

Tutorial:

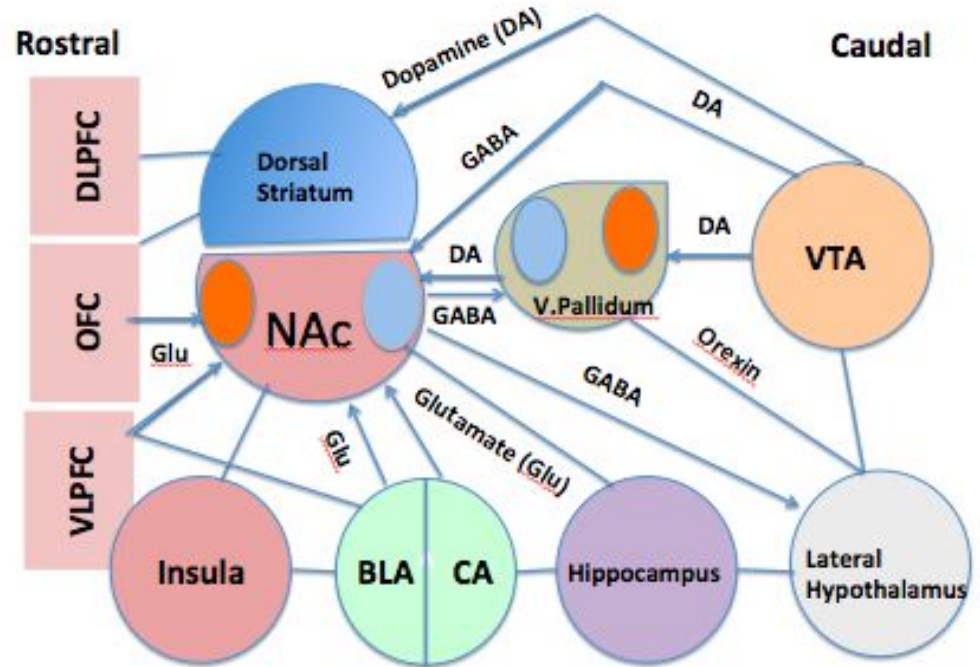
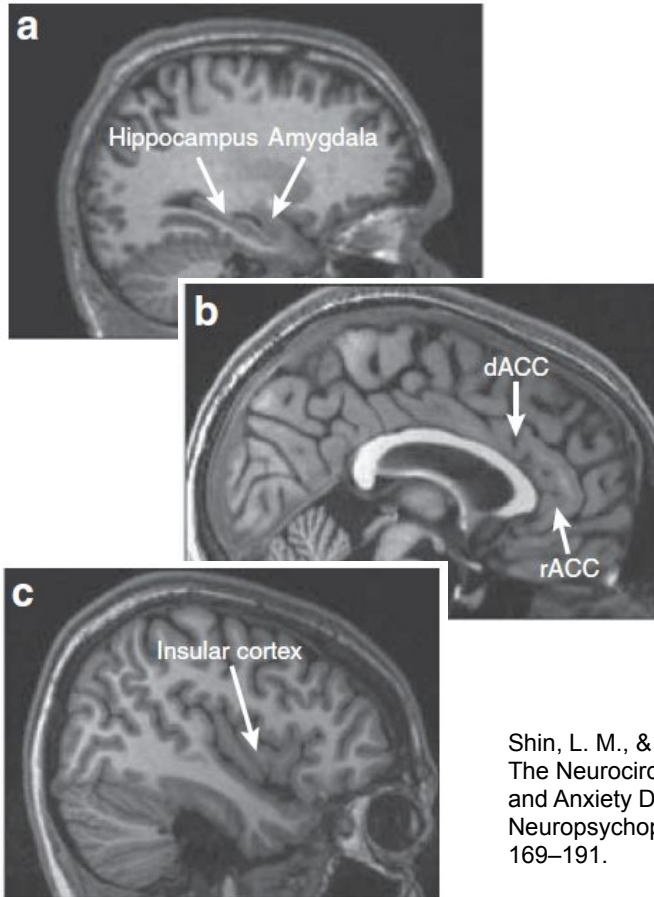
Optogenetic interrogation of neuronal circuits involved in fear and anxiety

Alana Darcher

Outline

- Background
 - Emotional circuits
 - Fear/Anxiety: Mouse models
- Designing an optogenetic experiment
- Methodological considerations
- Viral expression + Surgery
- Behavior + Optogenetics
- Analysis
- Histology + Microscopy
- Summary

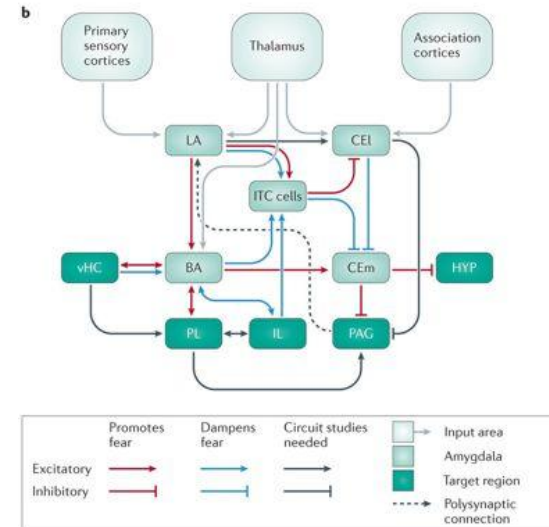
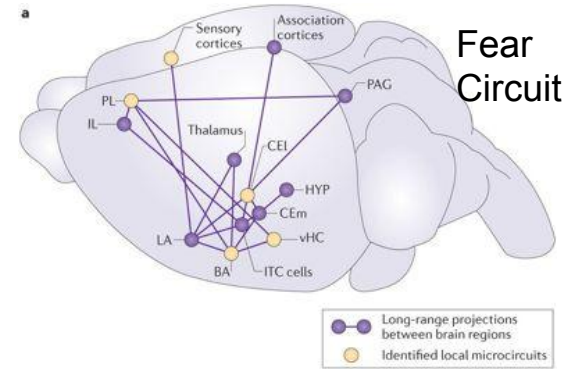
Emotion Circuits



Shin, L. M., & Liberzon, I. (2010).
The Neurocircuitry of Fear, Stress,
and Anxiety Disorders.
Neuropsychopharmacology, 35(1),
169–191.

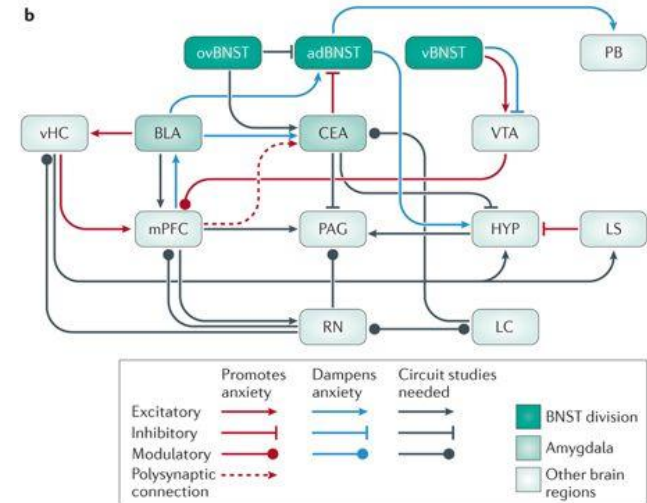
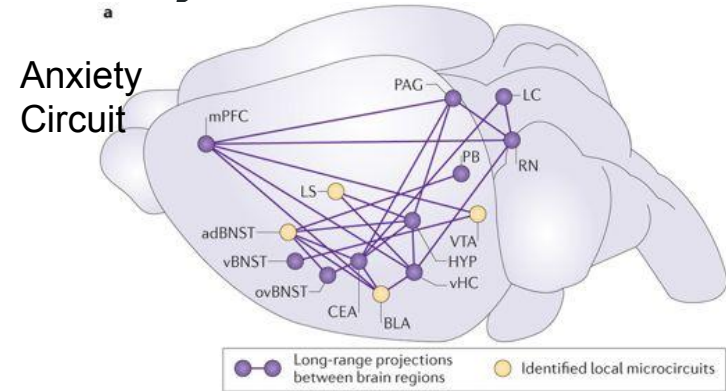
<https://www.drmanipavuluri.com/explaining-brain/reward-and-emotion-circuits-are-linked-in-the-brain-a-hot-meas-if-things-go-wrong/>

Animal models for studying fear/anxiety

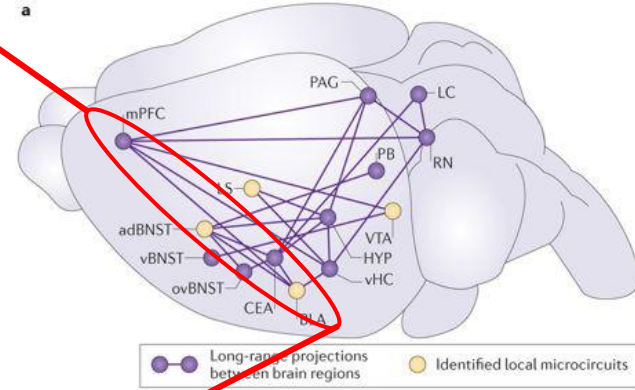
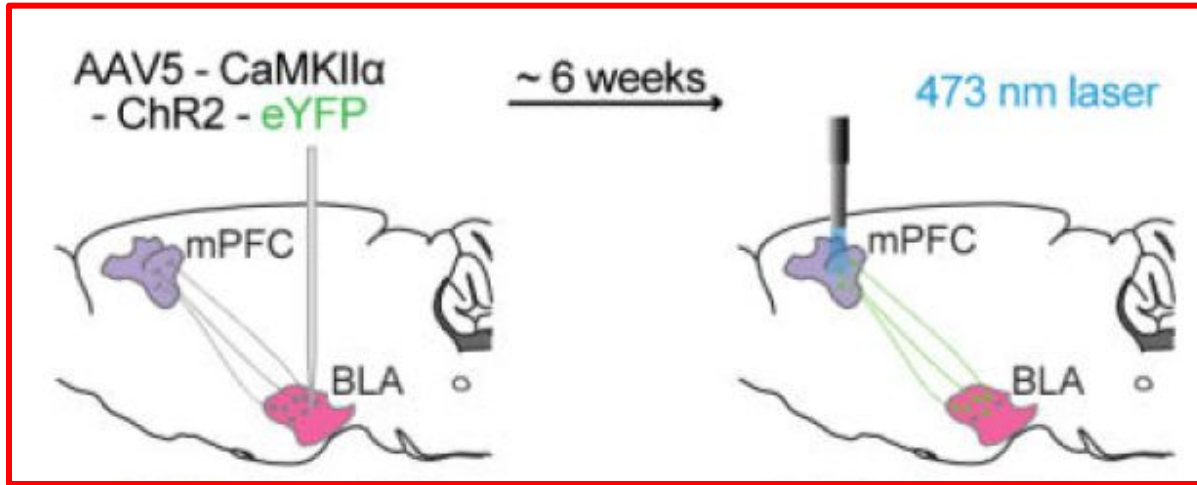


- Analogous behaviors in animals
- Network/circuitry involved in fear & anxiety highly conserved across species and evolution

Tovote P, Fadok JP, Lüthi A.
(2015) Neuronal circuits for fear and anxiety.



Elucidating circuitry with optogenetics



Optogenetics:

- Target not just nuclei, but also projections
 - Can establish causal roles of connections between areas
 - Highly complementary to imaging techniques
- More clear idea of circuit parts underlying behavior

A.C. Felix-Ortiz, A. Burgos-Robles, N.D. Bhagat, C.A. Leppla, K.M. Tye (2016)
Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex

Methods for investigating circuits

Classic:

- Lesions (patients/animals)
- fMRI

Modern:

- Optogenetics (virtual lesion/induced activity)
- Paired with behavioral paradigms!

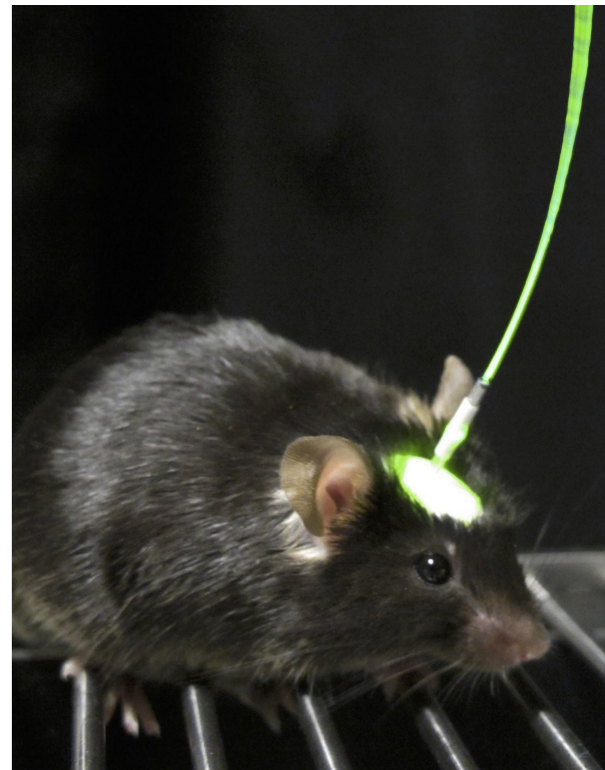


Image credit NSF, Inbal Goshen, Karl Deisseroth

Running an experiment

Optogenetics experiment: Timeline

Considerations:

- Region of Interest
- Method of transduction, serotype
- Obtaining animals

Build optic fibers

Surgery: inject viral cassette, implant fibers

Aftercare

Viral expression

Handling

Behavioral Assays

Histology

Analysis

1-2 days

1-2 days

~3 days

4-8 weeks

~3 days

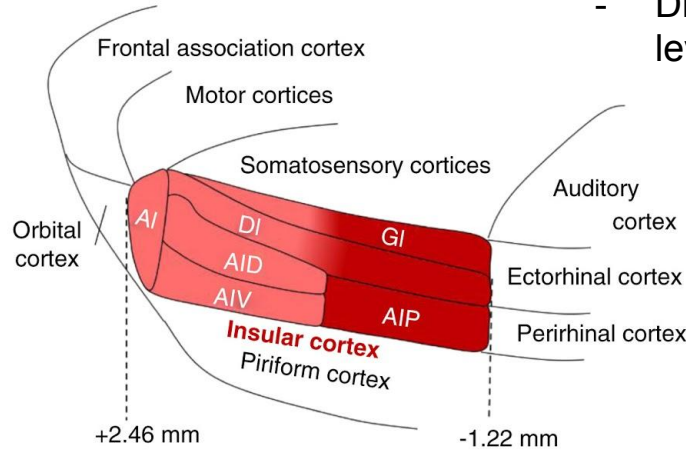
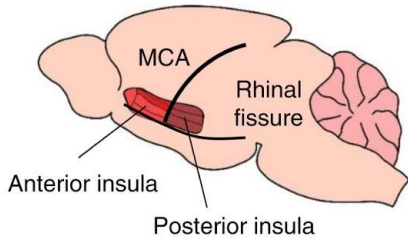
~days-months

1-2 days

Choosing a region of interest & viral serotype

B

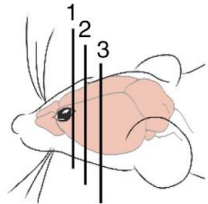
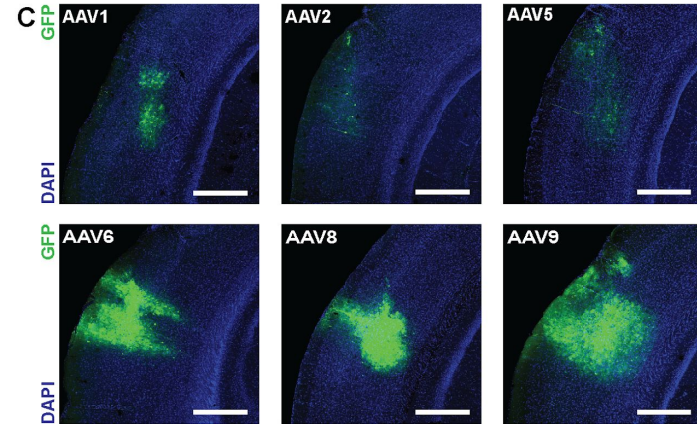
Mouse brain



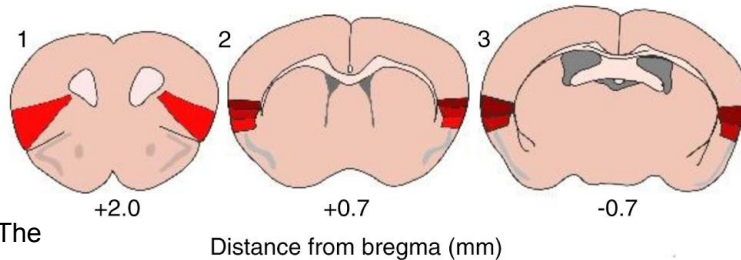
Adeno-associated virus:

- Not very pathogenic
- Differences in capsid proteins alters expression levels based on cell type
- Serotype efficacy varies with brain region

Shown: AAV-variant expression in auditory cortex



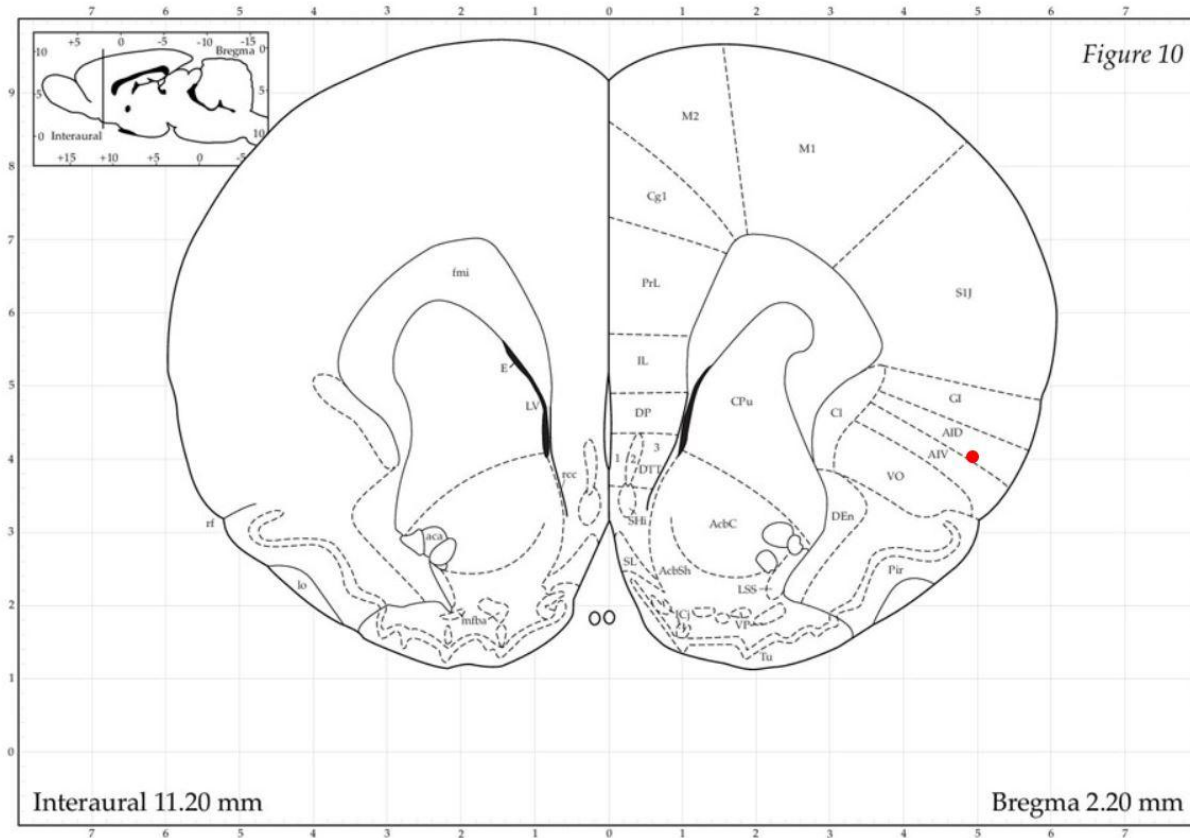
Gogolla (2017) The insular cortex



Current Biology

Aschauer DF, Kreuz S, Rumpel S (2013) Analysis of Transduction Efficiency, Tropism and Axonal Transport of AAV Serotypes 1, 2, 5, 6, 8 and 9 in the Mouse Brain. PLoS ONE 8(9): e76310.

Choosing stereotaxic coordinates

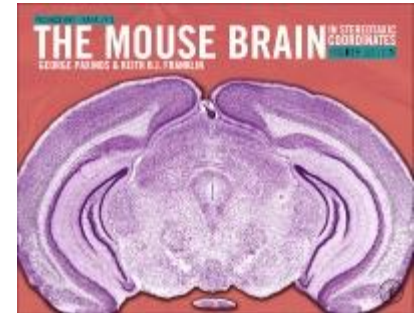


AI cortex= agranular insular cortex

- AIP: posterior
- AID: dorsal
- AIV: ventral

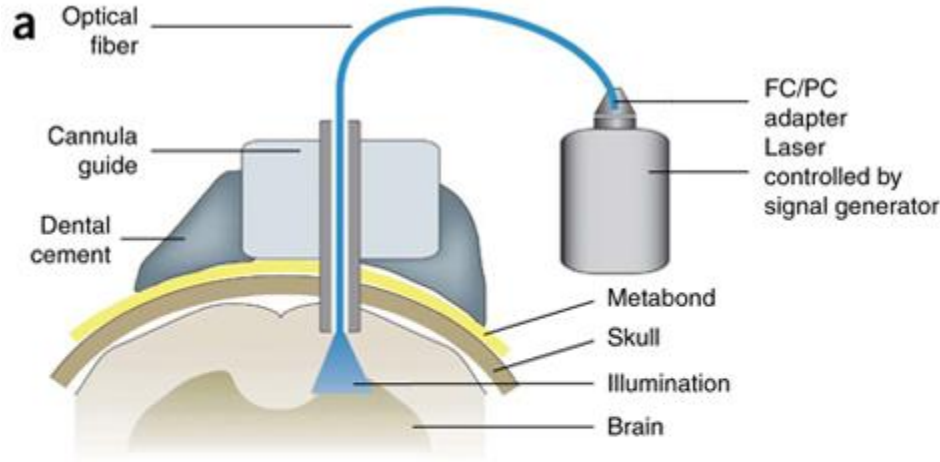
GI = granular insula

Paxinos
Atlas



Paxinos, George, and Keith B.J. Franklin. The mouse brain in stereotaxic coordinates: hard cover edition. Access Online via Elsevier, 2001.

Optogenetics: materials and implanting

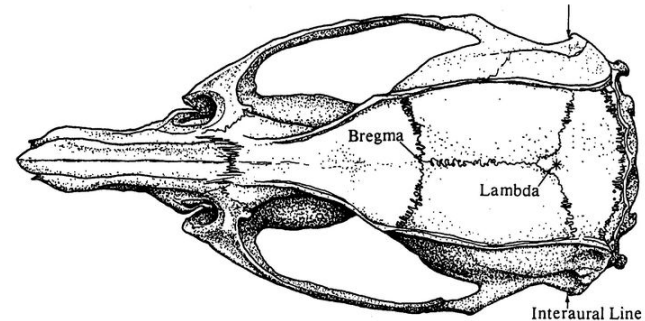


Optic fiber components:

- Glass rod, cannula, coupler

Laser light delivery:

- Calibrated to λ preferred by opsin, & behavioral factors
- Titrated to every experimental animal
 - Don't know how much viral expression there



<https://web.stanford.edu/group/dlab/optogenetics/hardware.html>

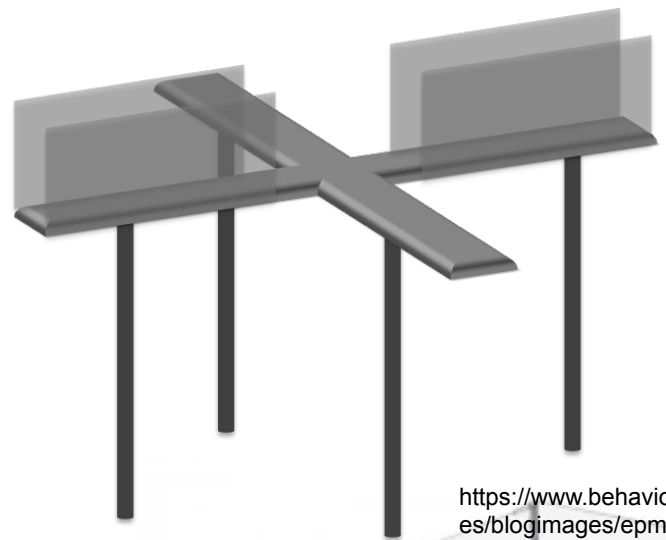
<https://www.leicabiosystems.com/pathologyleaders/navigator-through-the-brain-stereotaxic-atlases-for-neuroscience-research/>

Behavioral paradigms



← Fear conditioning box

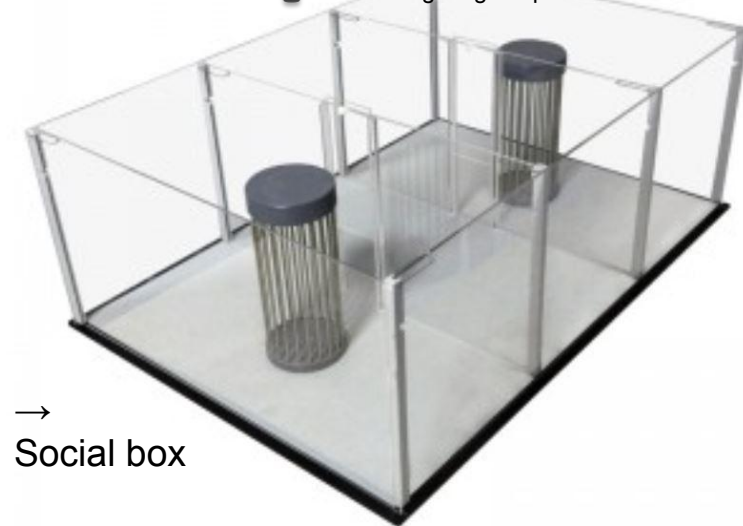
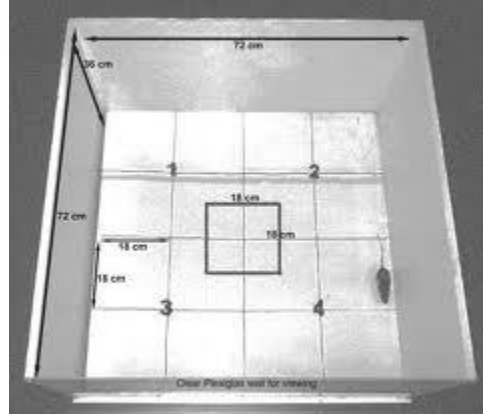
Elevated plus maze →



<https://www.behaviorcloud.com/images/blogimages/epm-1e0364d1>

Source: Harvard Apparatus

Open field test →



→
Social box

Behavior assay + Optogenetics

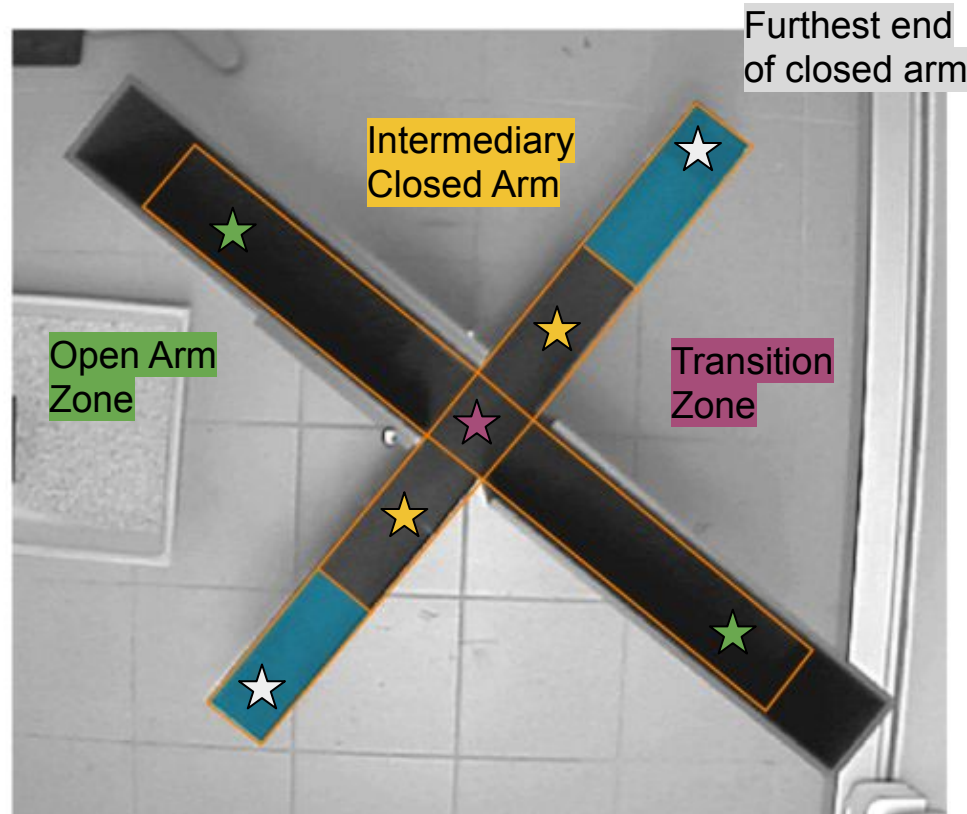
Selectively modulate neural activation according to conditions of the behavioral assay

Modulate according to:

- General time blocks
- Free-roaming behavior
- Discrete actions (e.g. head-dipping)

→ Some behavior can be automatically coded, some by hand

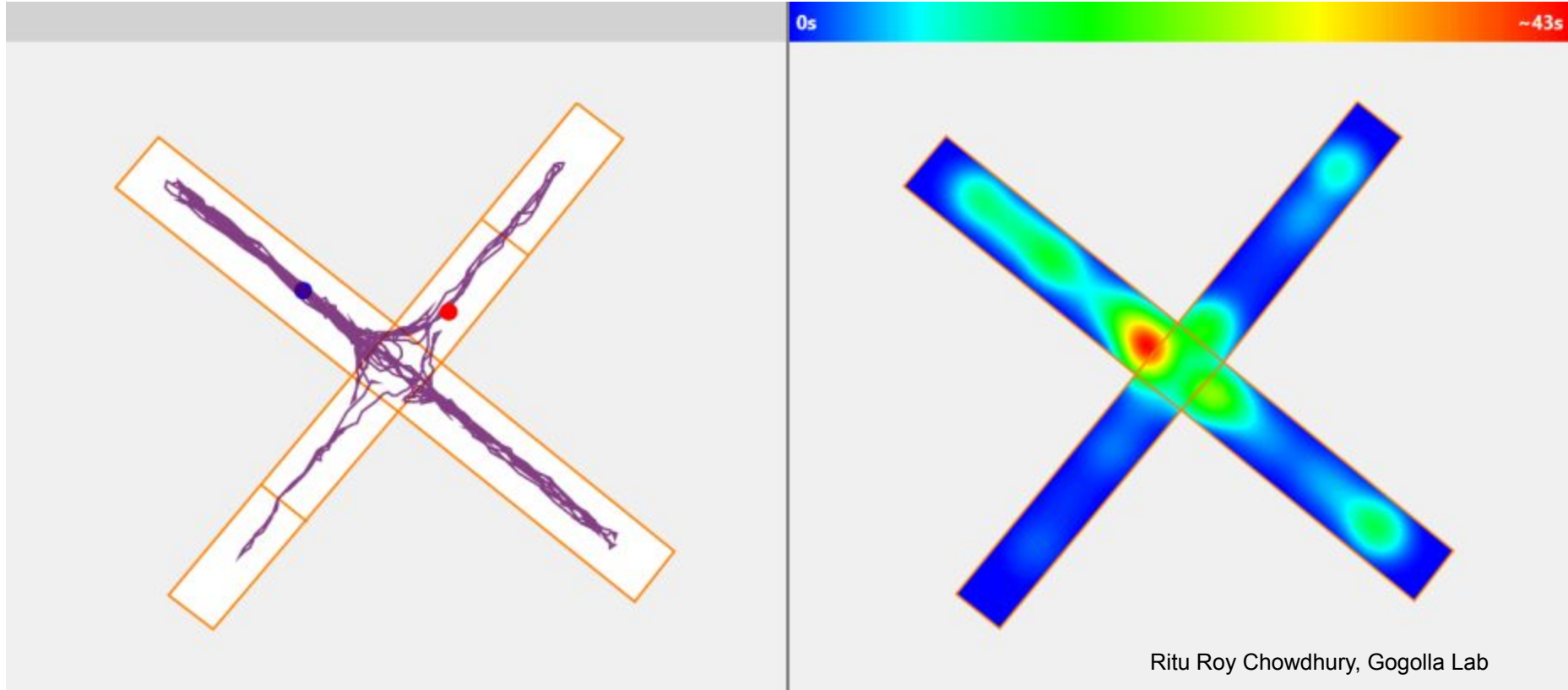
→ Modulation blocks differ in statistical sensitivity



Behavioral analysis



ANY-maze
Behavioural tracking software



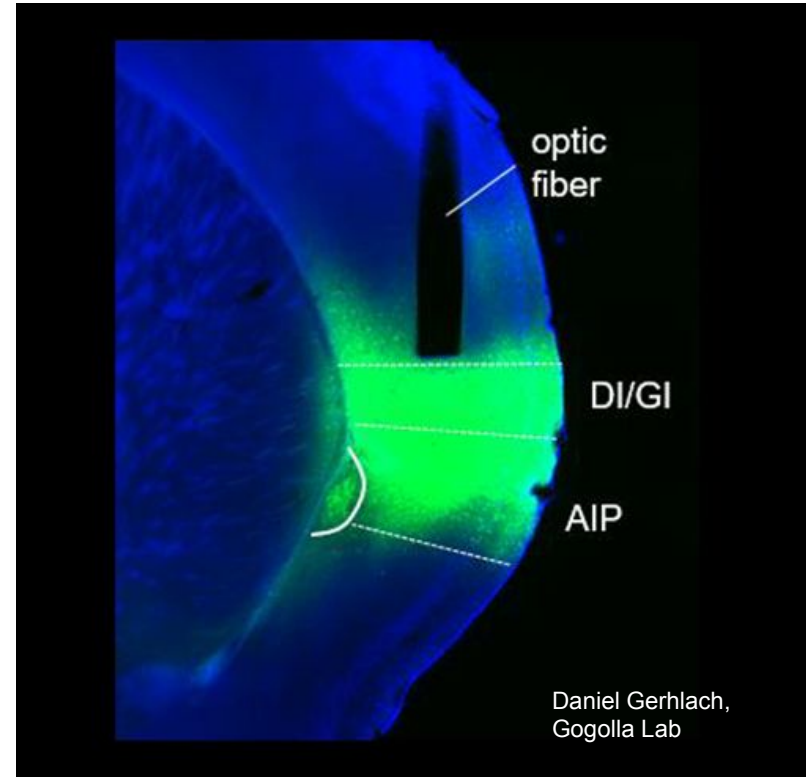
Ritu Roy Chowdhury, Gogolla Lab

Histological analysis

Did you actually implant where you wanted?

How intensely was the opsin expressed?

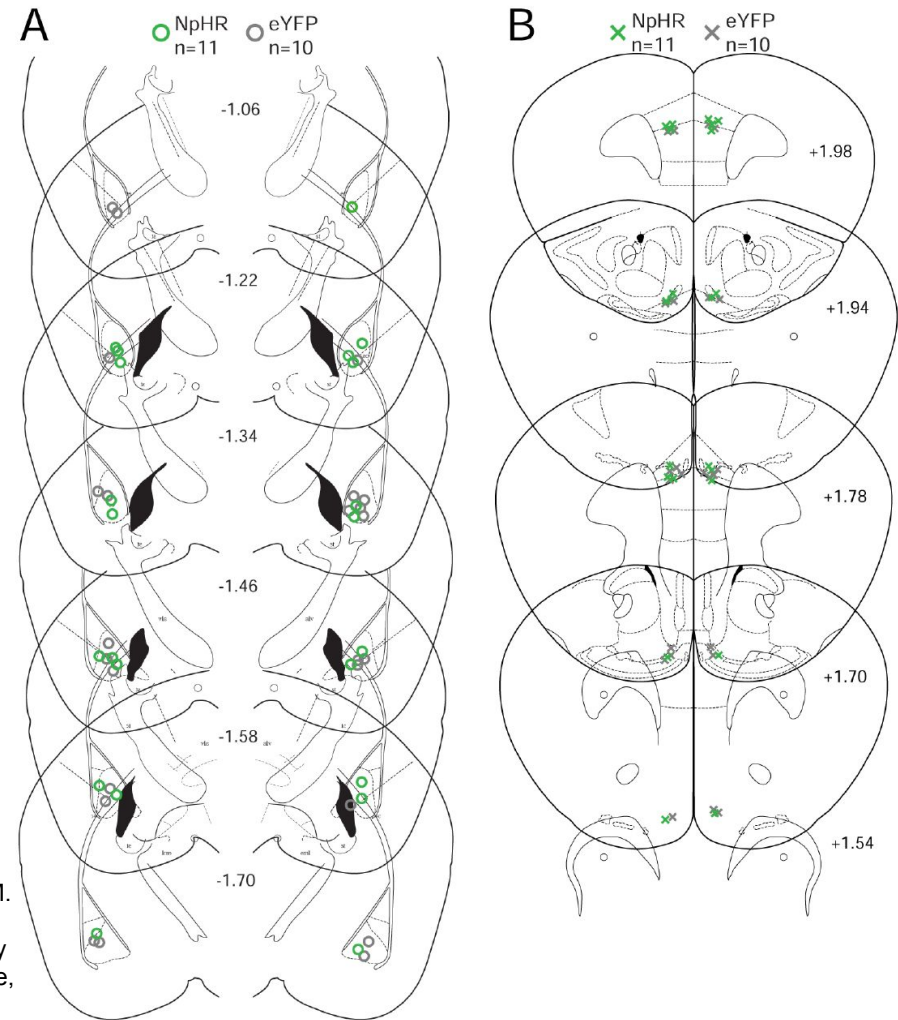
Where there any unexpected projections passing through the transduction site?



Shown: sites of local viral
infusion/fiber placement

- Each circle represents the implantation point for a different animal
- Range of test sites \neq failed experiments
- Still investigating local circuits, getting a broader picture of the function

A.C. Felix-Ortiz, A. Burgos-Robles, N.D. Bhagat, C.A. Leppla, K.M. Tye (2016)
Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex *Neuroscience*, 321: 197-209.



Summary

- Modern imaging and optic techniques provide a robust line of investigation into neural circuits underlying fear and emotion
- Optogenetics offers a toolbox for determining causal roles of nuclei and projections involved in complex behaviors
 - Great flexibility in experimental design
 - But, many potential confounds
- Obtaining meaningful results requires thoughtful analysis