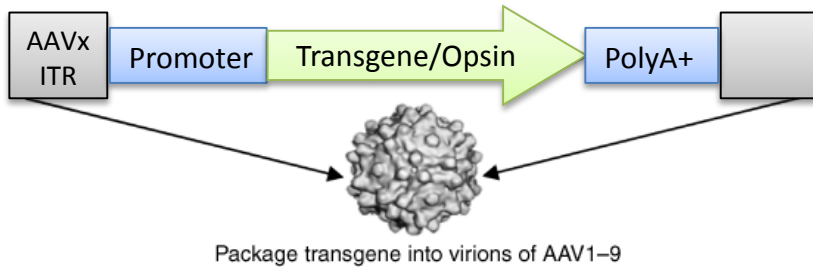
b



Functional Mapping with Optogenetics for cell-type specific control of activity

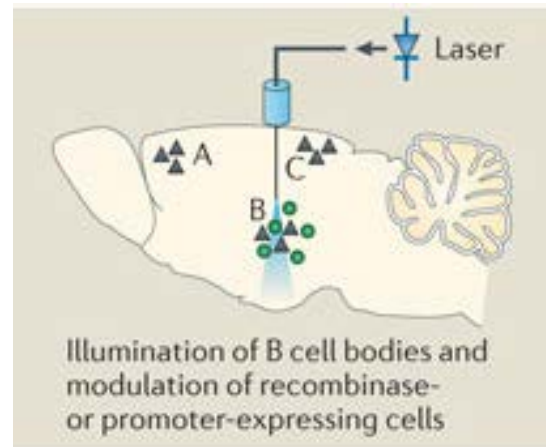
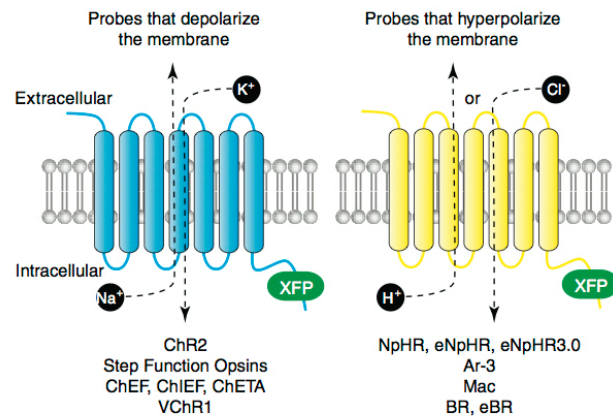
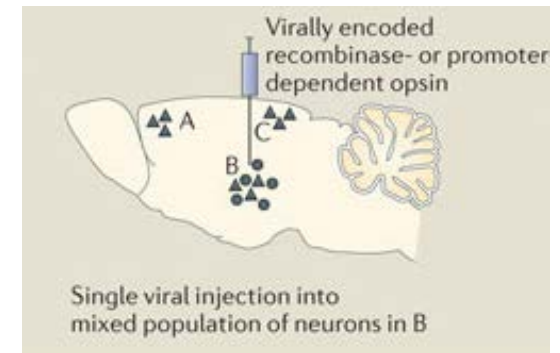


“Intersectional” strategies for circuit-specific control

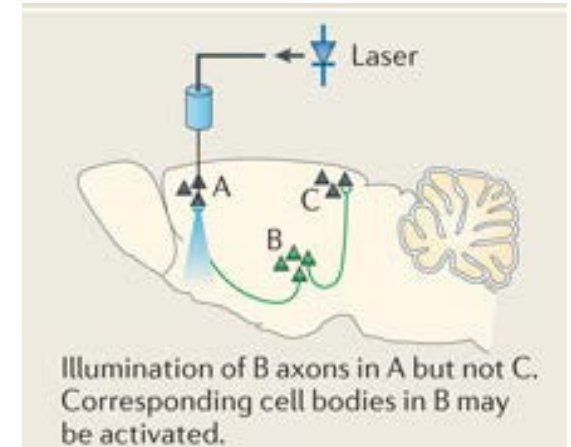


Cell-type specificity

- Promoter based
- Cre recombinase (dual vector delivery)



Cell body illumination



Axon terminal illumination

State-of-the-art in Optogenetics

Stimulation in Periphery

Excitation of Motor Neurons

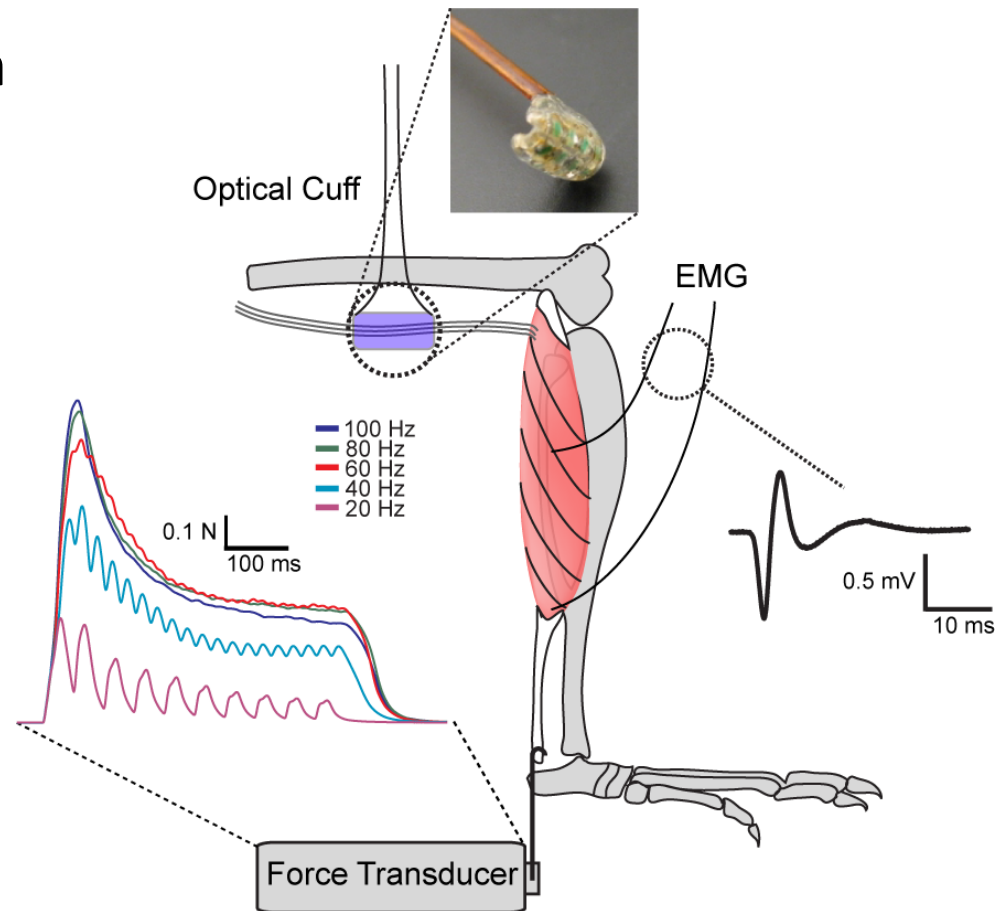
- Striated Muscle Contraction

- Thy 1::ChR2 mouse
- Optical control of sciatic nerve
 - Medial gastrocnemius muscle
 - Achilles tendon (force)

- Physiologic Activation

Fibers: orderly recruitment

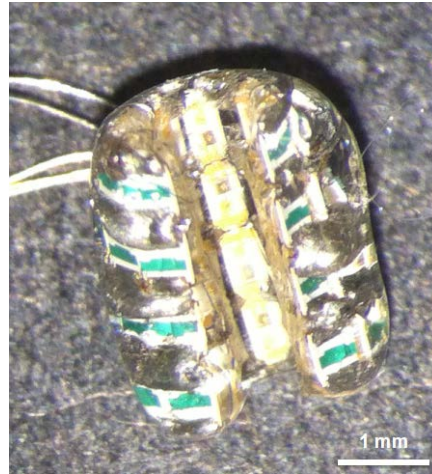
- Small→Medium→Large
- Force is “tunable”



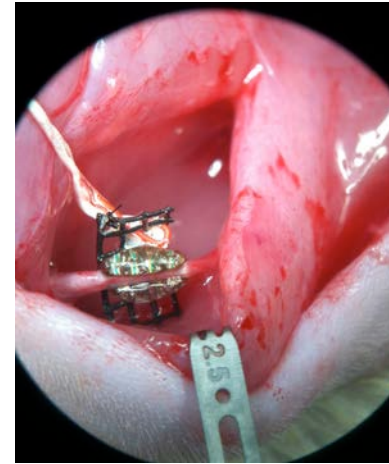
Optogenetic Hardware for Peripheral Nerve Stimulation

28 microLEDs attached to outside of glass capillary pipette

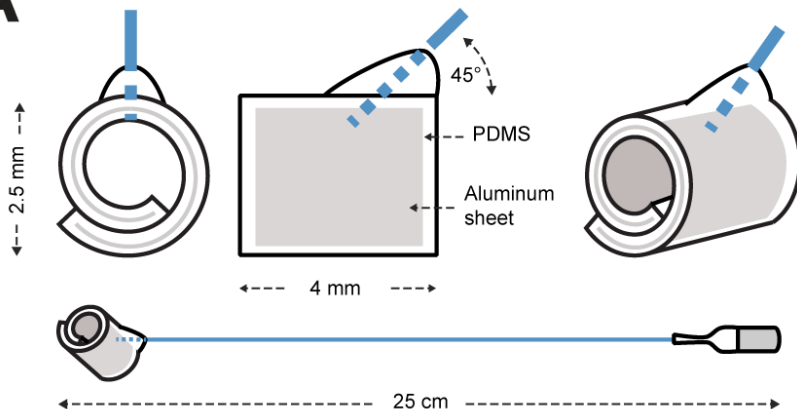
Soldered and insulated



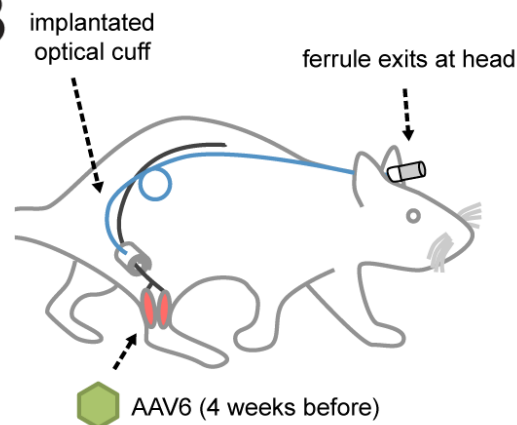
LED cuff around rat sciatic nerve



A



B



Towne et al.,
PLOS One, 2013

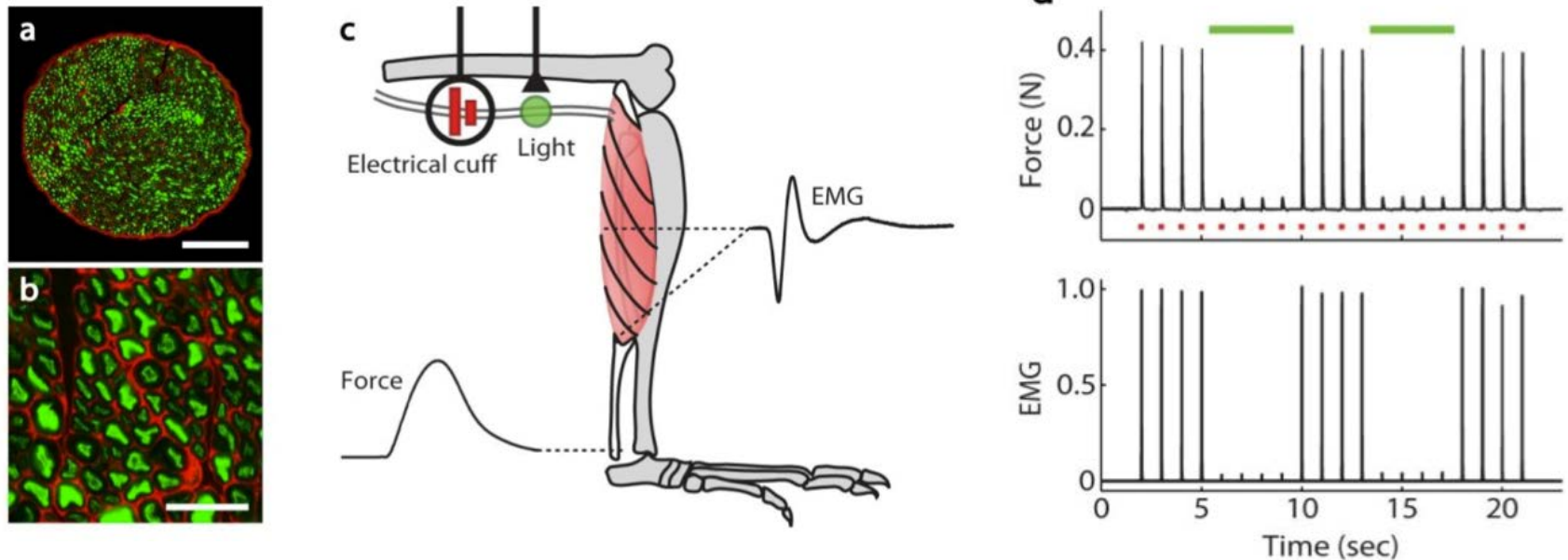
State-of-the-art in Optogenetics

Stimulation in Periphery

Inhibition of Motor Neurons

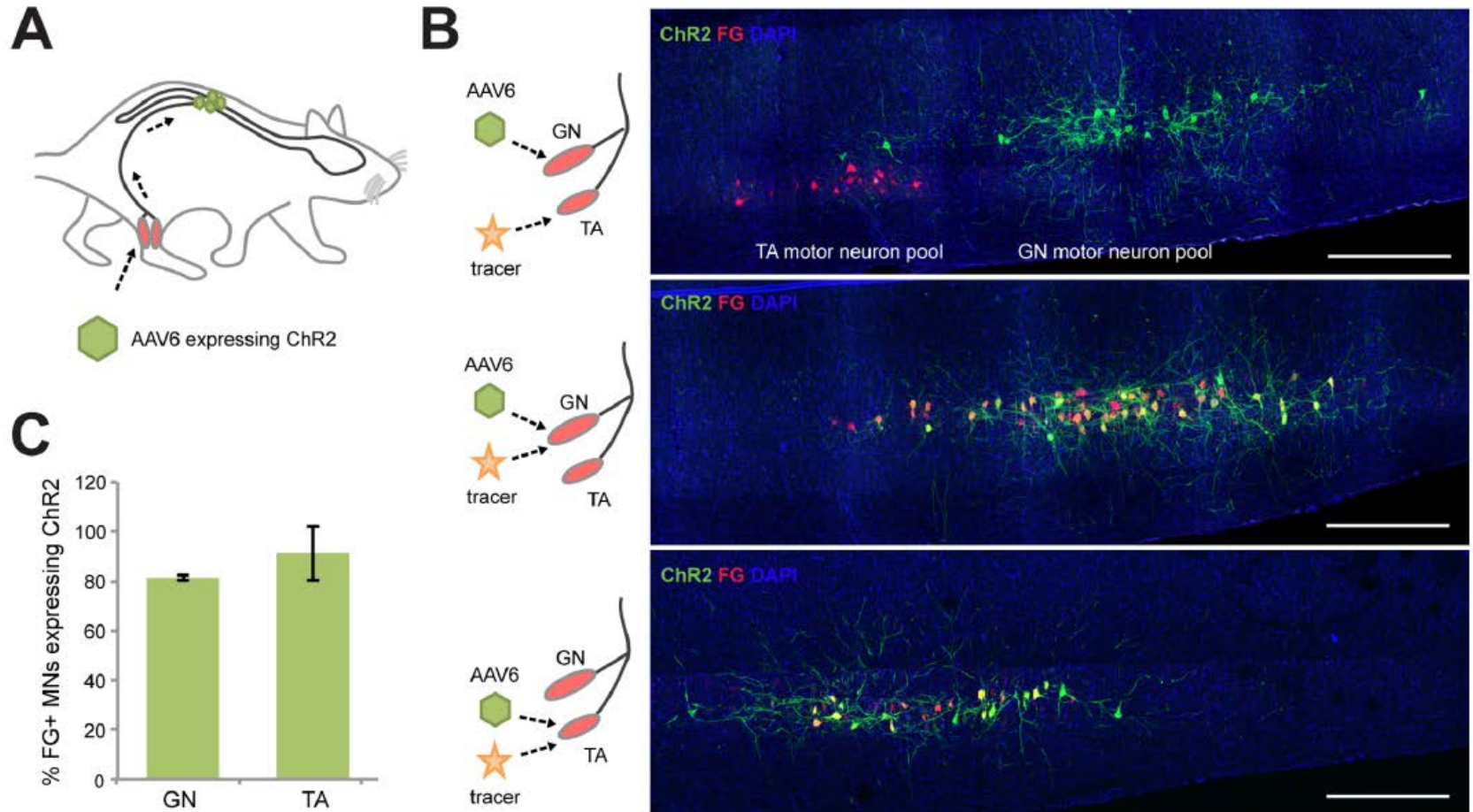
Thy1.NpHR2.0-YFP transgenic mice express Halo2.0 in MN axons

Green light blocks propagation of electrically-induced sciatic nerve action potentials



Liske et al., Muscle and Nerve, 2013

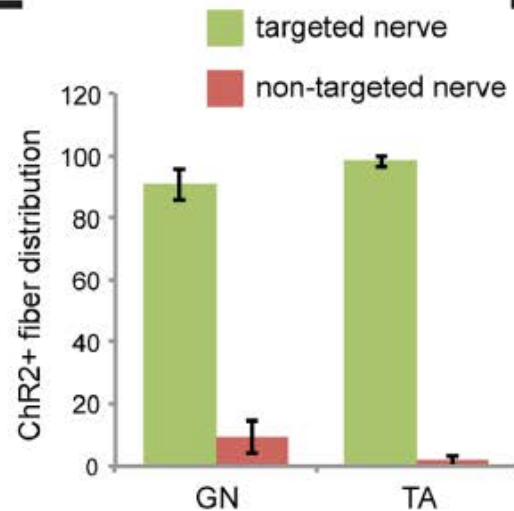
Opsin delivery to peripheral nerves through retrograde viruses injection in the target organ



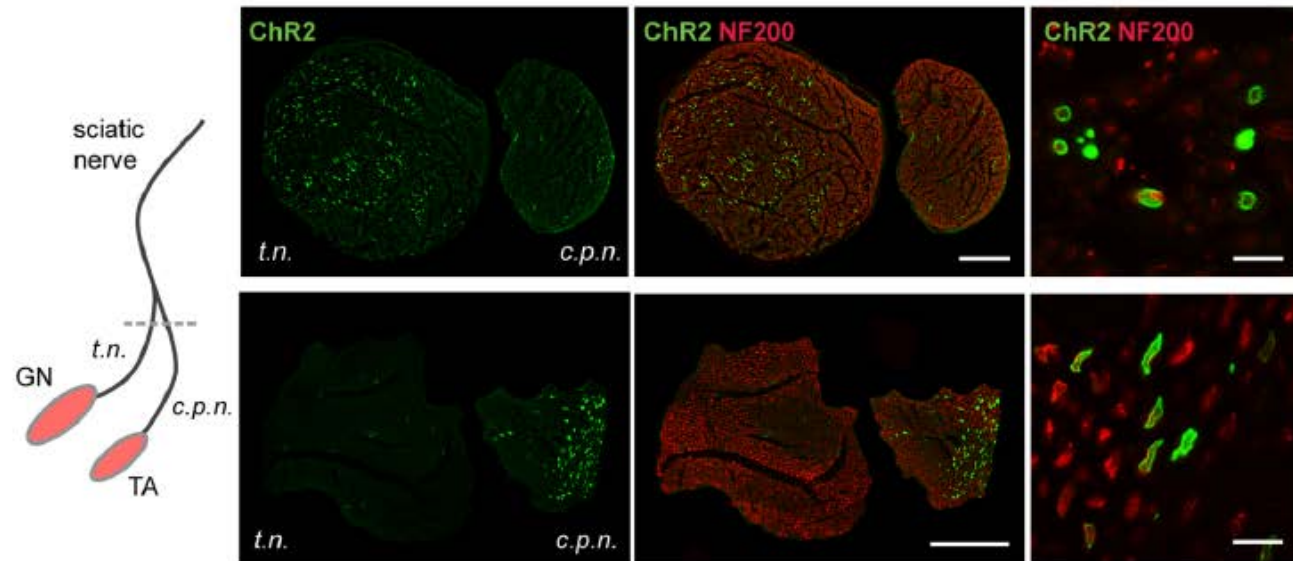
GN = gastrocnemius muscle; TA = tibialis anterior muscle; FG = Fluoro-Gold, retrograde tracer; MN = motor neurons
Longitudinal sections of lumbar spinal cord 4 weeks following AAV6 intramuscular injection

Opsins traffic to peripheral nerves following retrograde virus delivery to the target tissue

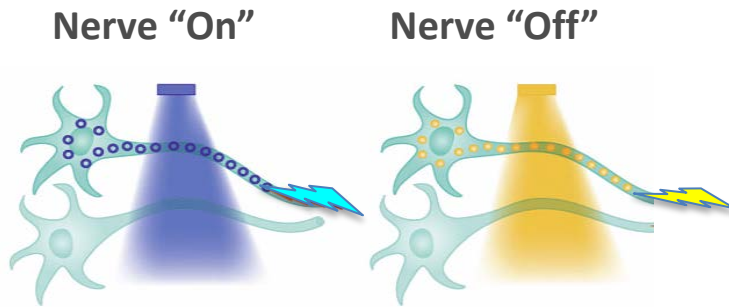
E



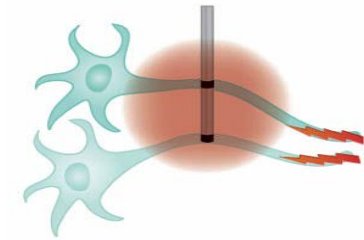
D



Optogenetic vs. Electrical Neuromodulation



Non-specific Excitation



Optogenetic Neuromodulation		Electrical Stimulation
Cell Type Specific	<i>Specificity</i>	Non-specific
Activate and/or Inhibit	<i>Cell Function Modulation</i>	Activate
Wavelength Specific	<i>Cell Type Orchestration</i>	Non-specific
Orderly: Small → Large	<i>Recruitment Order</i>	Reverse Order
Short → Prolonged	<i>Duration</i>	Short
Minimal Expected	<i>Side Effects</i>	Shock Sensation
Potential (similar as electrical)	<i>Implanted Device</i>	Electrode Implant

Challenges and Opportunities of Optogenetic Neuromodulation

- Safe retrograde trafficking via AAVs still an early area: needs to be confirmed in various systems
- Opsin trafficking to distant targets takes time
- Further red-shifted opsins (e.g. Reach, Crimson) would be beneficial for to maximize spread and minimize heating
- Light positioning and tissue compatibility (mice more challenging than rats due to size)

The tools for discovery need not be
the same as the tools for treatment

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