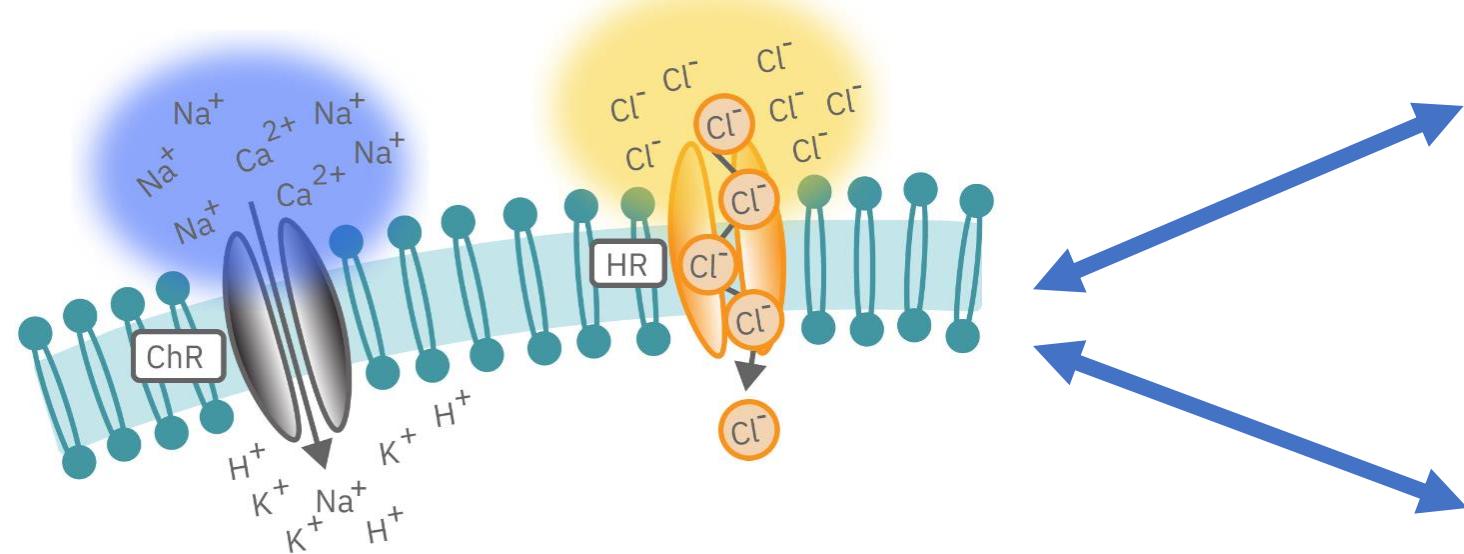


Experimental design: special considerations when manipulating neural activity



Lecture 12

Anita E. Autry, Ph.D.

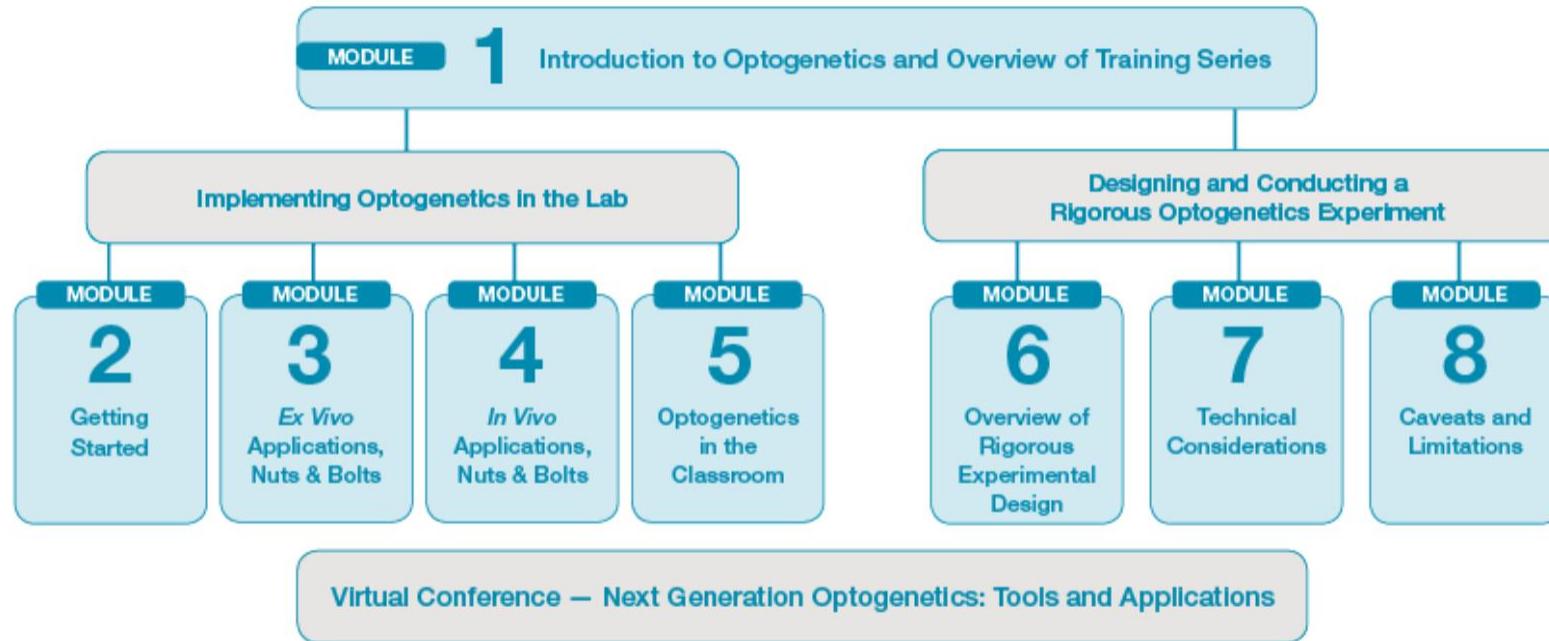


"I don't usually volunteer for experiments, but I'm kind of a puzzle freak."



"Of course I'm glad we escaped, I just wish I could say goodbye to cocaine button one more time."

SfN'S OPTOGENETICS TRAINING SERIES



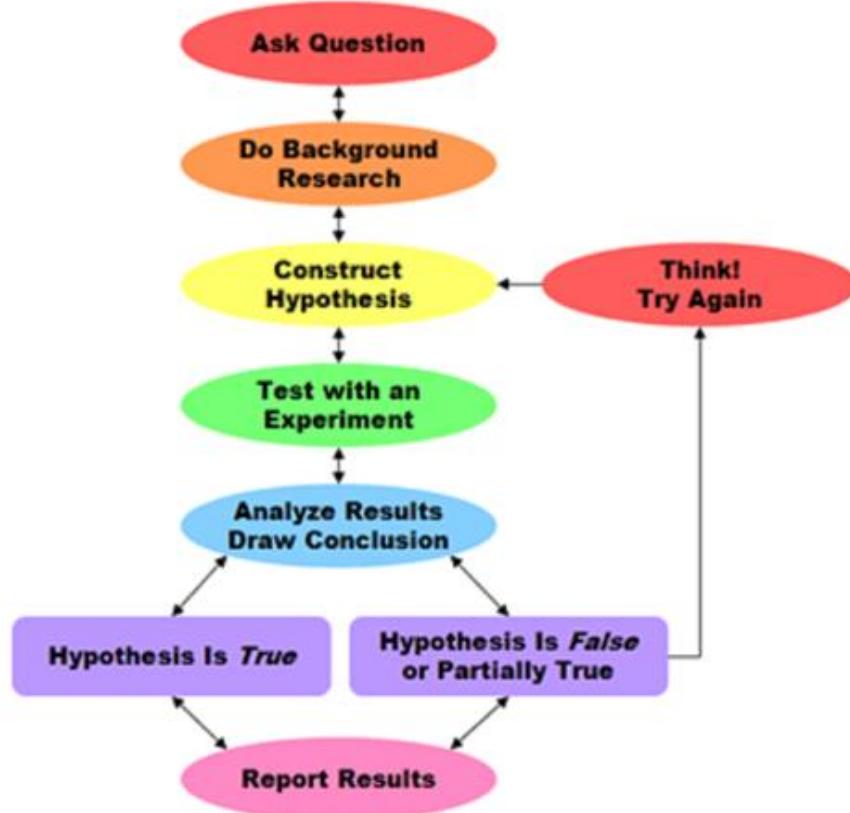
NEURONLINE.SFN.ORG/OPTOSERIES

- SfN's Optogenetics Training Series
- Optogenetics Resource Center (stanford.edu)

Experimental design

- Common designs for behavioral experiments
- Considerations for pitfalls that lead to challenges for interpreting and reproducing data
- Understand sources of variability
 - Technical
 - Biological

Scientific Method



Steps to follow to design experiment

- Form a specific and testable hypothesis
- Choose controls/comparisons
- Determine sample size
- Execute a rigorous experiment
- Analyze and interpret



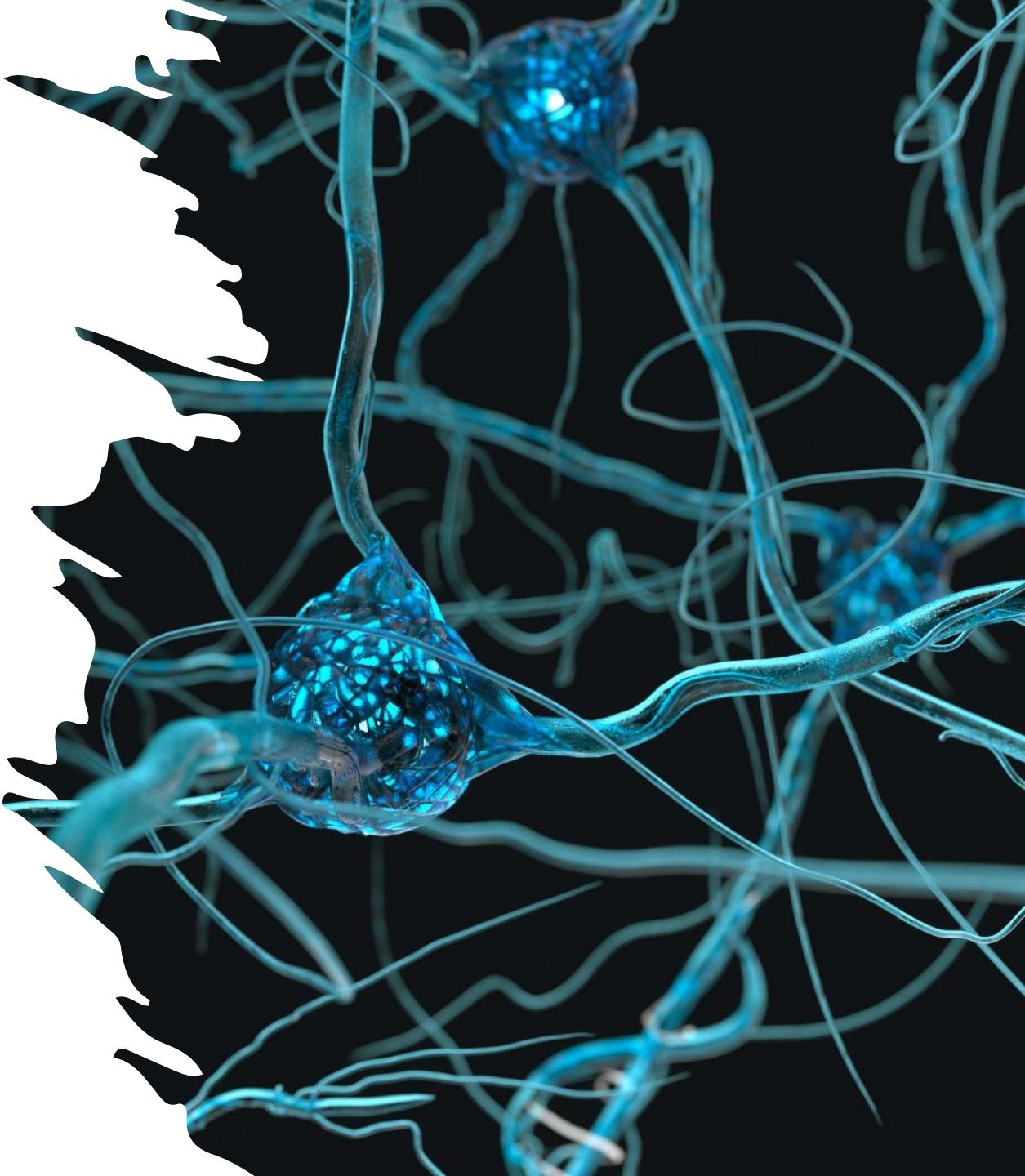
Components of F31 Research Strategy

- Significance
 - Hypothesis
- Innovation
- Approach
 - Aims
 - Experimental design (sample sizes, controls, outcome measure details)
 - Rigor and reproducibility
 - Data analysis
 - Anticipated results as well as interpretations and caveats, potential problems, alternative approaches



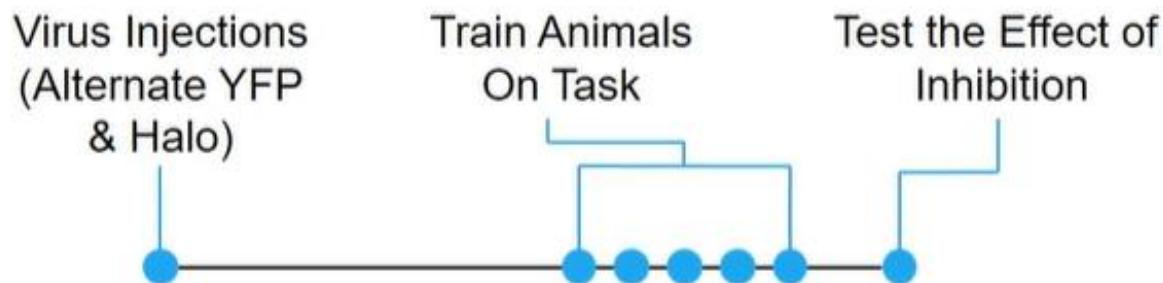
Develop a specific and testable hypothesis

- Are you testing necessity or sufficiency? Or both?
- What aspect of behavior is your target neuron/neurons/brain region involved in?
- When during the behavior do you want to manipulate (when is activity of your neurons of interest critical during the task)?
- Is there a specific cell type of interest?
 - Manipulate/record from individual neurons or a large population?
 - Deep or superficial areas?
 - Unilateral or bilateral?
 - Are physiological and/or molecular features of the cells already known?

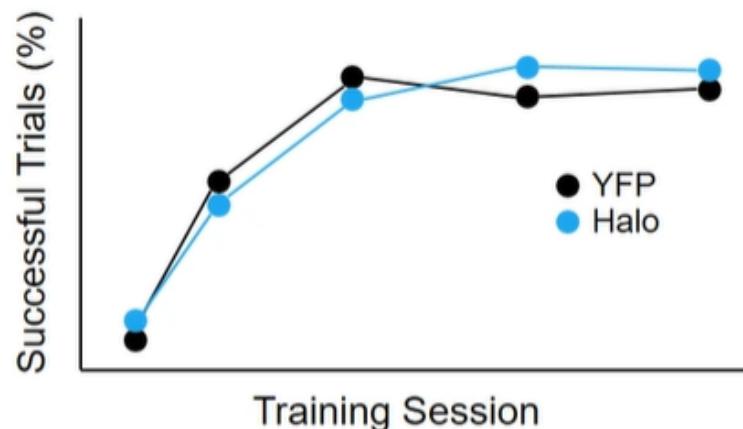


Choose controls/comparisons

- Typical experimental timeline:



- Typical behavioral outcome measure:



Reduce variability (group design):

- Balance groups by age/sex
- Alternate YFP/Halo (eliminate batch effects)
- Experimenter blinded to condition

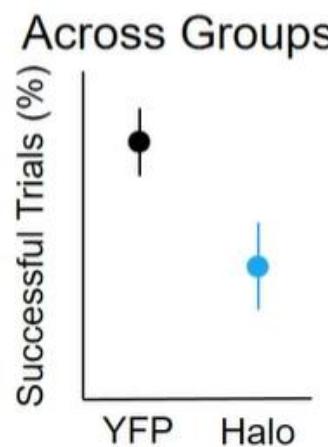
Reduce variability (behavior design):

- Habituate animal to tether
- Obscure light from animal
- Use minimal power for stimulation of opsin***
- (Consider using red-shifted opsin)

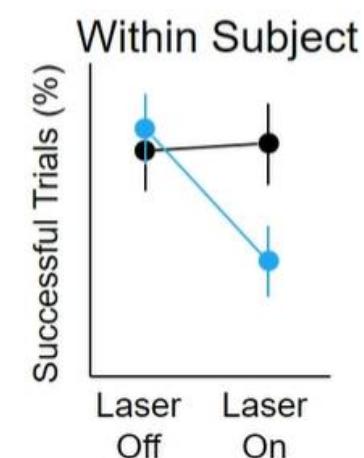
Choose controls/comparisons

- Typical experimental designs:

Between group/subjects design



Within group/subjects design



Randomized crossover/counterbalanced design

This design controls for:

- Virus infection
- Light delivery
- Fiber/cable insertion
- Behavior training/exposure

This design controls for:

- Heat/light of laser (photoelectric effect)
- Bonus: increases power

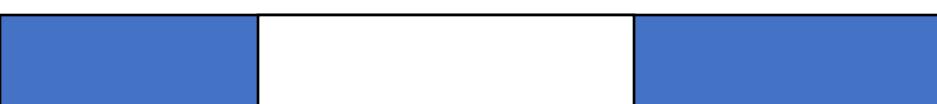
Choose controls/comparisons

- Typical trial designs:

Interleaved



OFF ON OFF



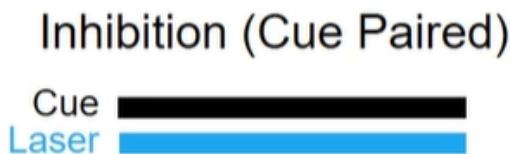
ON OFF ON

Repeated



Choose controls/comparisons

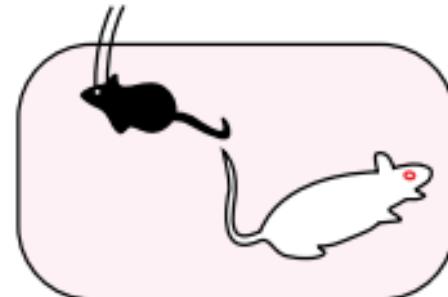
- Typical stimulation patterns (broadly):



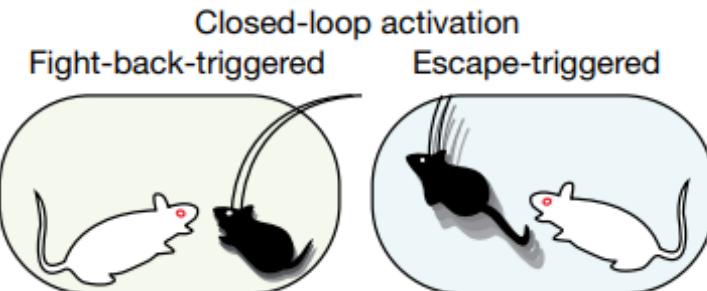
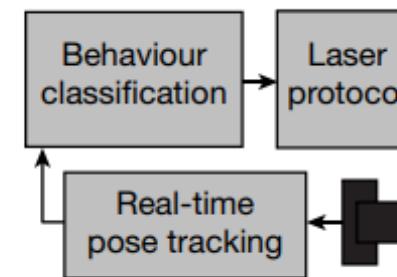
VERSUS



Open-loop activation

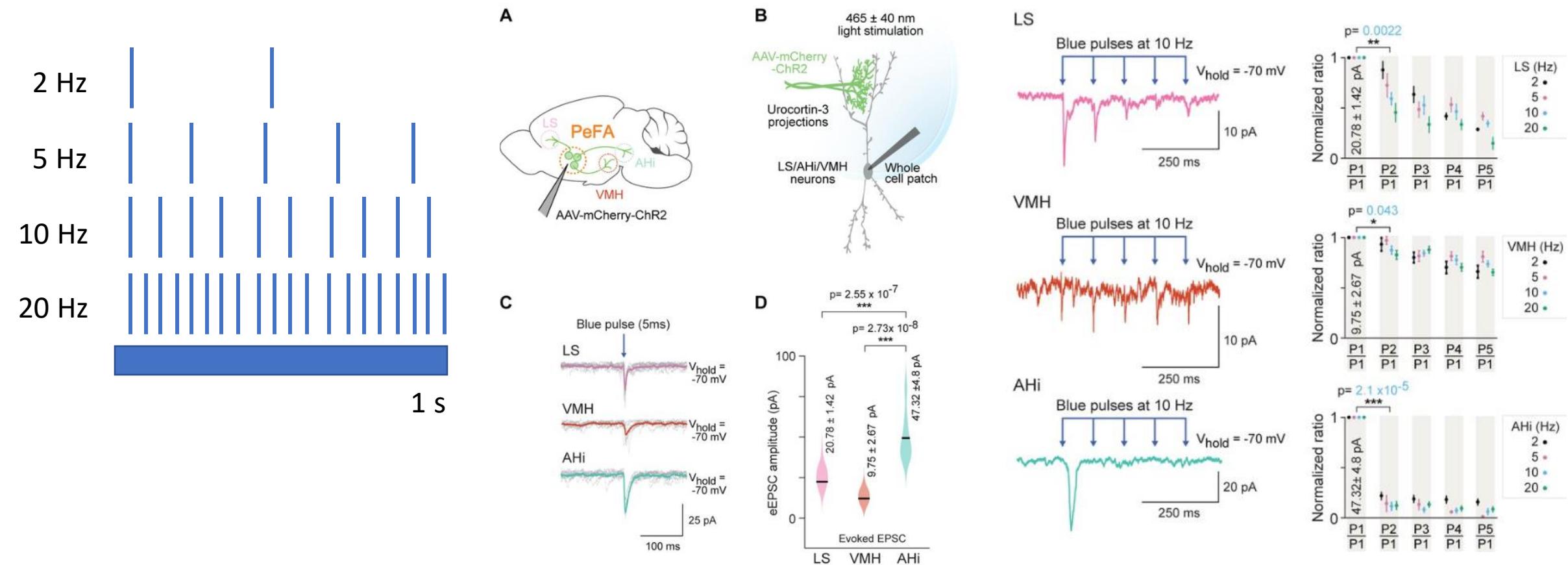


VERSUS



Choose controls/comparisons

- Typical stimulation patterns (fine-grain):





Determine sample size

- A good design has a high statistical power
- What is statistical power?
 - Probability that a test will detect an effect/difference
- Power calculation
 - Way to estimate the group size needed to achieve a certain power
- How/where to perform power calculation
 - Have relevant information: mean and standard deviation of outcome measures, effect size (based on pilot data or on literature), critical p-value (<0.05; also known as alpha), power (high is 0.8-0.9; also known as beta)
 - See [Sample Size Calculations \(IACUC\) | Research Support \(bu.edu\)](#)

Execute a rigorous experiment

01

Randomize group
assignment and
order of
surgeries/treatments

02

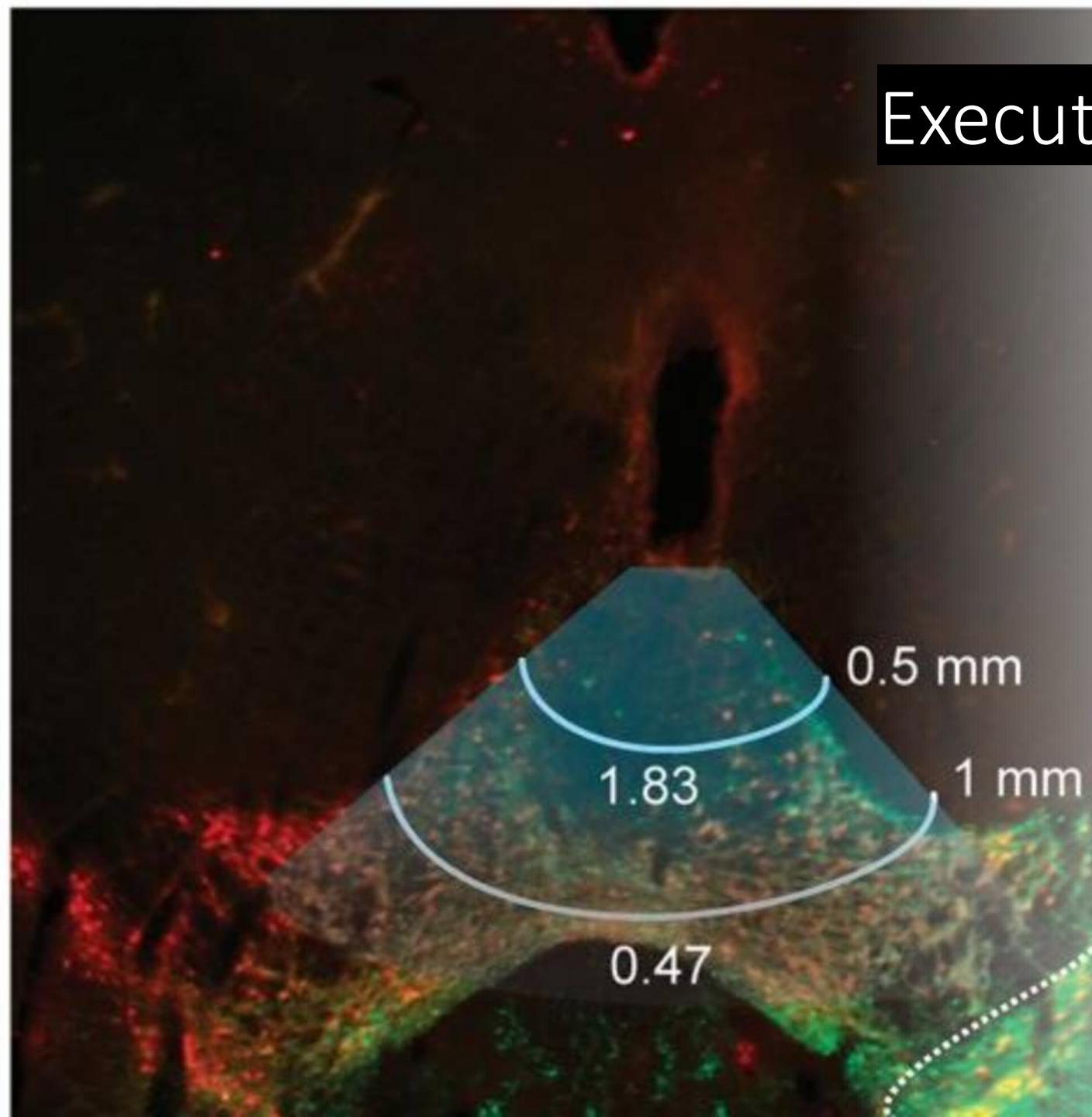
Keep experimenter
blinded to conditions
throughout data
collection and
analysis

03

Don't break the code
until after exclusions
made!!

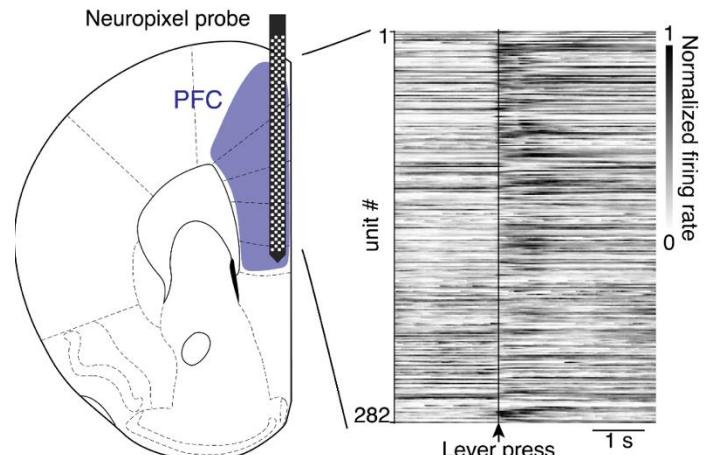
Execute a rigorous experiment

- **Typical exclusion criteria:**
 - **Evaluate specificity of viral targeting**
 - Use a predetermined cutoff for number of cells/area infected
 - Use a predetermined cutoff for on/off target of regions (within x mm by stereotaxic coordinates)
 - Done usually by histology of conjugated fluorophore
 - **Evaluate fiber placement**
 - Use a predetermined cutoff for on/off placement (within x mm of target region)
 - **Observe possible toxicity**
 - Abnormal morphology of cells or aggregation of fluorophore
 - Obvious difference in appearance of cells beneath fiber vs. elsewhere
 - **Behavior outlier data (QA within recordings not met)**
 - Grubb's outlier test



Execute a rigorous experiment

- Consider how to validate your manipulations (rigor)
 - Electrophysiological validations
 - In vivo during experiments
 - Validates if constructs works as predicted
 - Verifies activation kinetics
 - Reveals short or long term plasticity
 - Uncovers potential undesirable or network effects
 - Seizure/synchrony
 - Depolarization block
 - Disruption of chloride reversal
 - Post-inhibition rebound activity
 - Ex vivo by slice physiology
 - Validation as above (activation kinetics and STP/LTP)
 - Shows natural activity of cells
 - Determines if cells can “follow” stim trains



Breton-Provencher lab



Analyze and Interpret

- Typical data analysis steps:
 - For between group analysis:
 - Wilcoxon Rank Sum Test (non-parametric test with no matching)
 - Unpaired t-test (parametric measures with no matching)
 - Normality can be assessed using a Shapiro-Wilk test)—in some cases a one-sample t-test may be most appropriate if the ceiling/floor effects lead to no variability
 - For within group analysis
 - Wilcoxon Signed-Rank Test (non-parametric test with matching samples)
 - Paired t-test (parametric measures with matched observations)
 - For intersectional design
 - Two-way repeated measures ANOVA (parametric measures; virus vs. laser condition; repeated if applicable)
 - A lot more complicated if dealing with non-parametric measures so consult the Biostatistics Core! [Biostatistics | Department of Epidemiology & Population Health | Albert Einstein College of Medicine \(einsteinmed.edu\)](#)

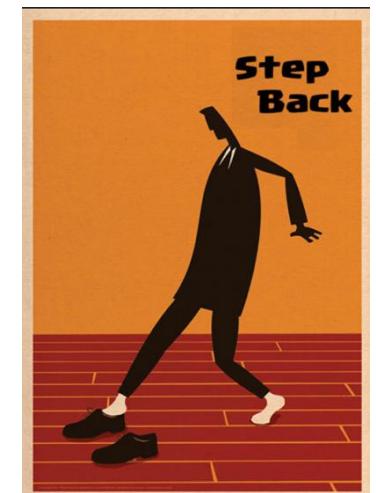
Walk-in Statistical Consulting Center

Investigators can visit the weekly walk-in statistical consulting centers to meet with a statistician without appointment and obtain quick advice on their projects. **Due to COVID, the walk-in center is operating virtually on Tuesday afternoons from 3 - 5 pm. Please contact the Division of Biostatistics Administrator, Ms. Maureen Delouise (maureen.delouise@einsteinmed.edu), for further information.**



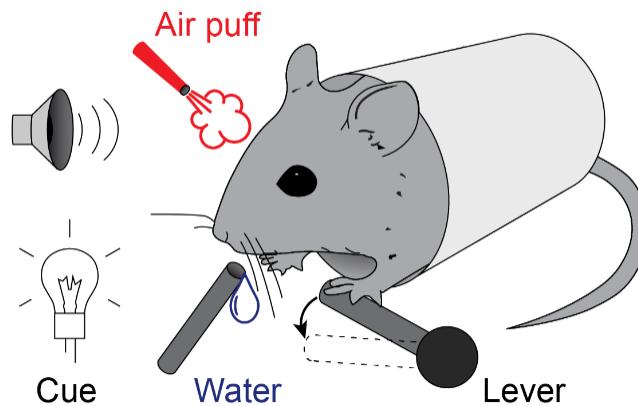
Analyze and Interpret

- Cellular/circuit/behavioral considerations (caveats) for interpretation of effects:
 - Behavioral/sensory confounds
 - Locomotion
 - Laser light, noise of shutter
 - Circuitry confounds
 - Recurrent circuits
 - Response latency
 - Backpropagation/collateral activation
 - Complex neurochemistry
 - Physiological confounds
 - Can your neurons follow your train?
 - Can your opsin follow your train?
 - Can your light source follow your train?

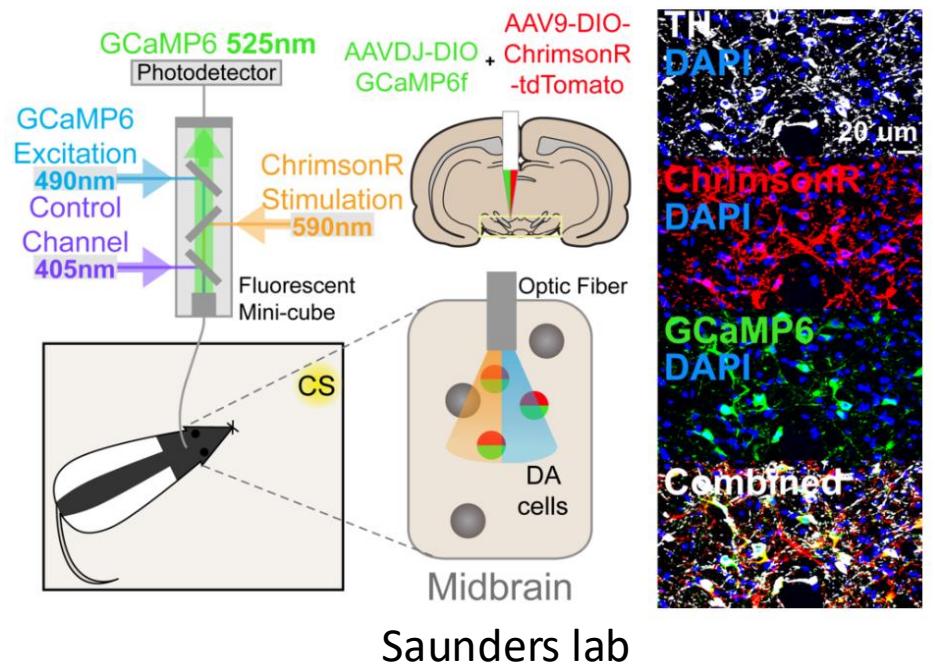
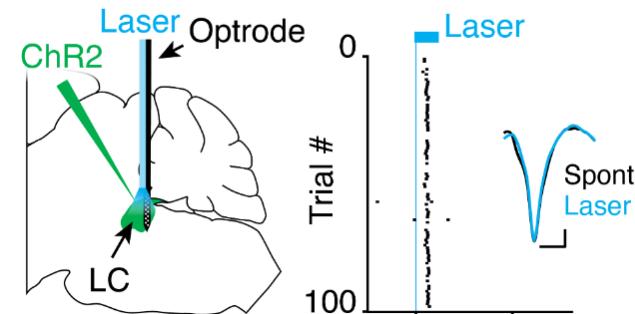


What is optogenetics used for?

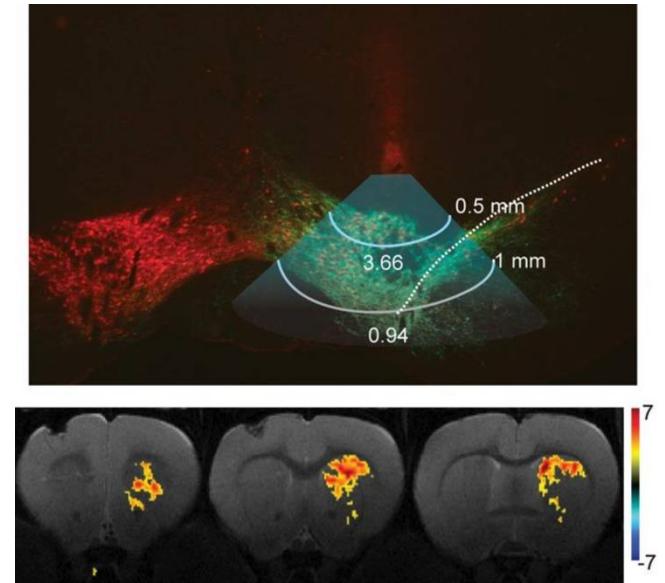
- Manipulate behavior
- Test functional connectivity
- Combine optogenetics and imaging/recording
- Translational applications



Breton-Provencher lab



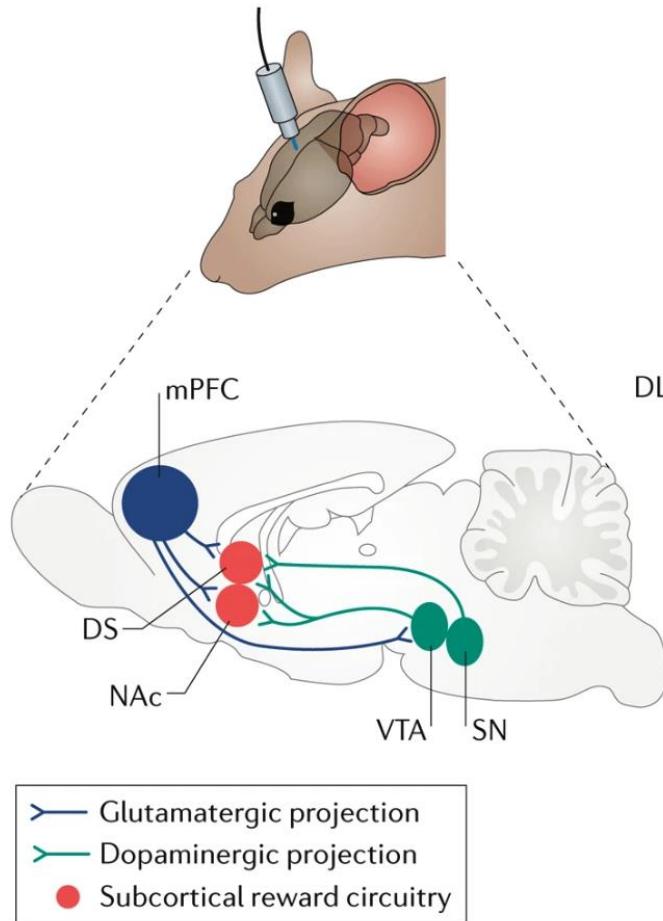
Saunders lab



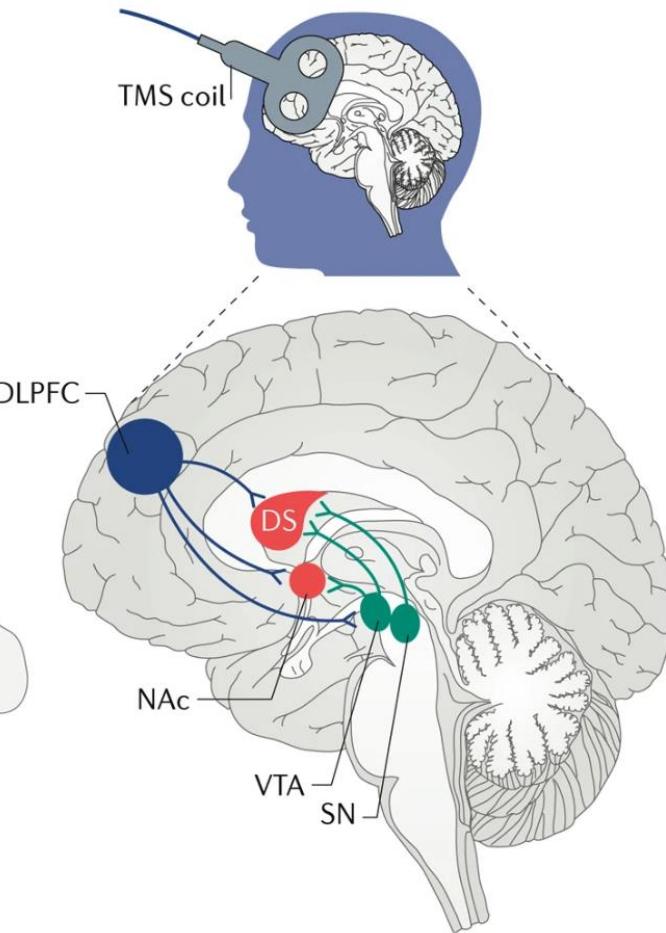
Lohani et al, Mol Psych. 2017

Translational applications: Opto vs. TMS

a Optogenetic modulation



b TMS modulation

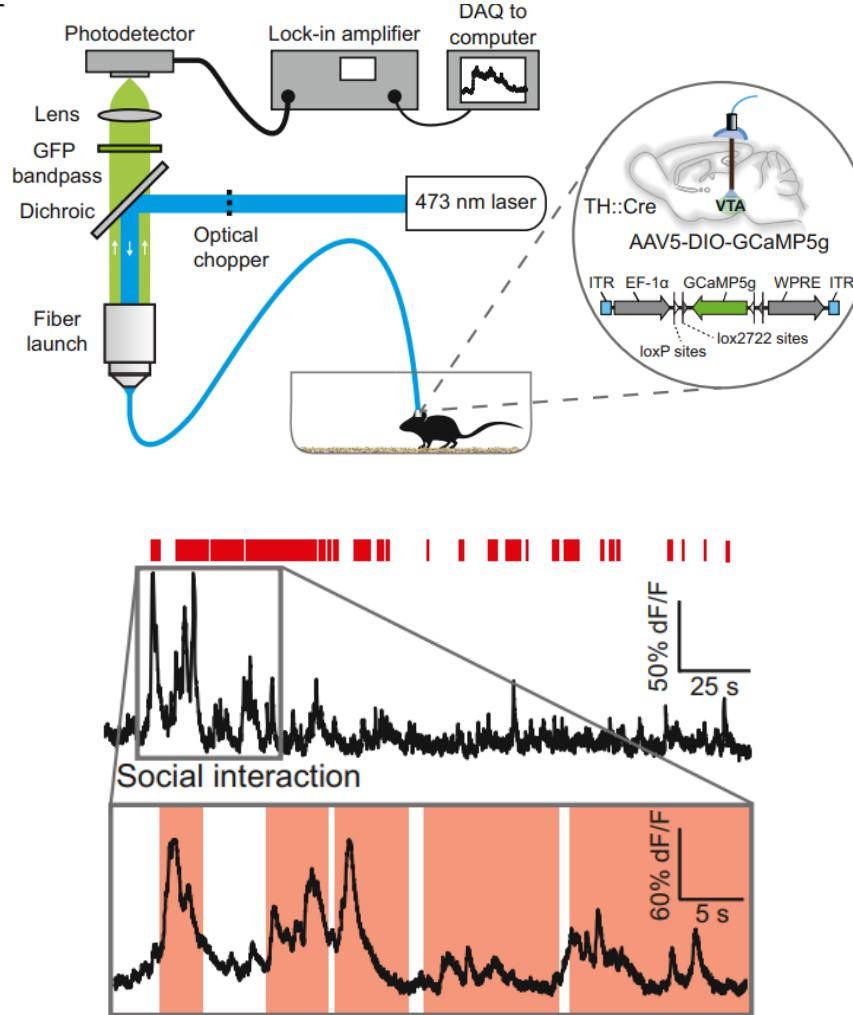


Nature Reviews | Neuroscience

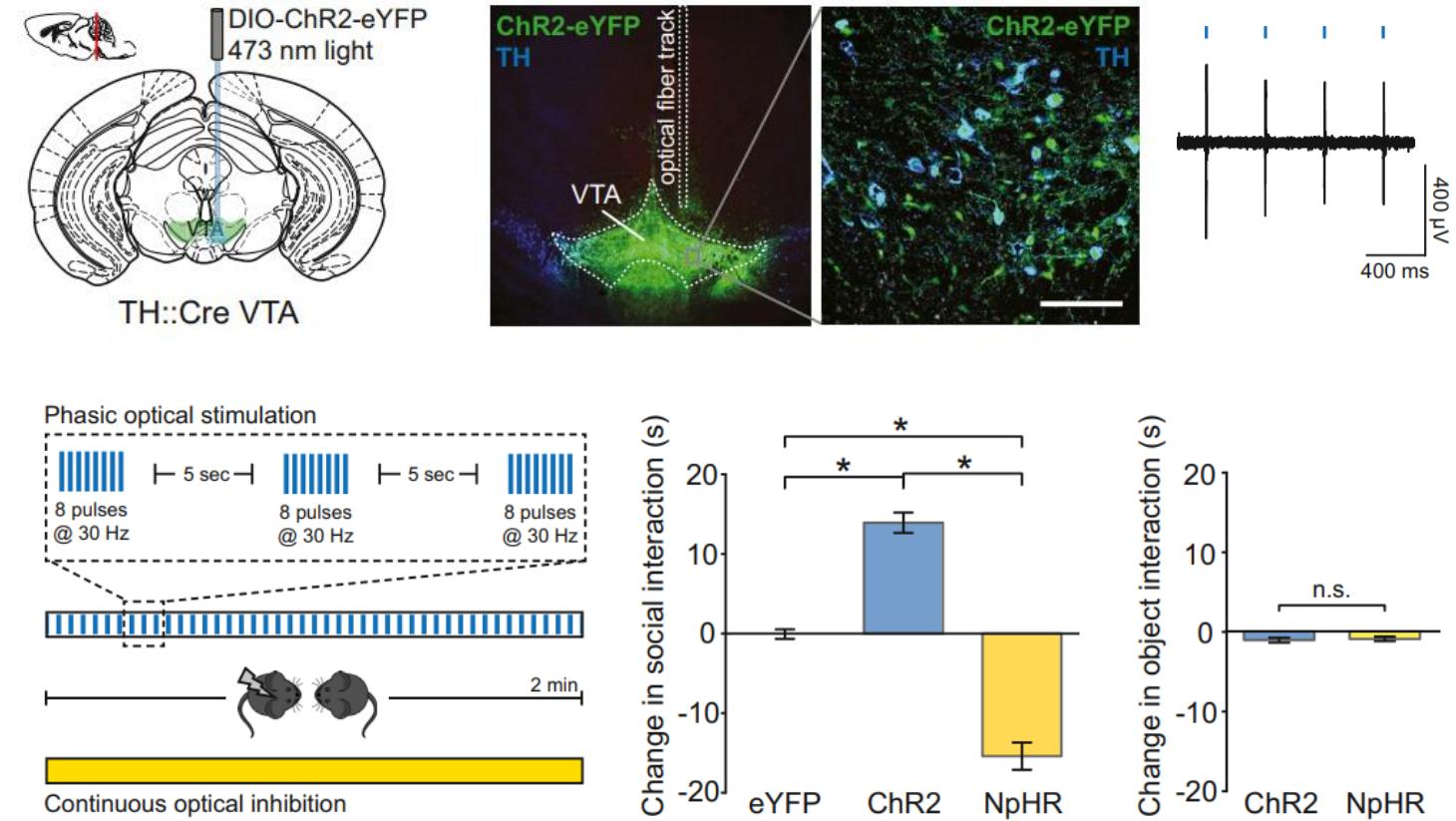
Diana et al Nat Reviews Neuro 2017

Example of behavioral study

Record during behavior

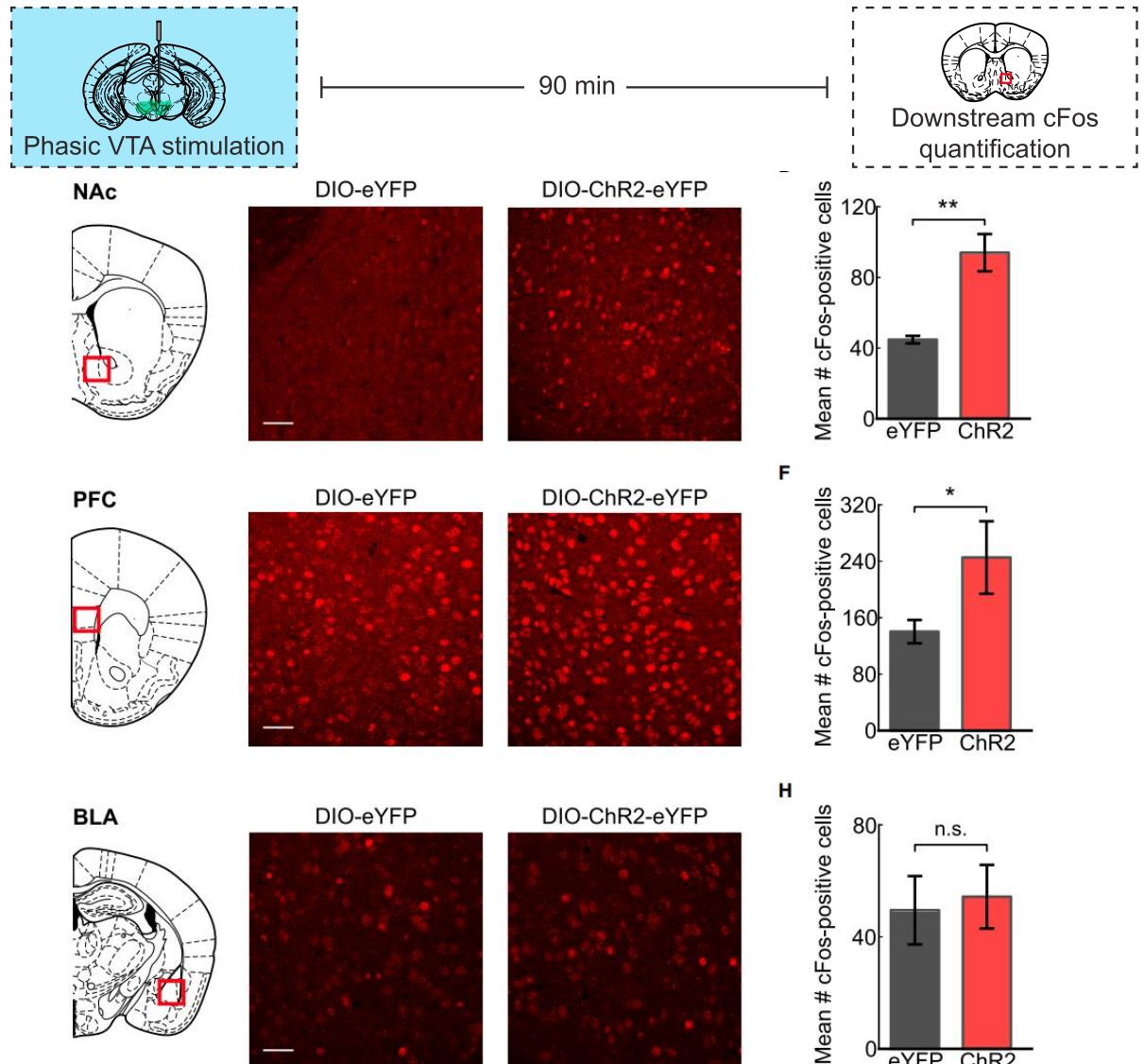


Stimulate/inhibit during behavior

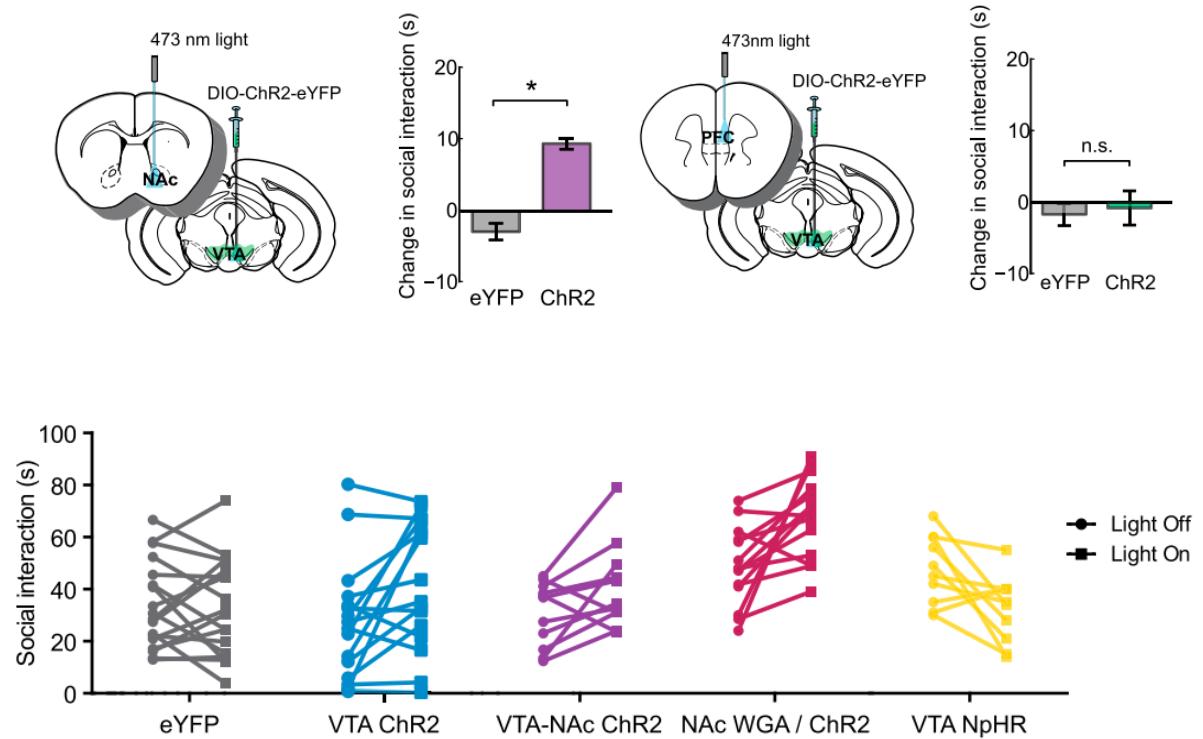


What about the circuit?

Activity mapping

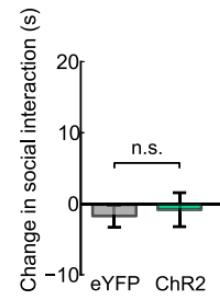
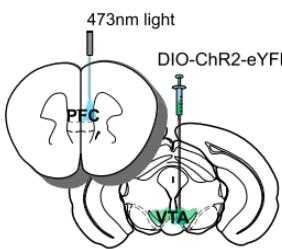
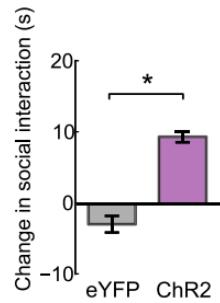
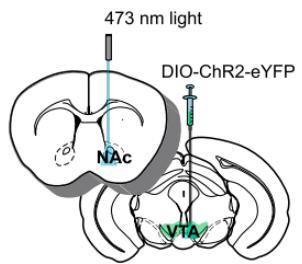


Projection-specific manipulation

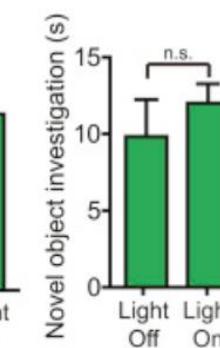
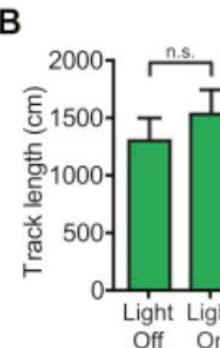
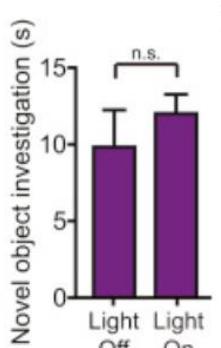
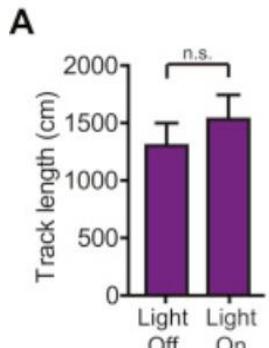


“Double dissociation” using projection stim

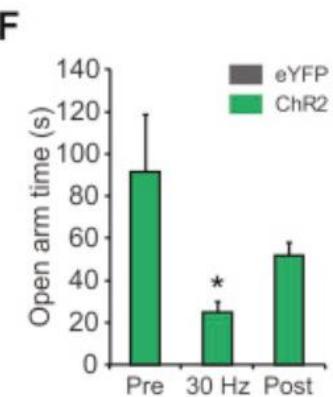
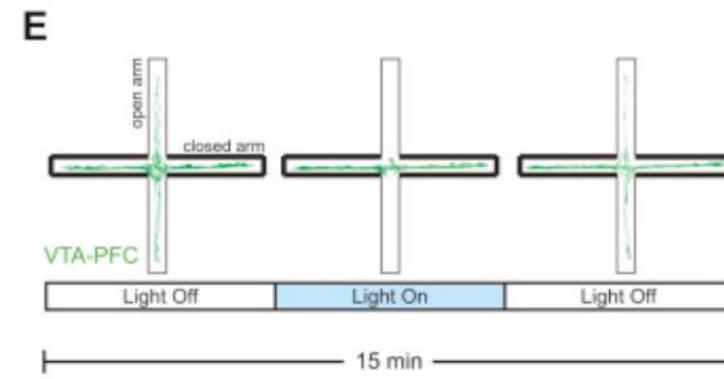
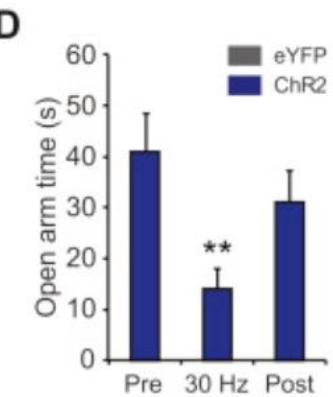
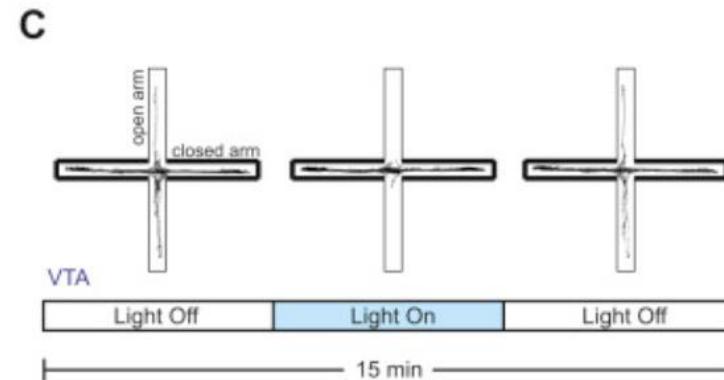
Social behavior affected by
VTA-all and VTA-NAc Stim



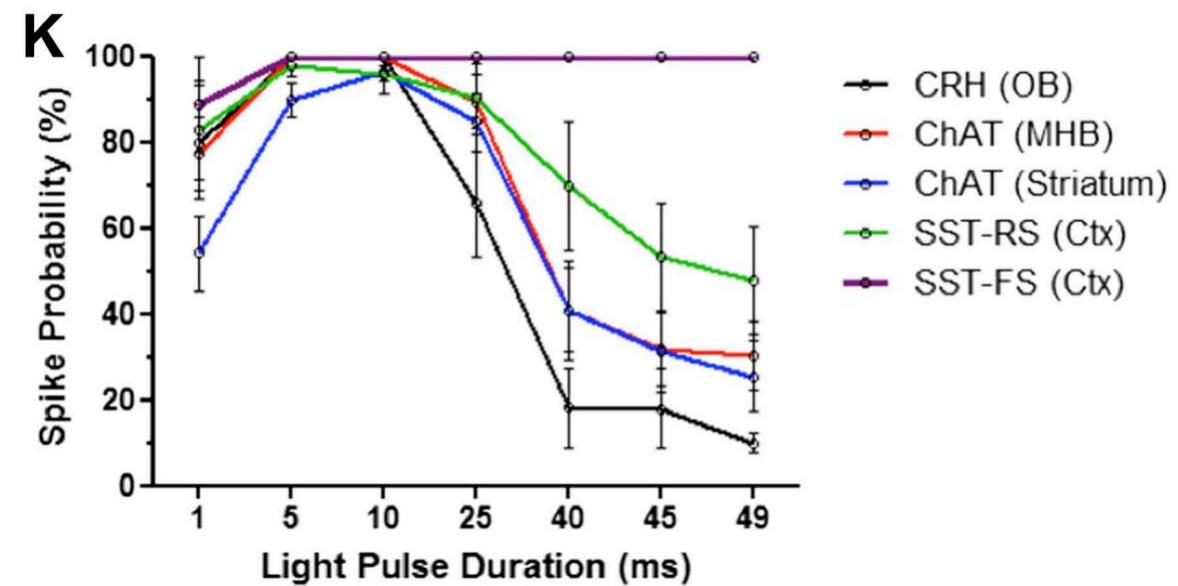
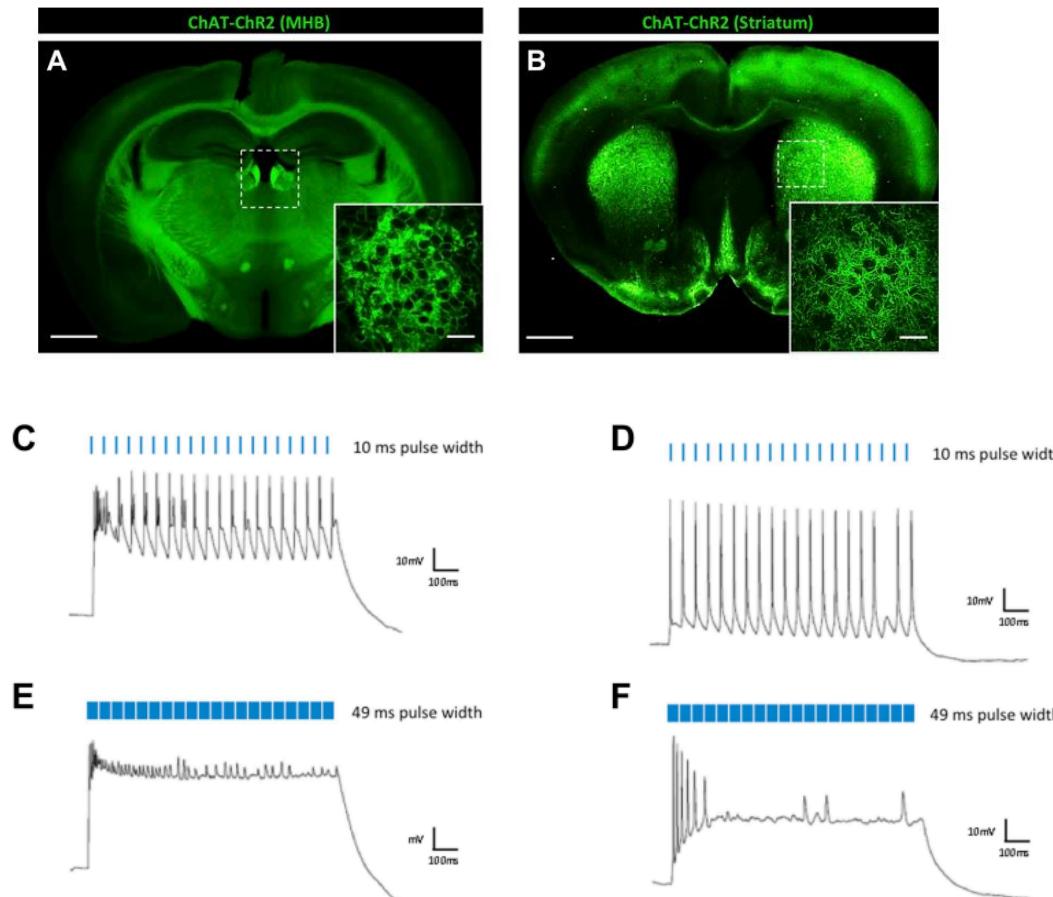
No impact on locomotion



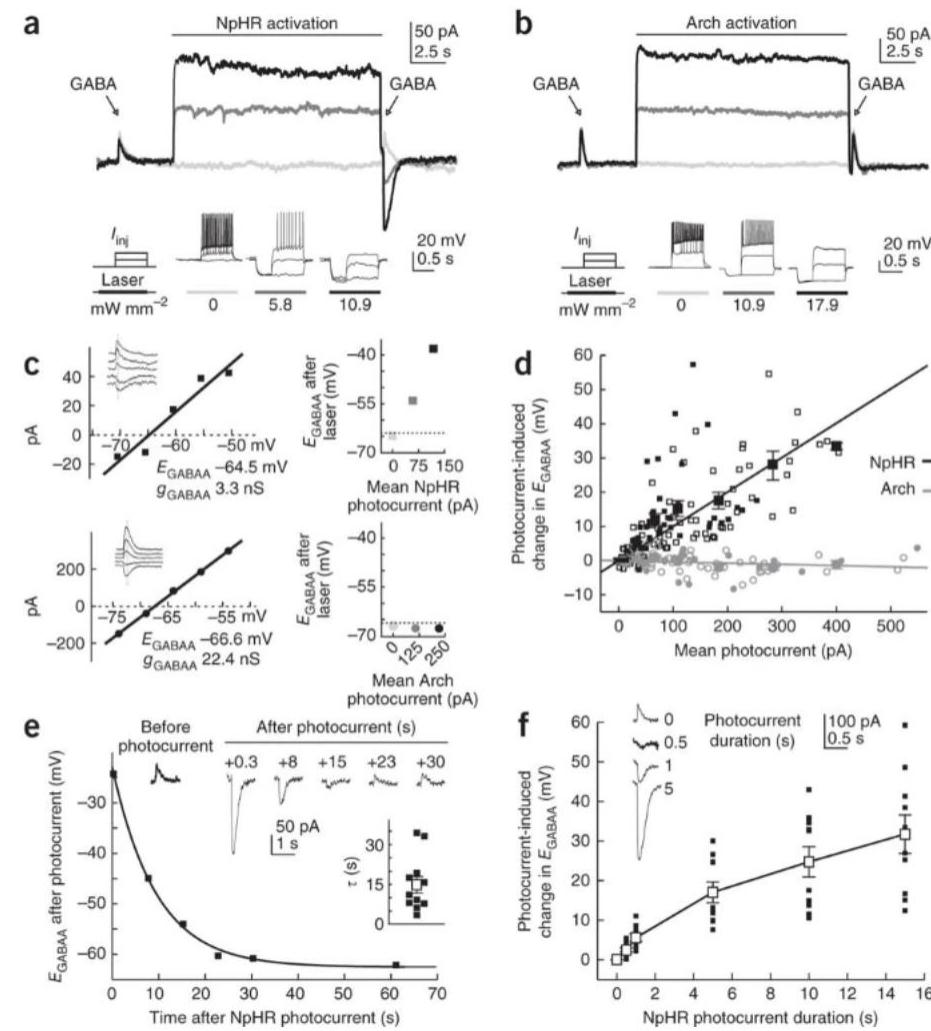
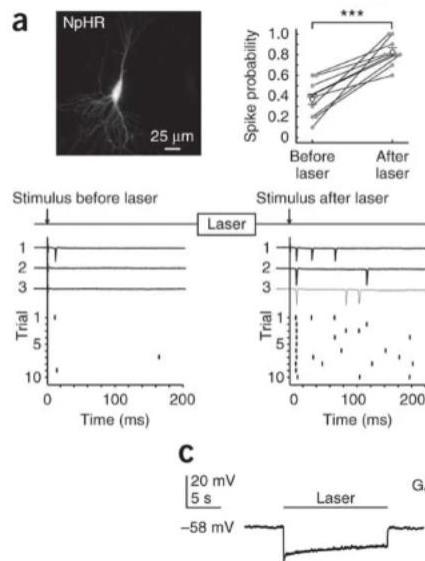
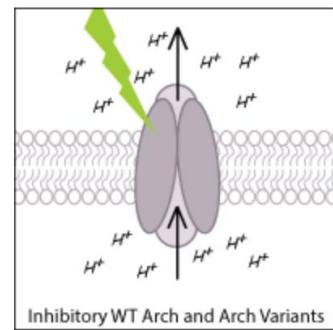
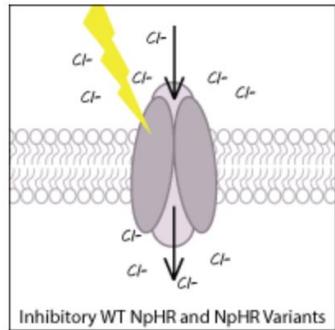
EPM behavior affected by
VTA-all and VTA-mPFC Stim



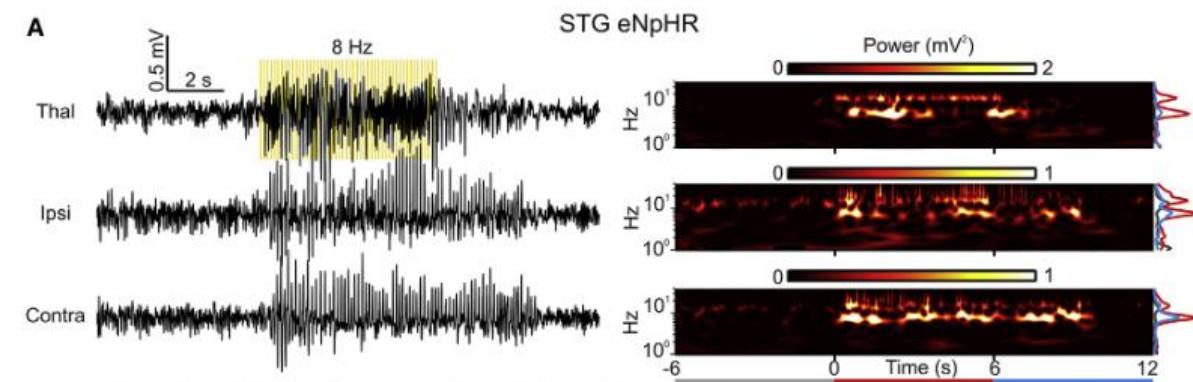
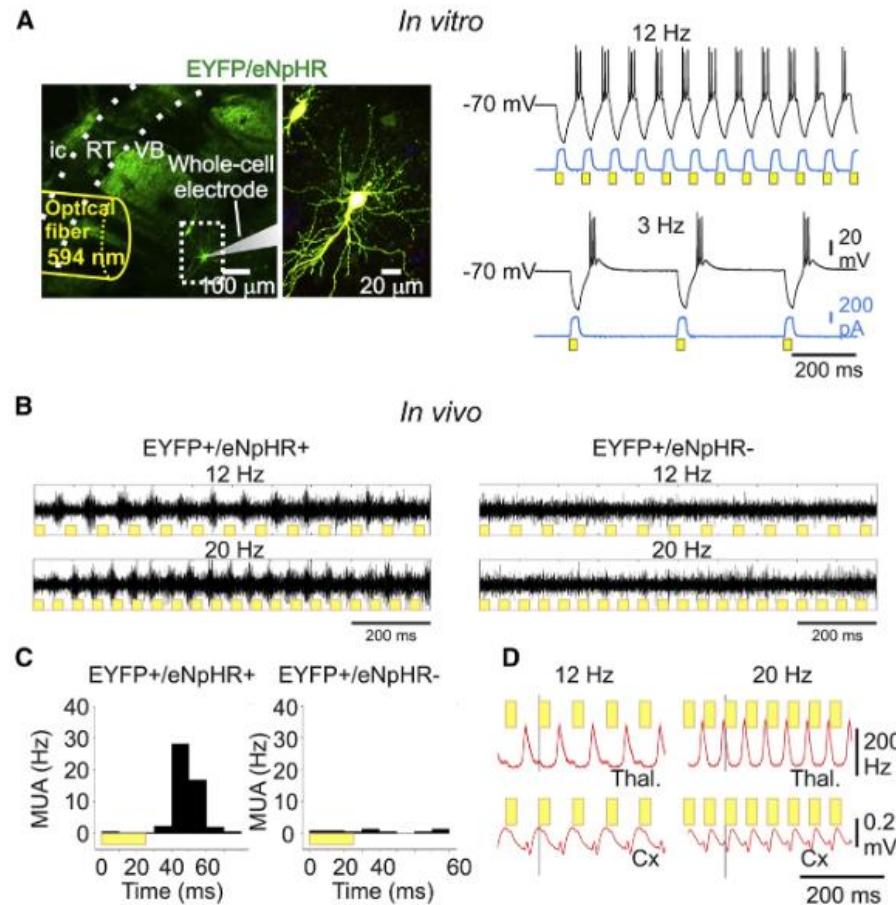
Depolarization block



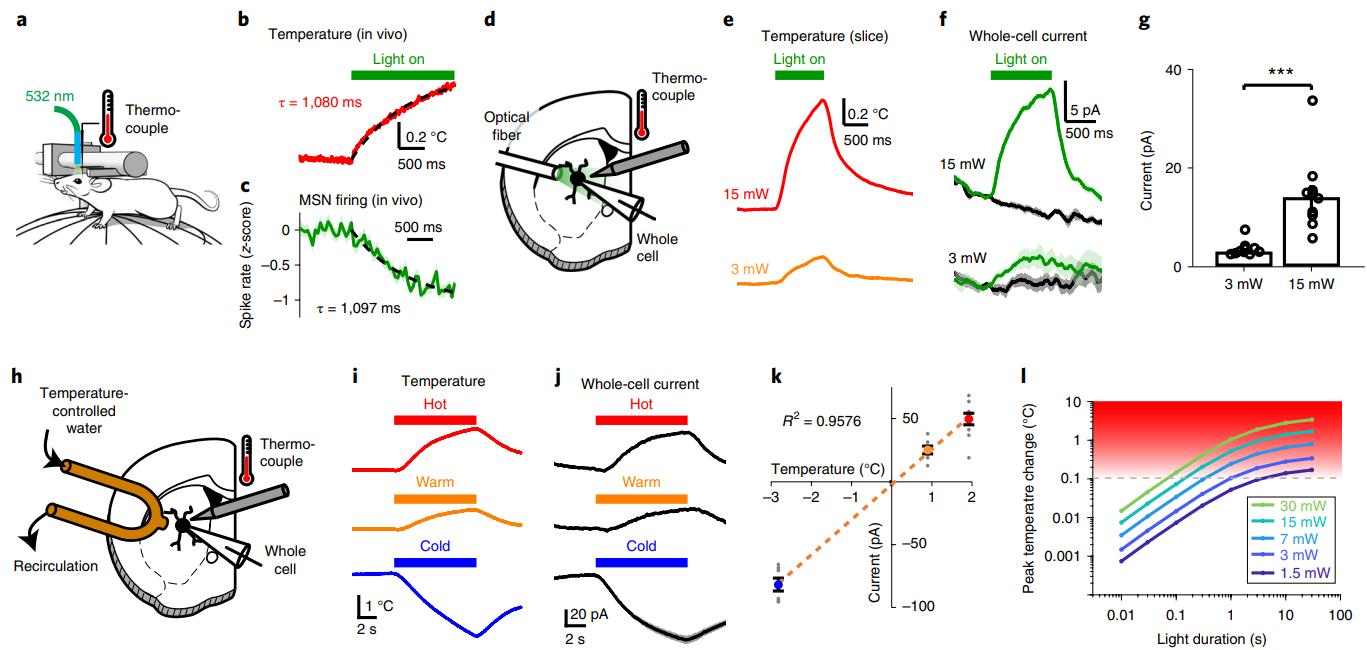
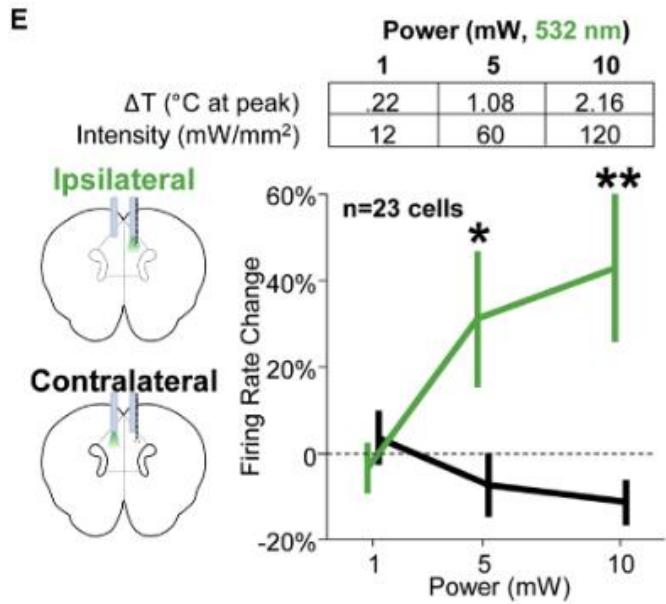
Disruption of GABAergic signaling



Rebound after inhibition → network synchrony

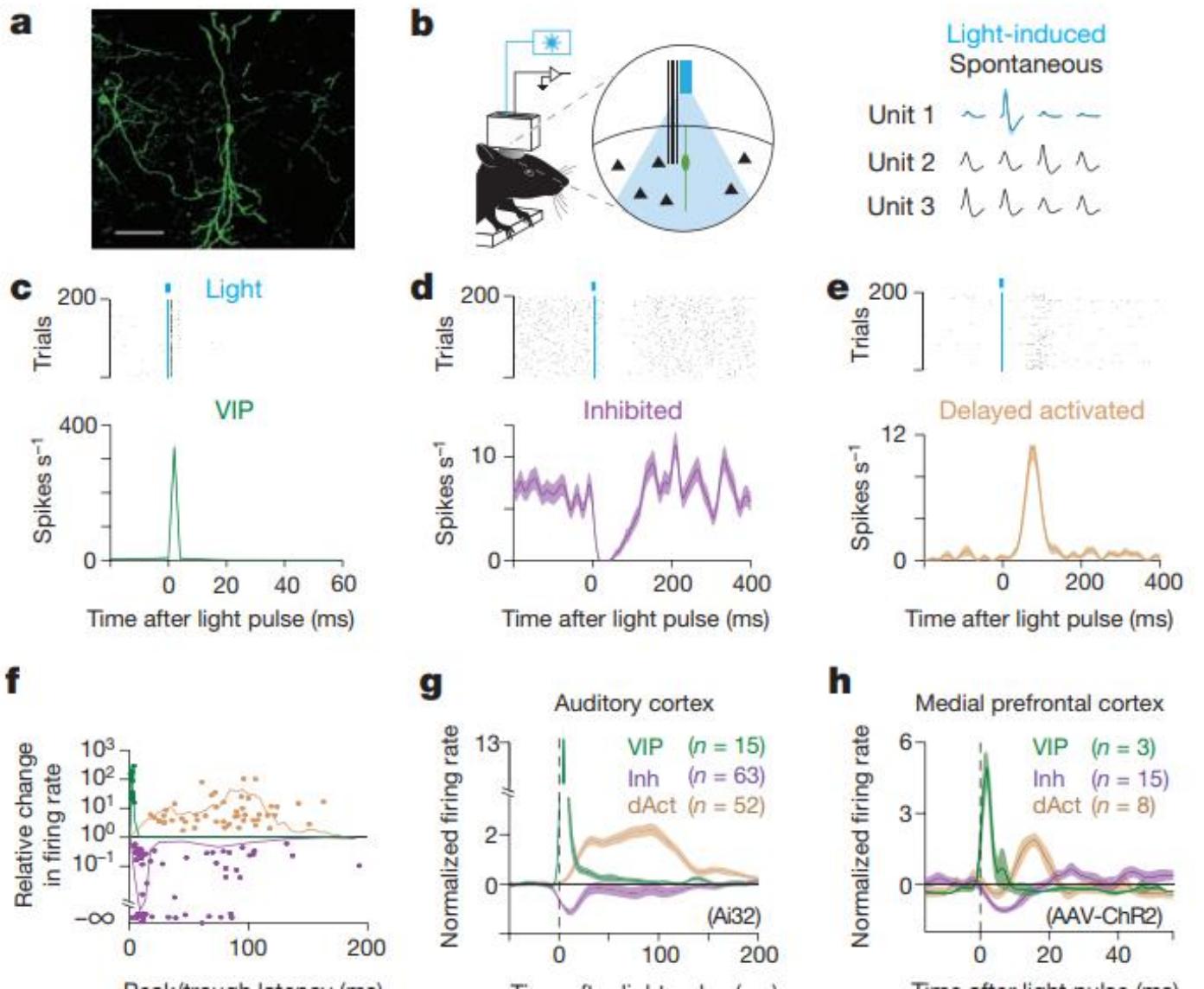


Tissue heating



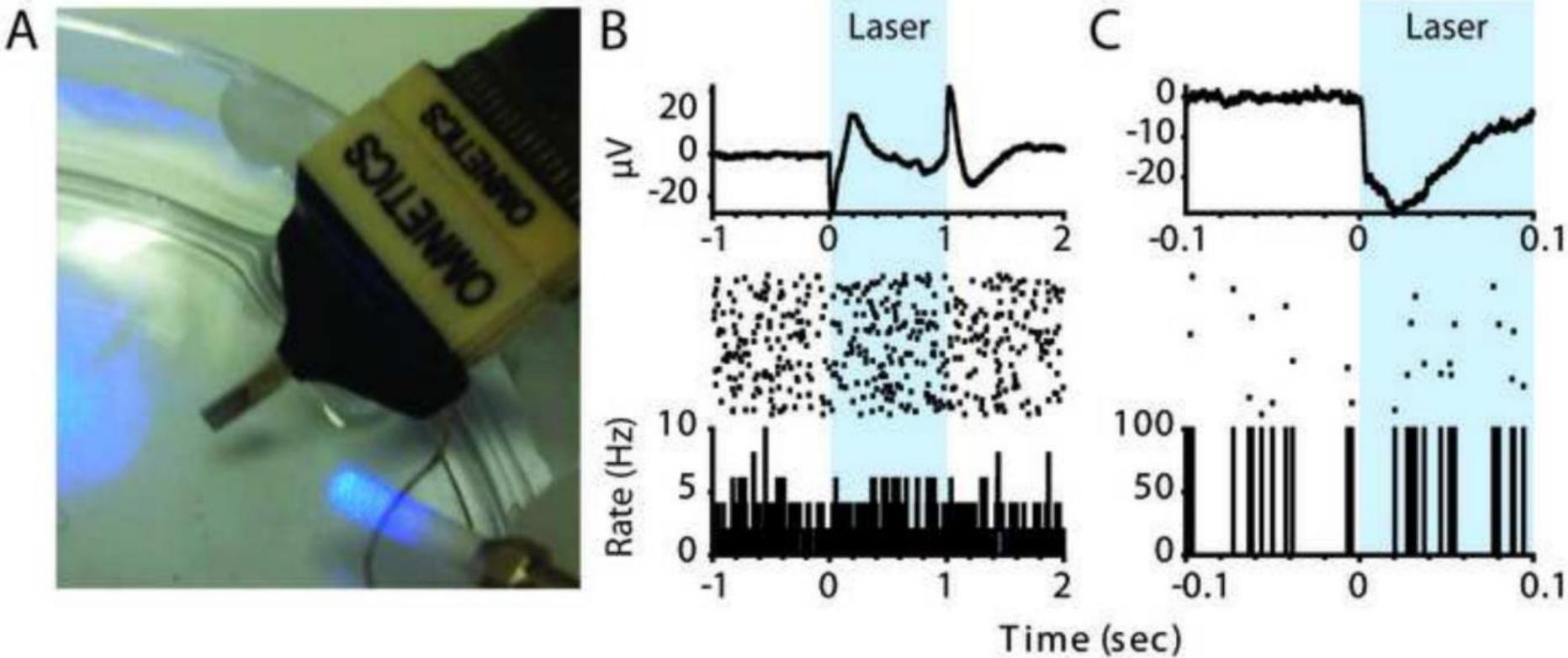
Recurrent circuit activation example

- Pi et al Nature 2013

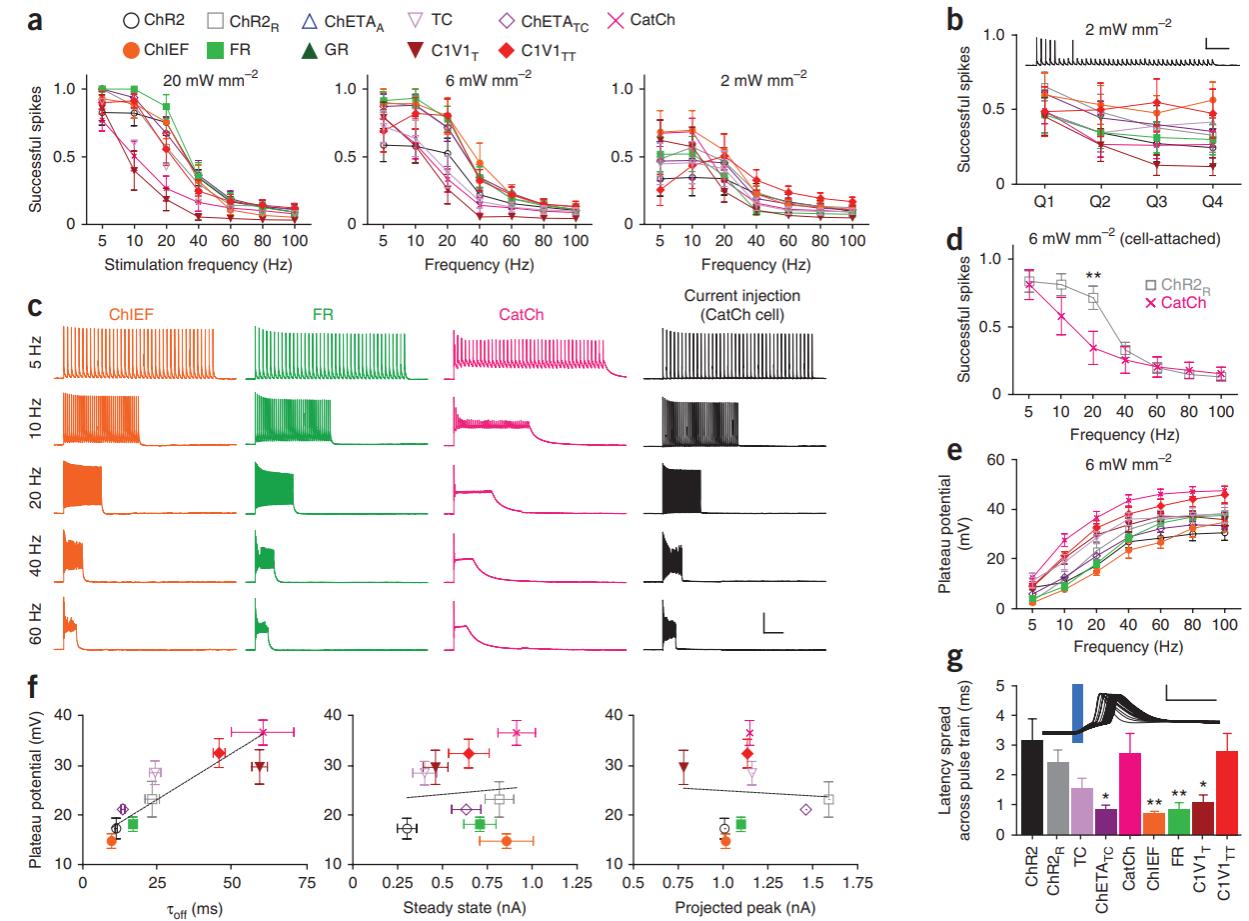
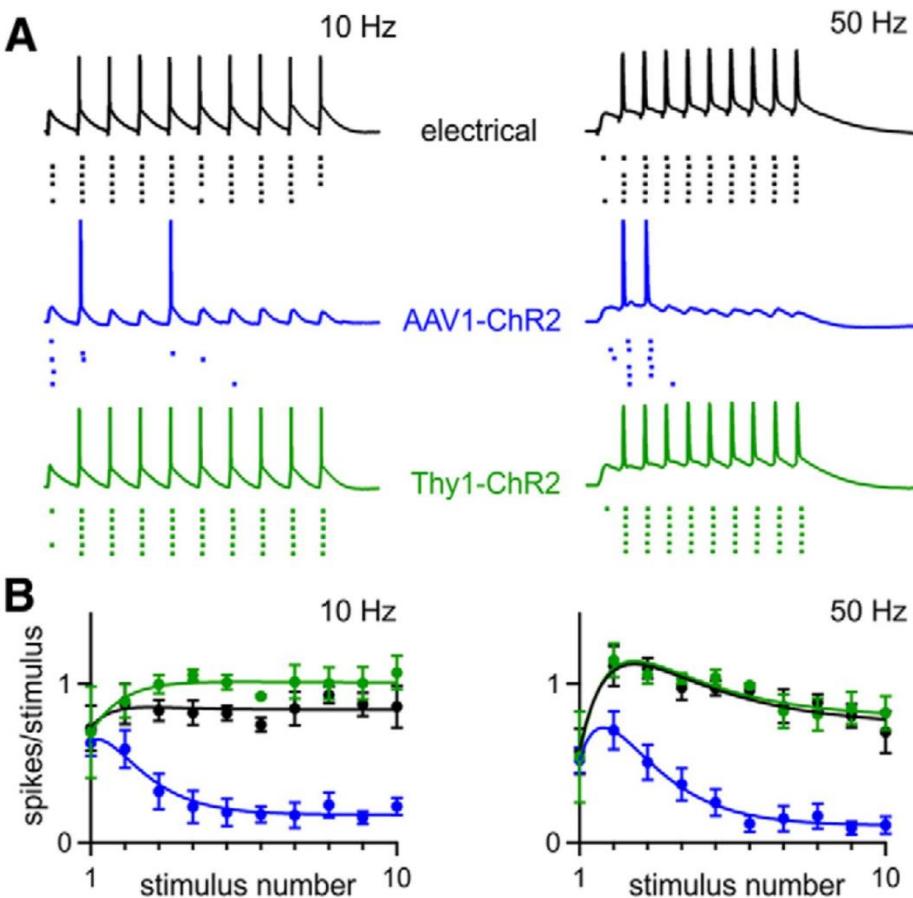


Photoelectric effect

- Kravitz et al Brain Res. 2013

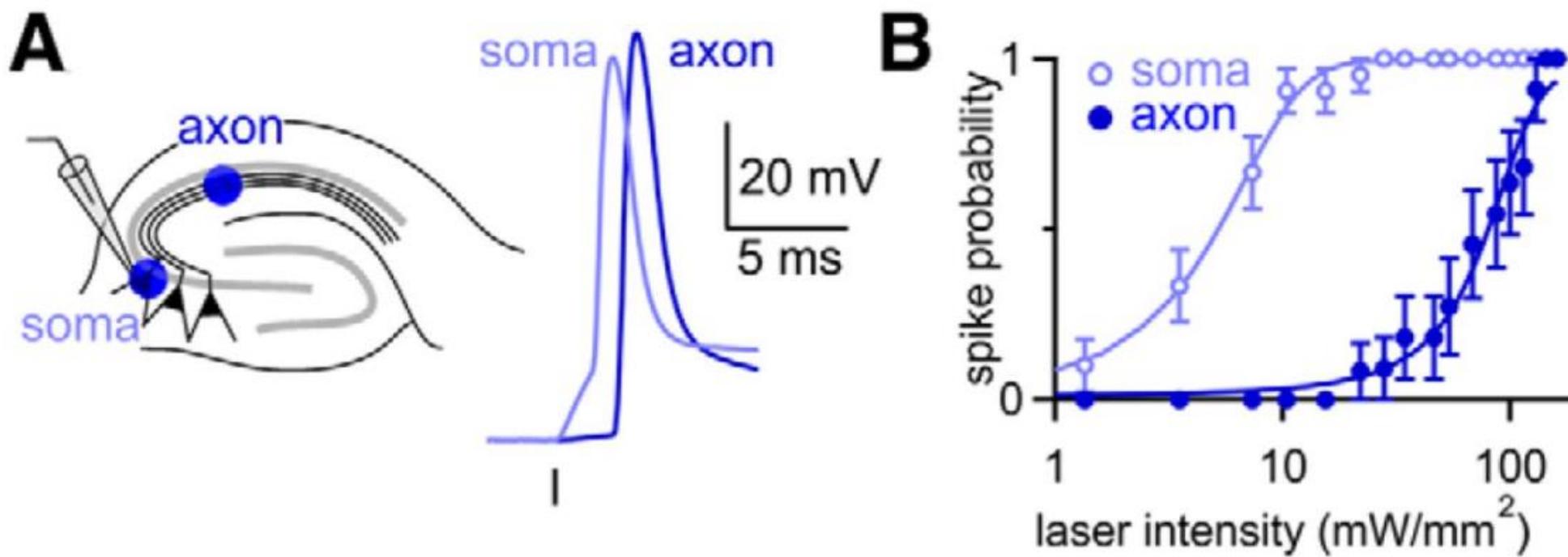


Pulse train caveats

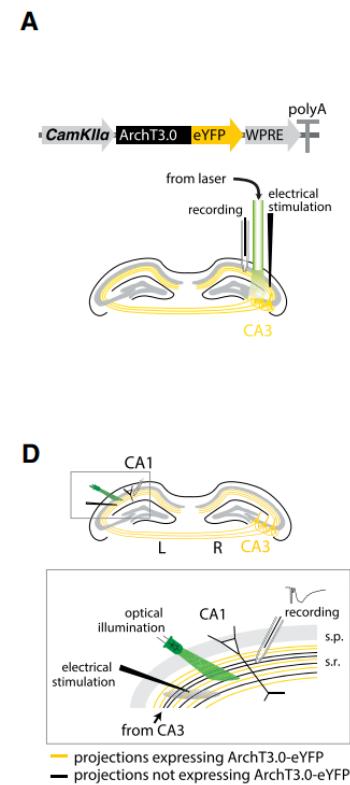
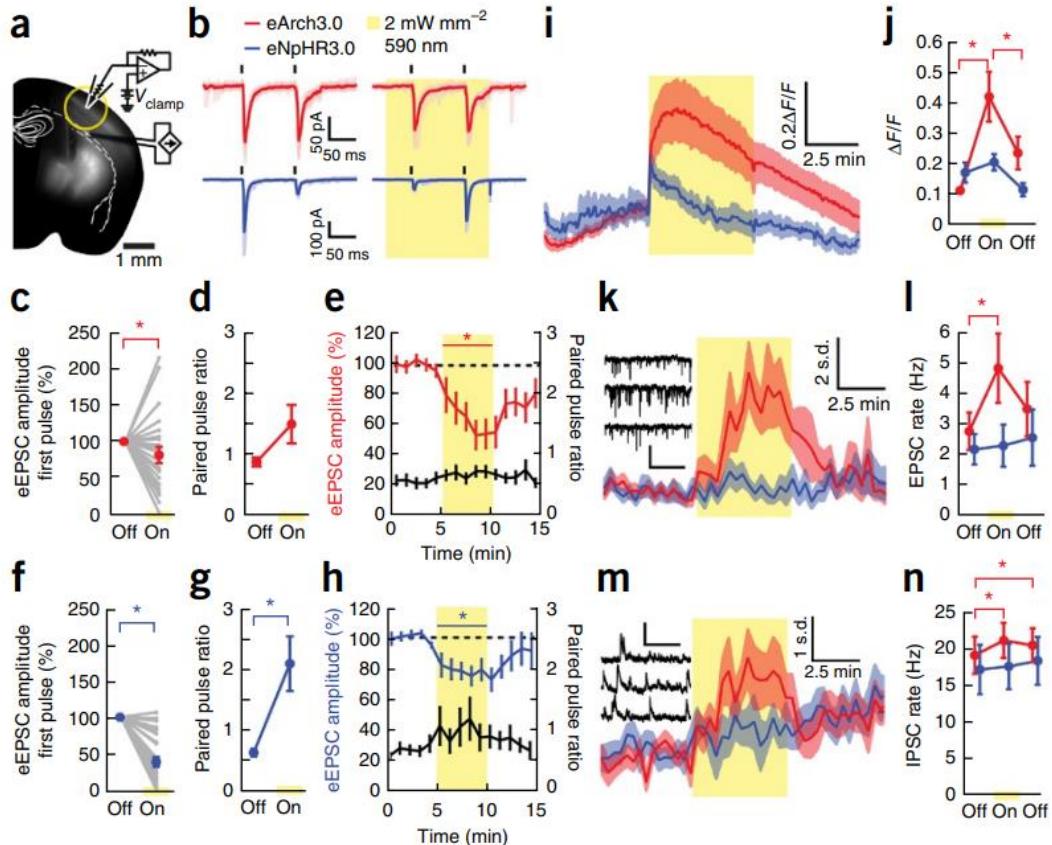


Soma vs axon terminal activation

- Jackman et al J Neuro 2014



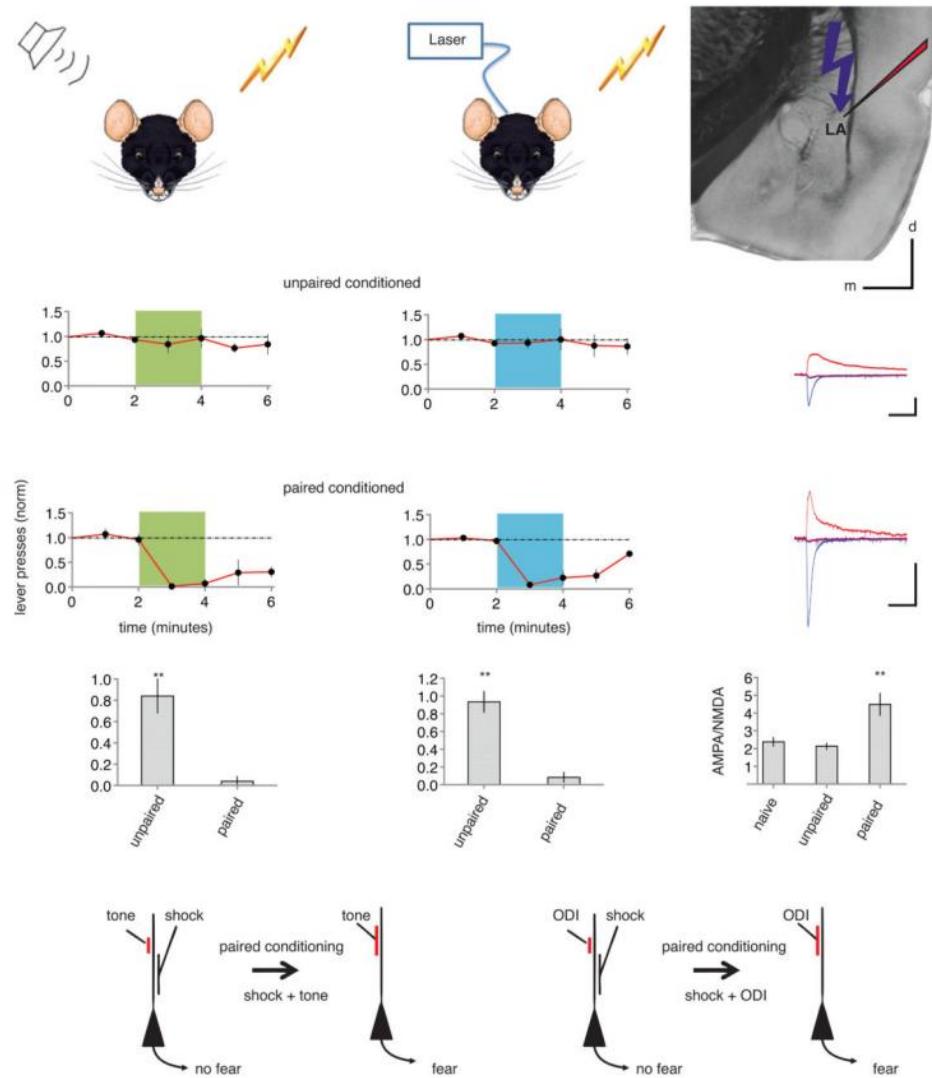
Terminal inhibition caveats



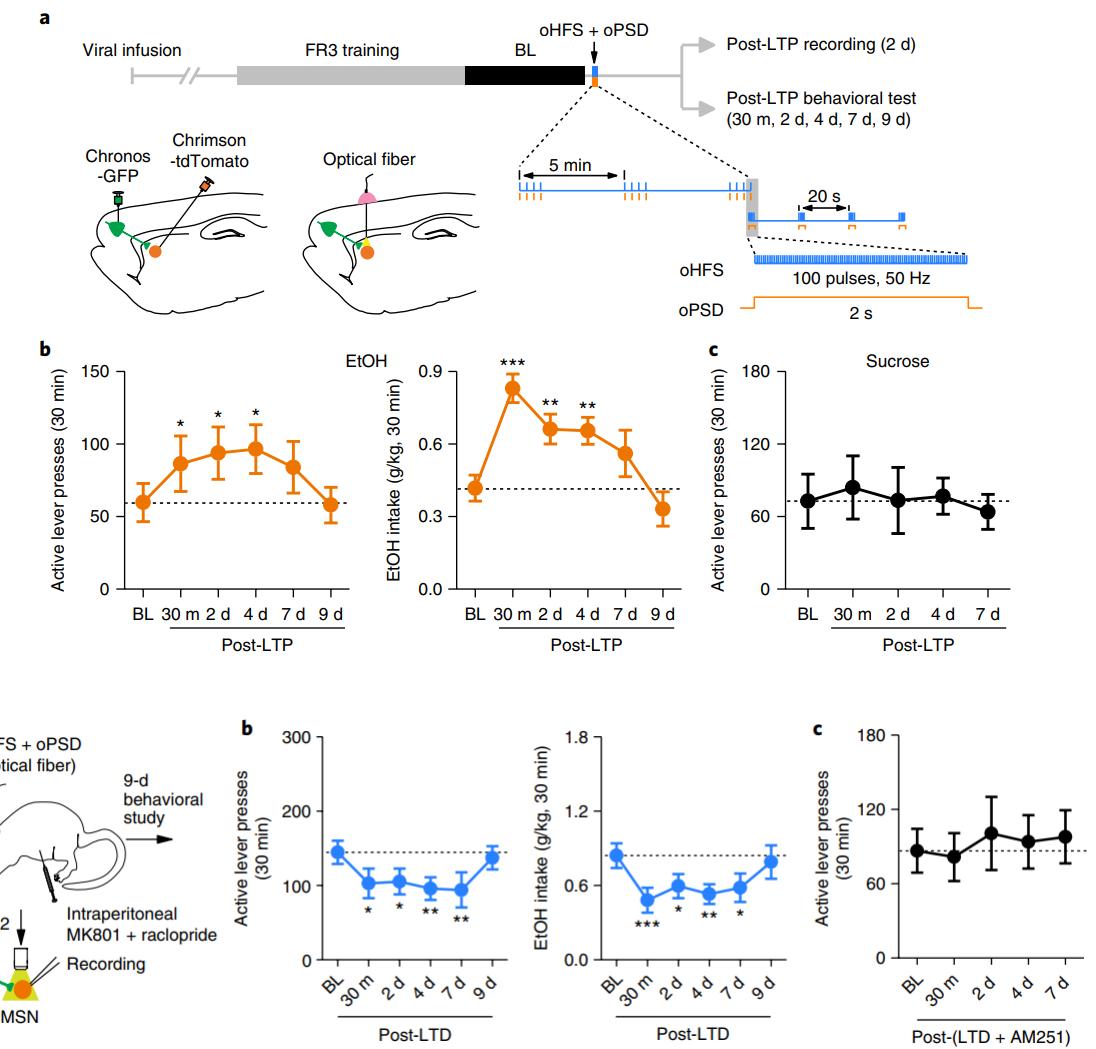
Mahn et al Nat Neuro 2016

El Gaby et al Cell Reports 2016

LTP/LTD examples



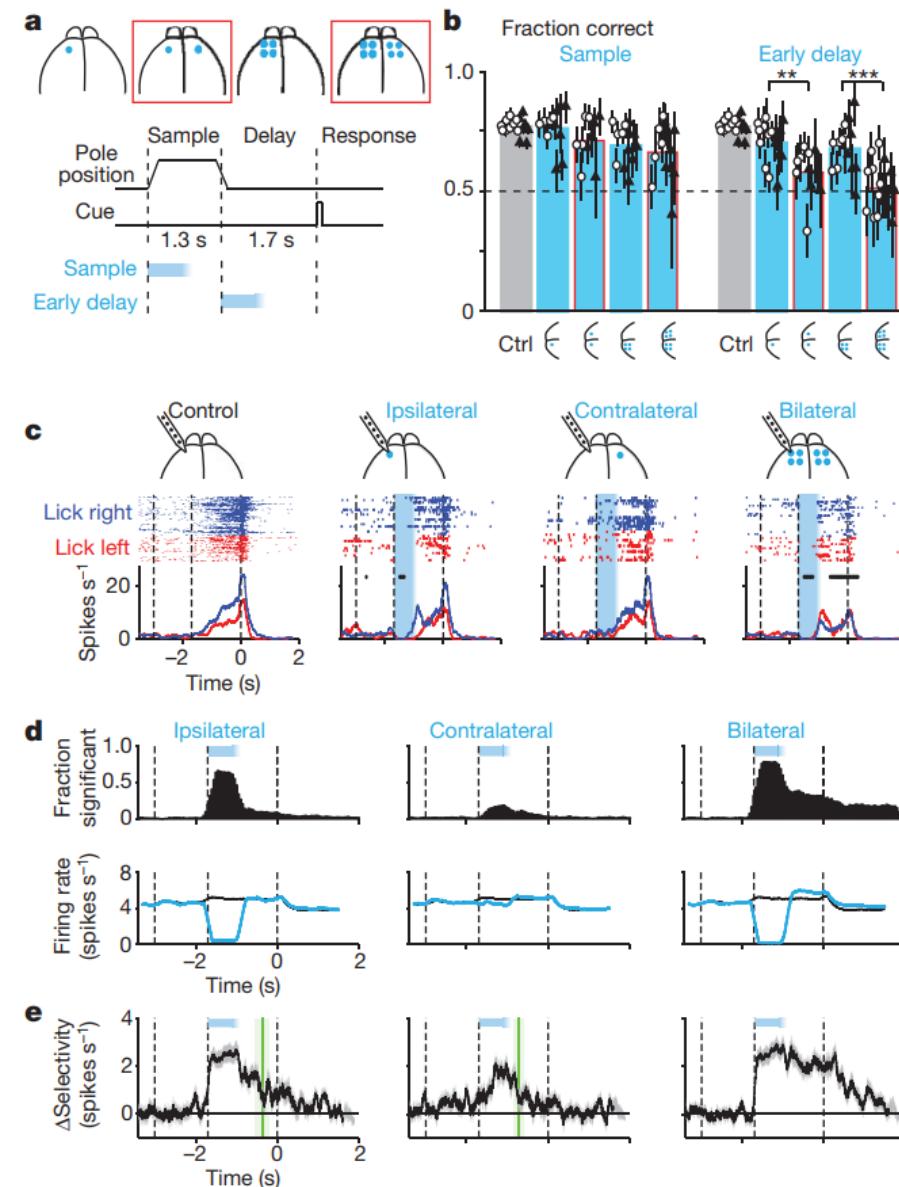
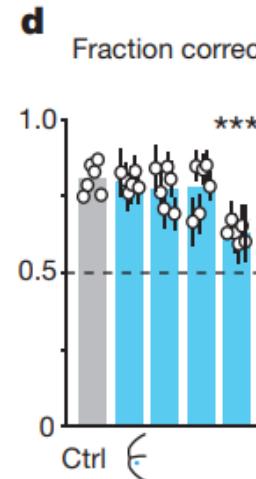
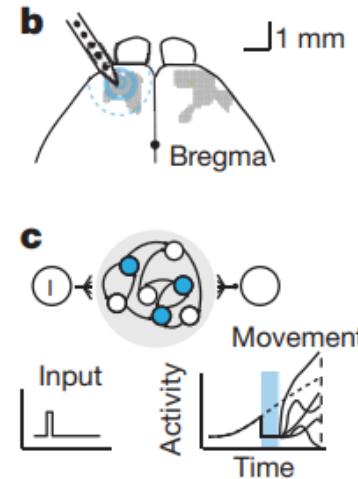
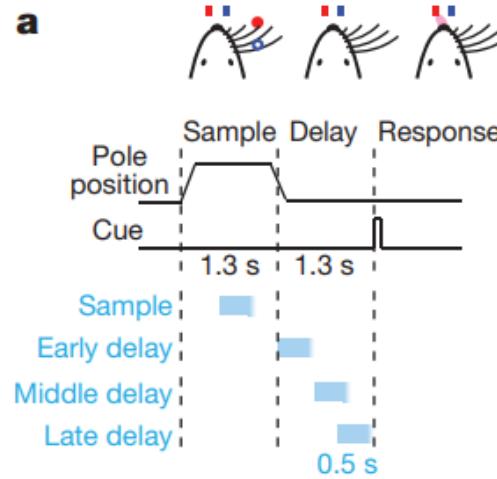
Nabavi, Fox Nature 2014



Ma et al Nat Neuro 2018

- State-dependent plasticity
- Disease related plasticity
- Homeostatic plasticity

Unilateral versus bilateral example



Complex neurochemistry example: DA → Str

