

In Vivo Optogenetics and Chemogenetics

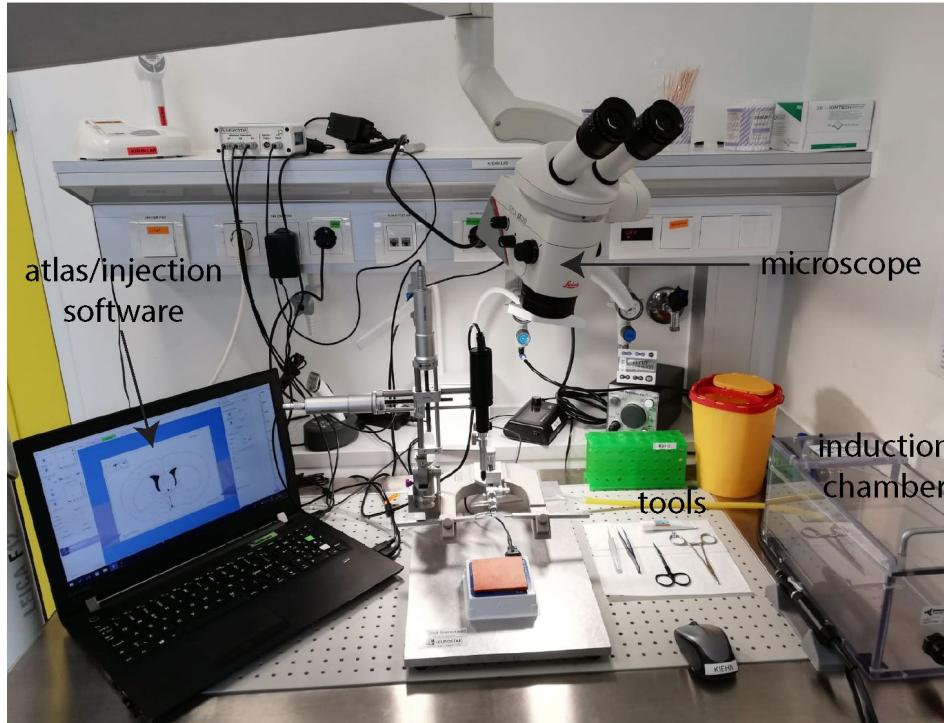
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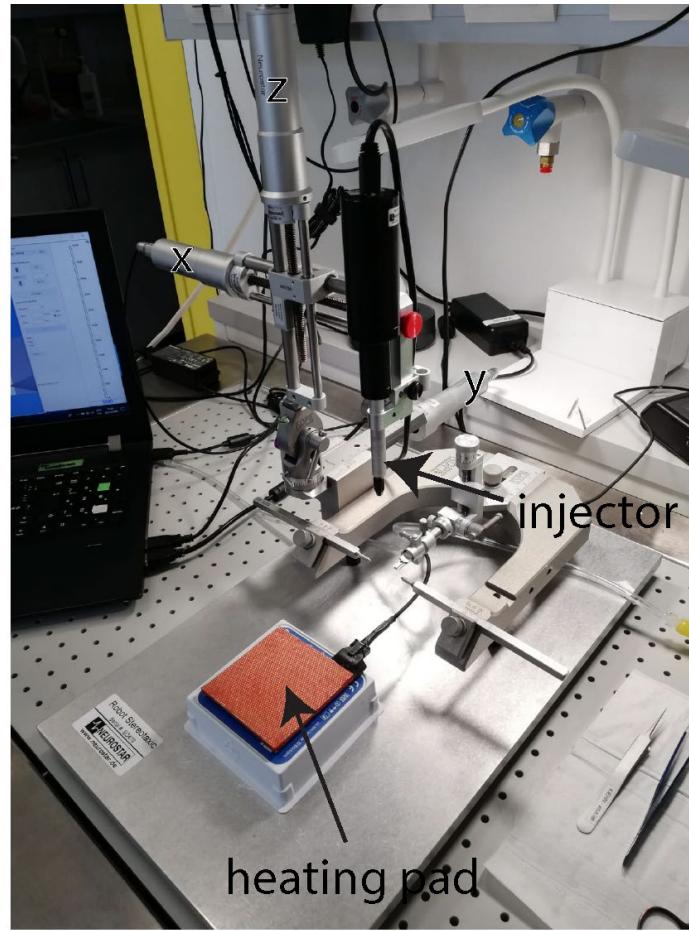


Surgical Setup



- computer has injection software, which contains brain atlas in 3D coordinate system
- computer controls the 3 arms of the stereotaxic setup, and nanoliter injector
- surgeries are performed under >10x magnification

Stereotax



- anaesthesia delivered via a nose cone
- animal's temperature is maintained on a heating pad
- 3 arm (x, y, z) robotic stereotaxic system
- nanoliter volume injector

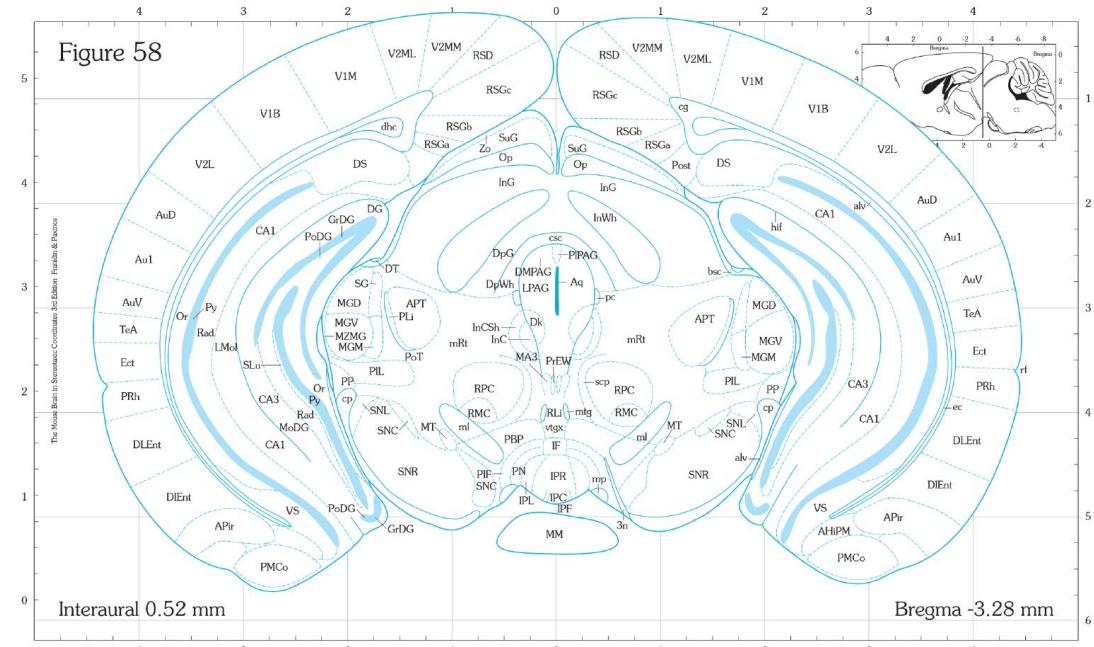
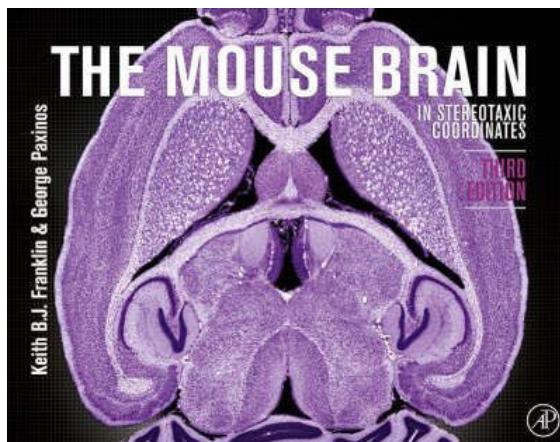
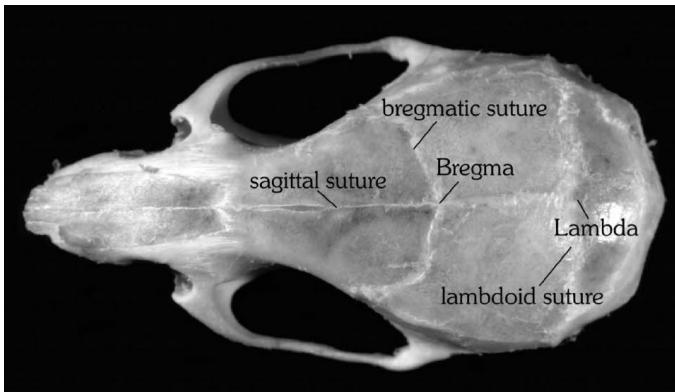
Anaesthesia



anaesthesia system

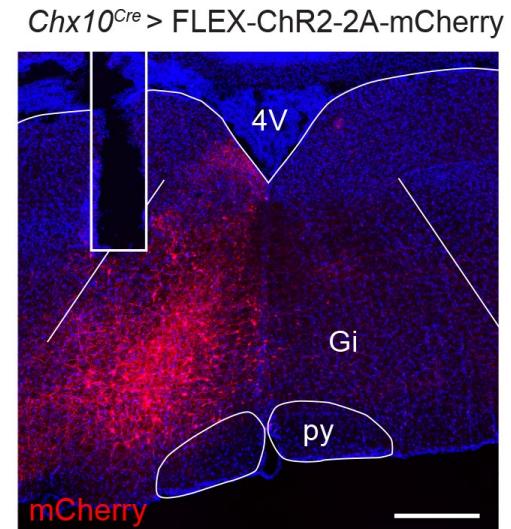
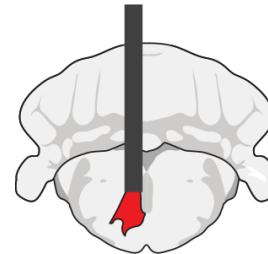
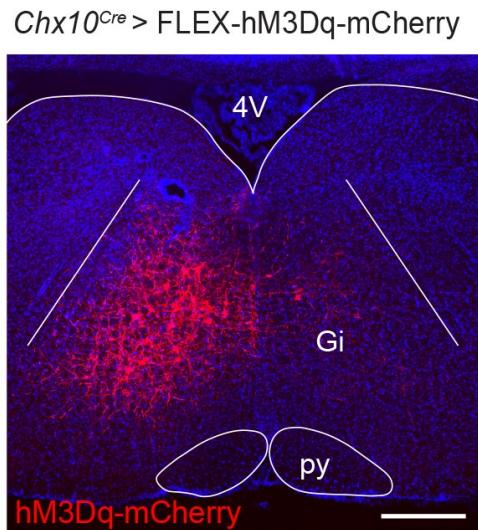
4% isofluorane for induction, 1.5-2% isofluorane for maintenance

Targeting a Specific Brain Region



Franklin and Paxinos – The Mouse Brain in Stereotaxic Coordinates

Viral expression 3 weeks after injection

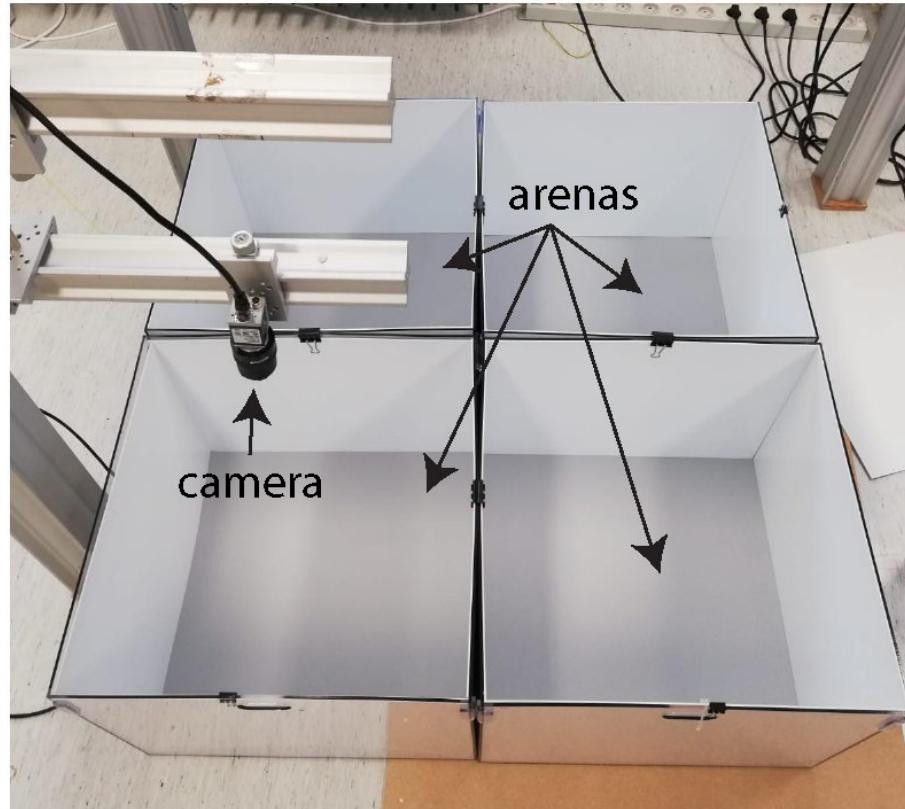


3 weeks after viral
injection in the Gi

viral injection + optical
fiber implantation

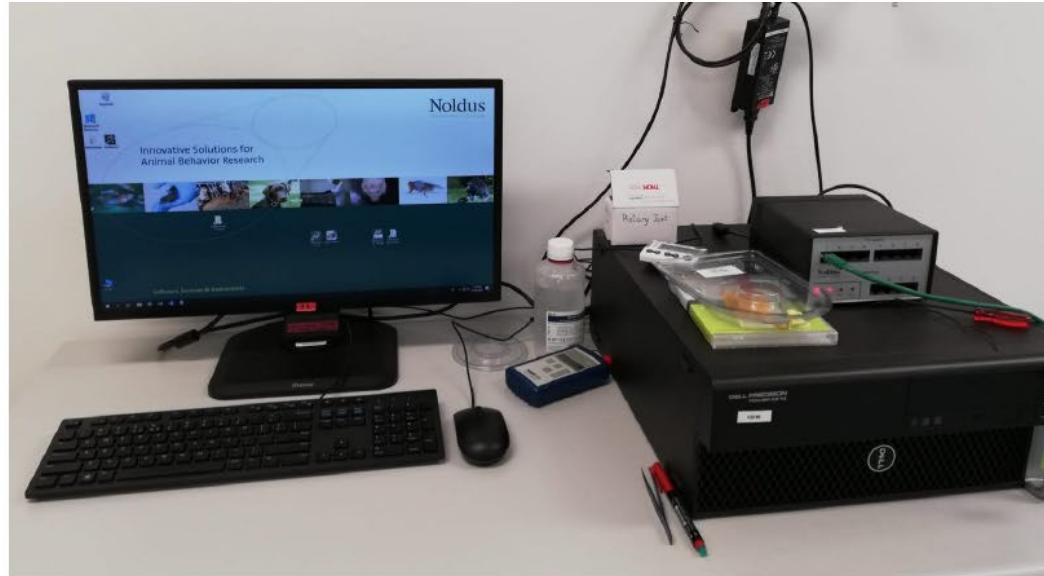
- Virus takes approximately 3 weeks for expression
- Fibers are implanted and attached to the skull with dental cement
- Expression is visualized post mortem using tissue immunocytochemistry

Behavioral Arenas



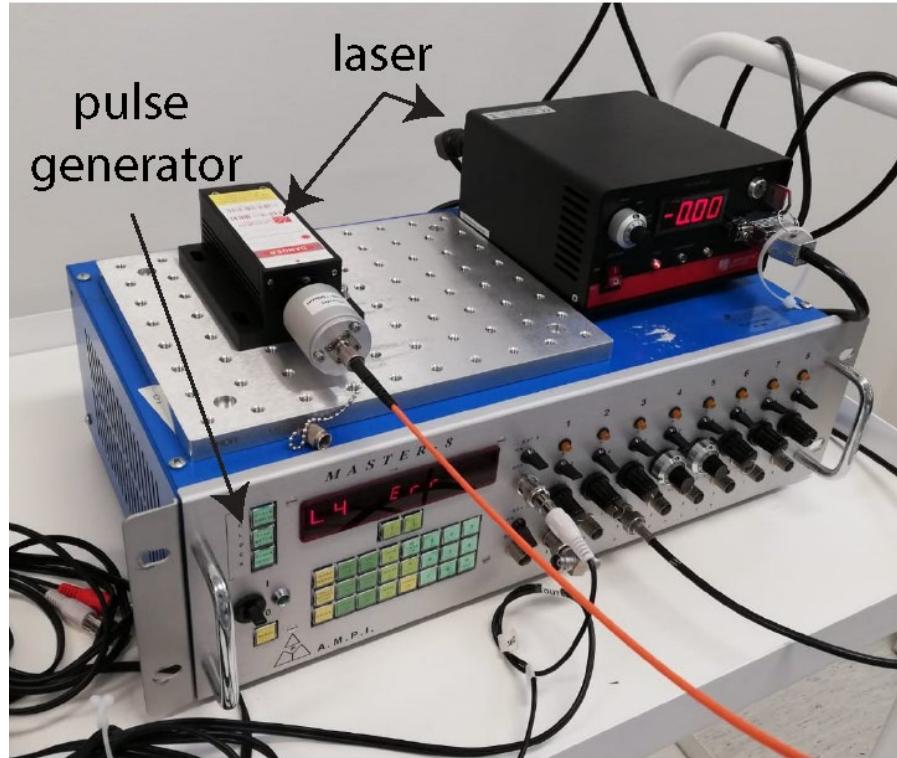
- Camera records freely behaving mice in the open field arena
- Neutral background color of the arena allows visual contrast
- Multiple arenas allows tracking

Live Behavioral Tracking



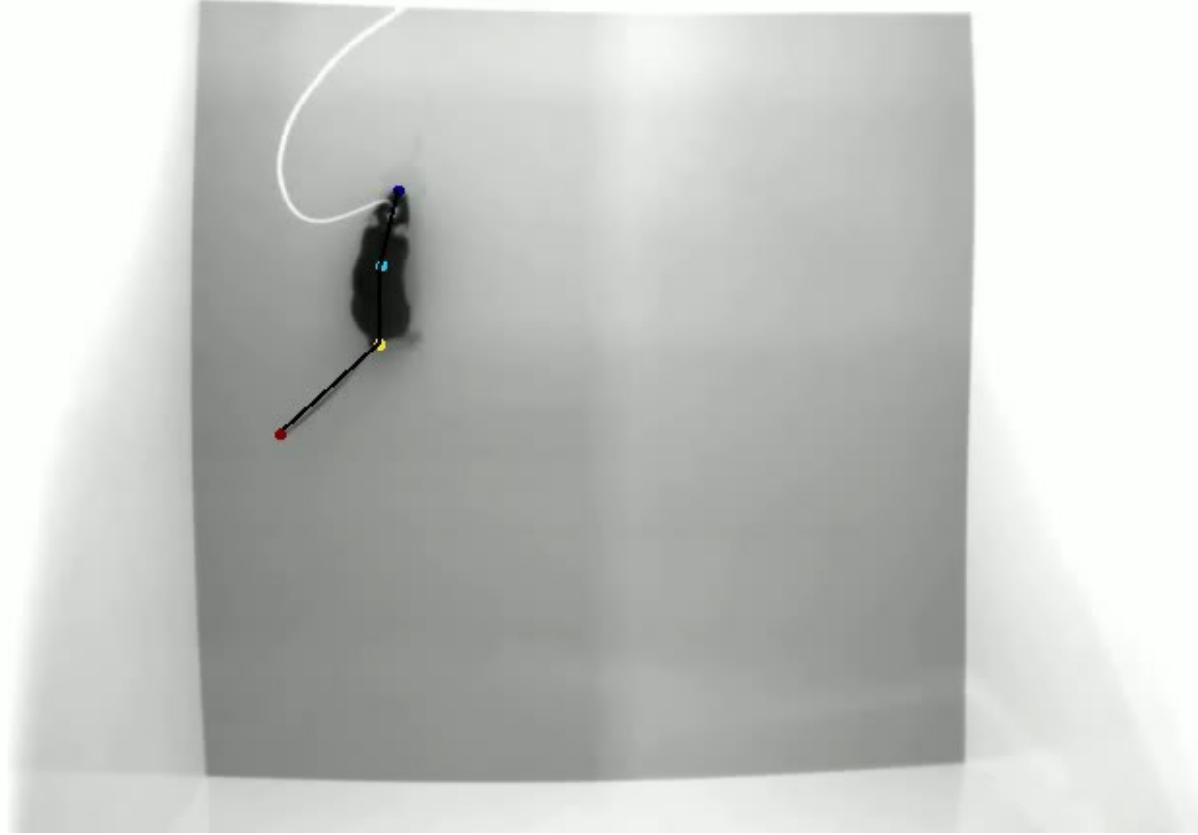
- Ethovision behavioural tracking software
- Live behavioural tracking and TTL triggering
- TTL triggering allows optogenetic stimulation in the framework of the behavioural tracking software

Optogenetic Stimulation



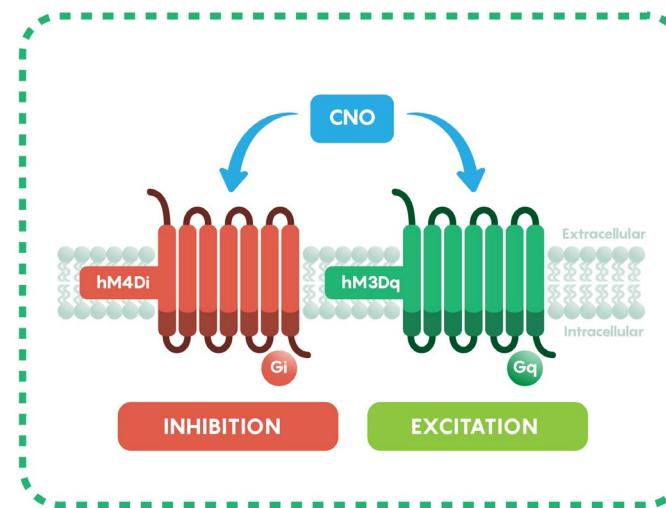
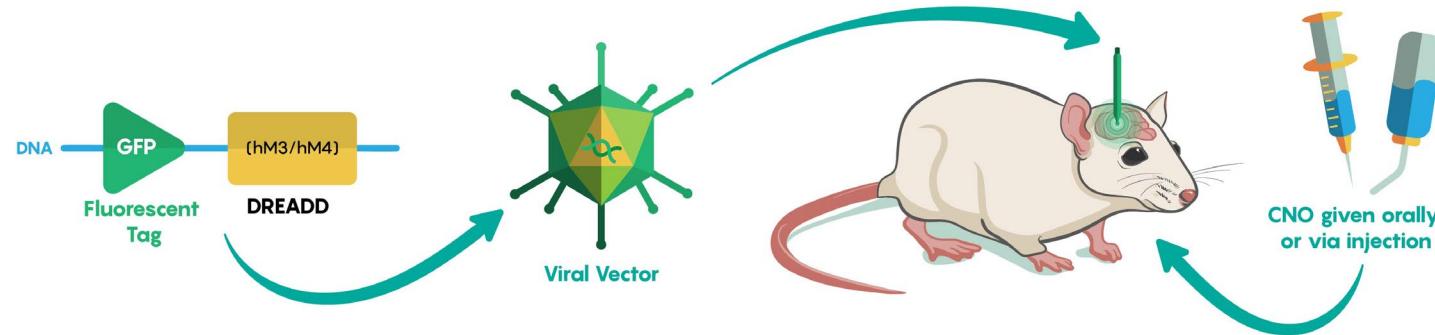
- TTL trigger from the behavioural tracking software engages the pulse generator
- the pulse (waveform) generator (Master-8) delivers voltage waveform to the laser
- the laser fires based on the input waveform

DeepLabCut Tracking of Mouse Behavior

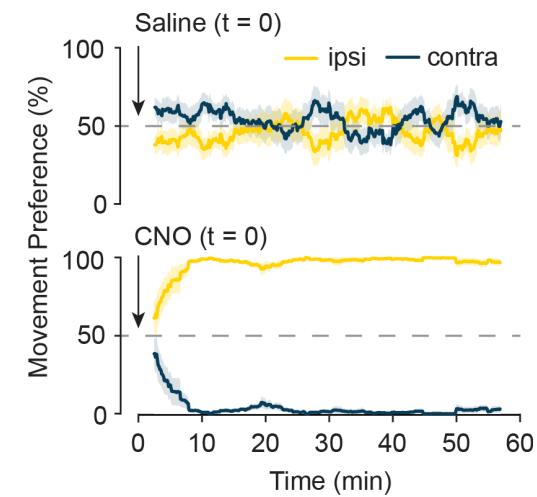
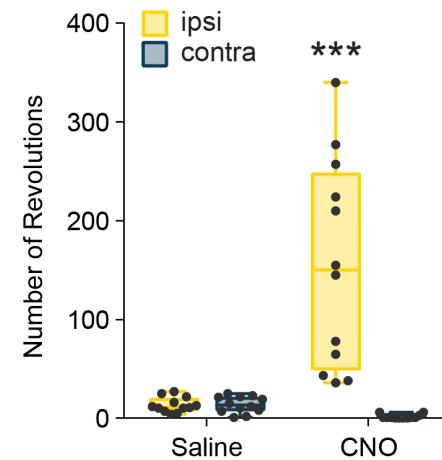
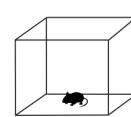
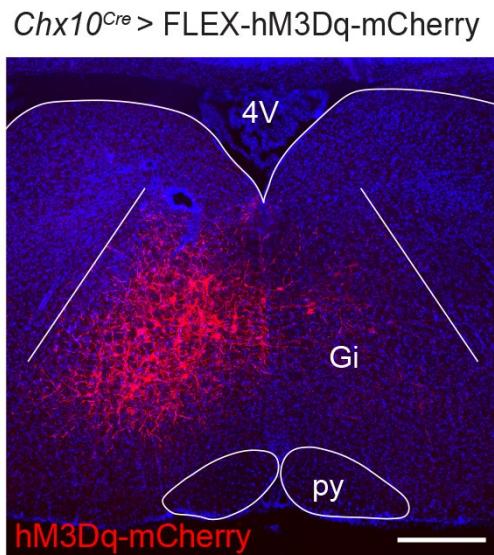


- A deep learning software (DeepLabCut) can be trained to recognize mouse features
- Automatic tracking of distinct mouse features

Chemogenetics



Chemogenetics: Unilateral Excitation of *Chx10* Gi Neurons in Freely Moving Mice



Unilateral excitation of *Chx10* Gi neurons causes ipsilateral turn

$Chx10^{Cre} > \text{FLEX-hM3Dq}$ (left Gi)

0 min

25 min

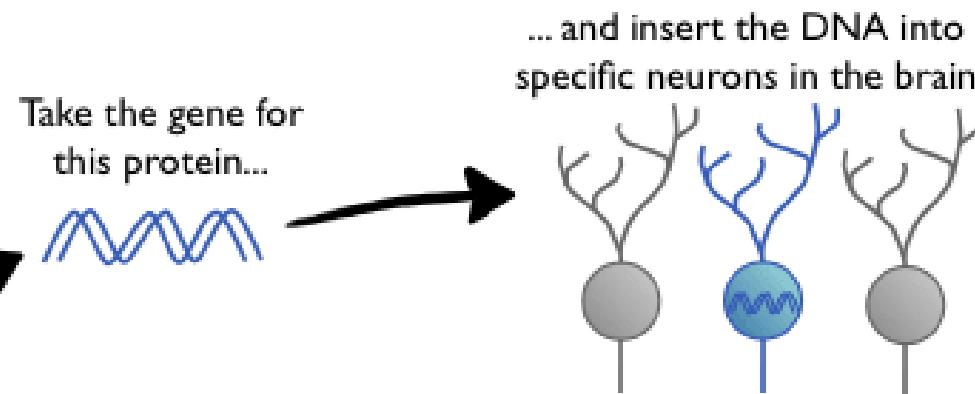
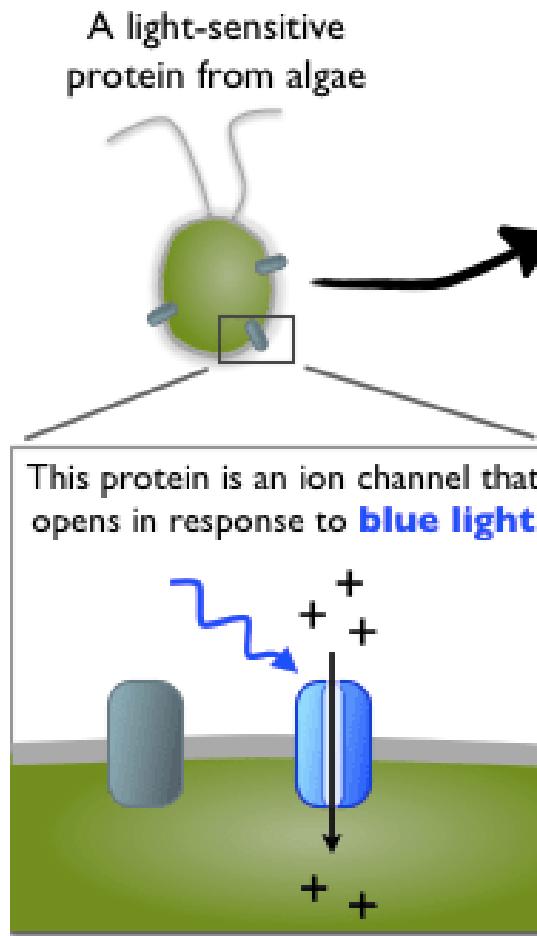


no left/right preference



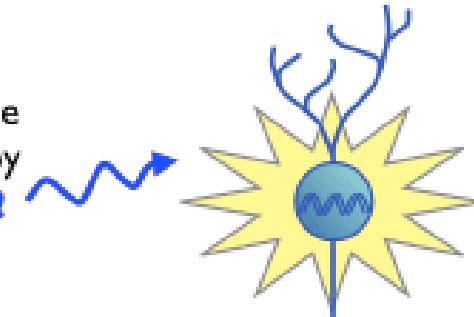
turning to the left

Optogenetics



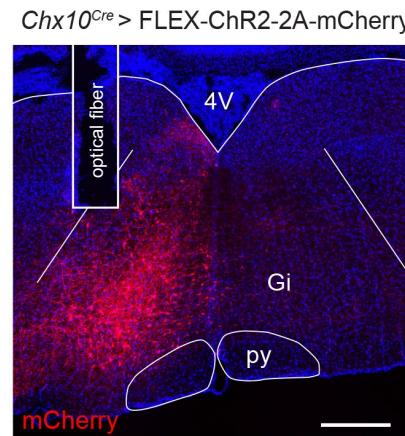
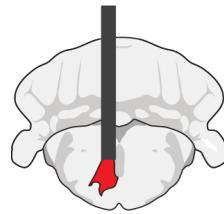
Neurons communicate by “**firing**.” This is an electrical signal created by opening & closing ion channels.

So now you can cause neurons to fire just by flashing **blue light!**



With the right combination of neurons, you can activate an entire brain circuit to control specific behaviors (like movement)

Unilateral Stimulation of *Chx10* Gi Neurons in Freely Moving Mice



$Chx10^{Cre} > \text{FLEX-ChR2}$ (left Gi)

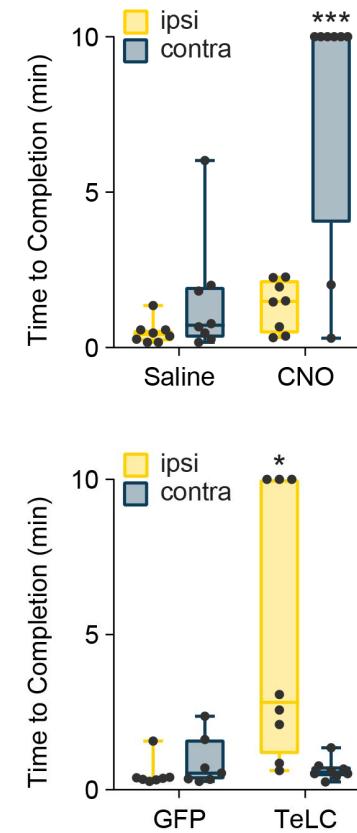
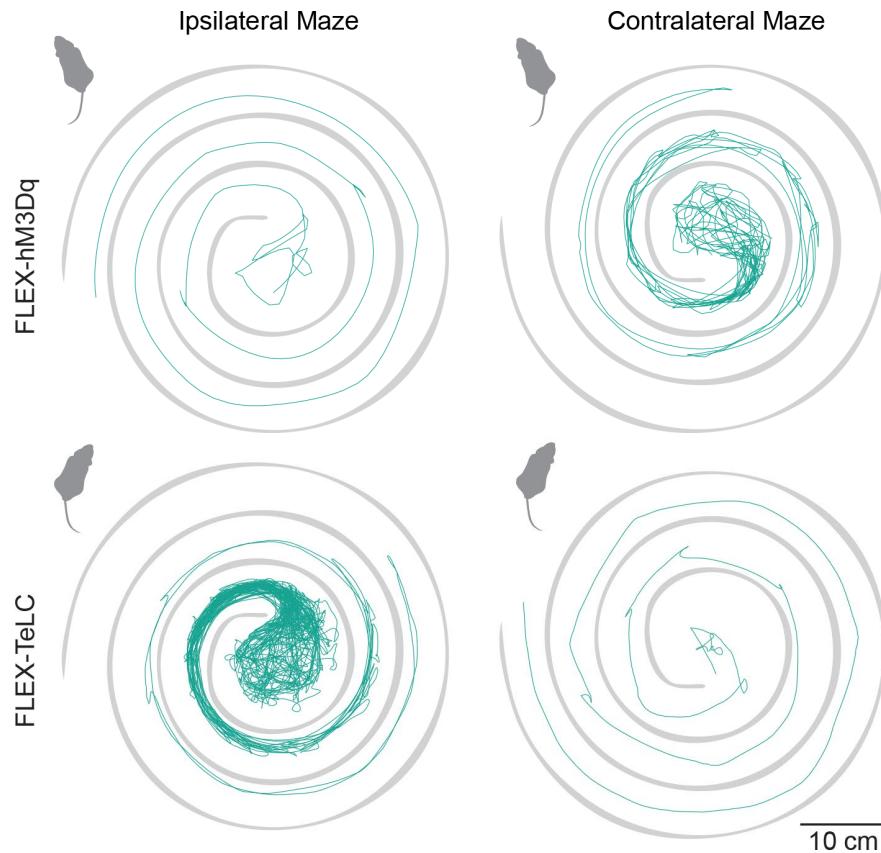
Example 1

Example 2



Light **ON** = turn left

Other behavior paradigms – the 'Spiral Maze'



Summary

- Stereotaxic viral injection allows specific types of neurons to be infected with specific neuronal actuators (DREADDs, opsins)
- Mouse behavior can be monitored using video recording and behavioral tracking software
- Manipulation of specific types of neurons in the brain allows causal link between neuronal function and behavior
- Chemogenetics allows chronic activation or inactivation of distinct neuronal subtypes
- Optogenetic manipulation allows acute manipulation of neuronal subtypes with millisecond-scale precision

Questions?