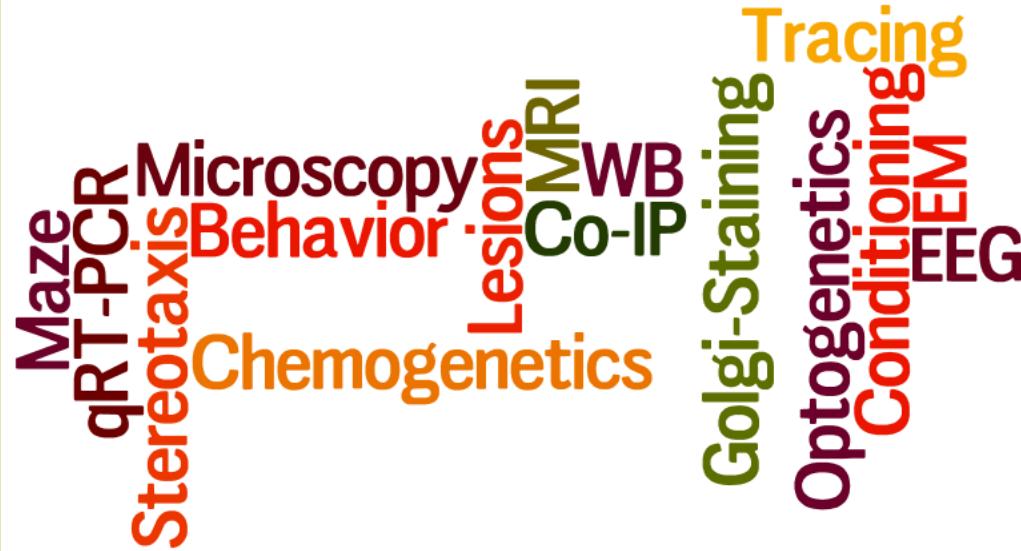


WEEK 1

HOW TO STUDY THE NERVOUS SYSTEM?

Learning objectives

- Know how to study brain structural changes
- Know how to study cellular changes in structure/function
- Know how to manipulate cellular/brain function



Maze
qRT-PCR
Stereotaxis
Microscopy
Behavior
Chemogenetics
Lesions
MRI
WB
Co-IP
Golgi-Staining
Tracing
Optogenetics
Conditioning
EM
EEG

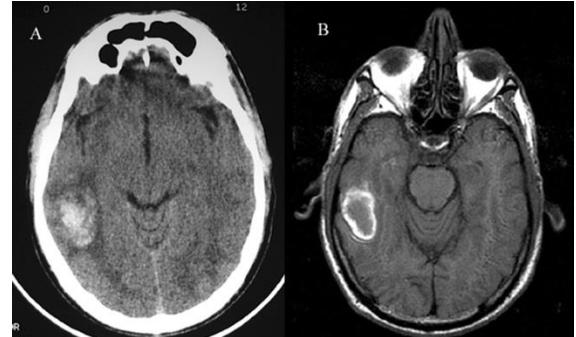
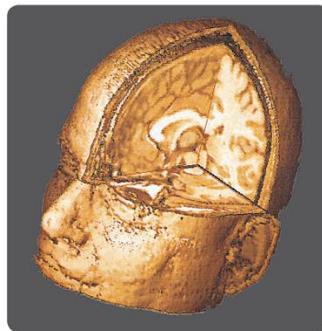
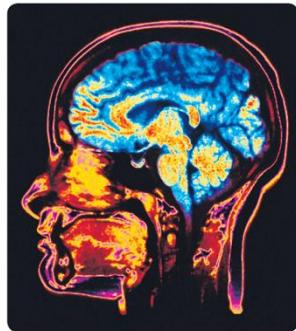
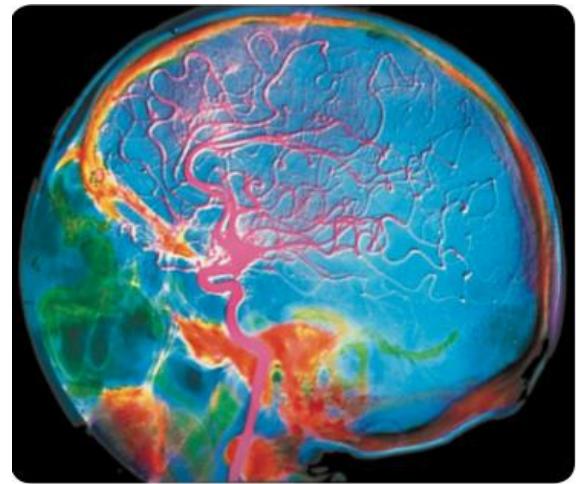
How would **you** study the nervous system?



- Structure
- Function

1) Brain structure

- Dissections (invasive, *post-mortem*)
- Imaging techniques (non-invasive, *in vivo*)
 - Contrast X-ray technologies
 - X-ray Computed Tomography (CT)
 - Magnetic Resonance Imaging (MRI)



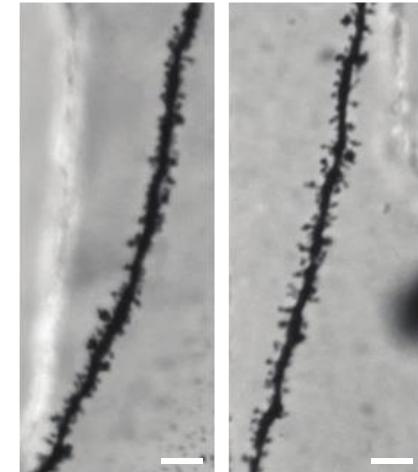
2) Brain/cellular structure

- **Neuroanatomical techniques**

- **Golgi stain**

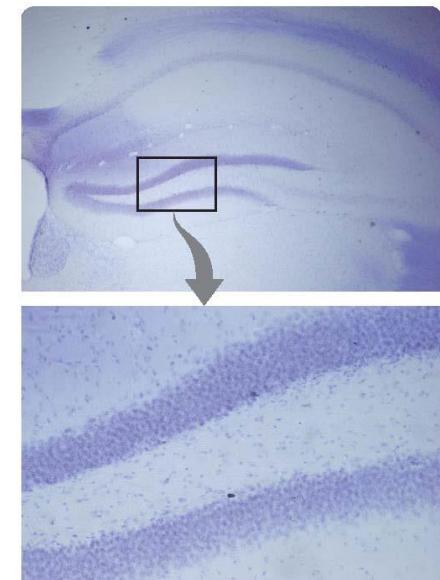
- Potassium dichromate and silver nitrate
 - Stains entire neurons (but not all) black
 - Useful for structural investigations

Mouse with neurodegeneration
Mouse with neurodegeneration on caloric restriction



- **Nissl stain**

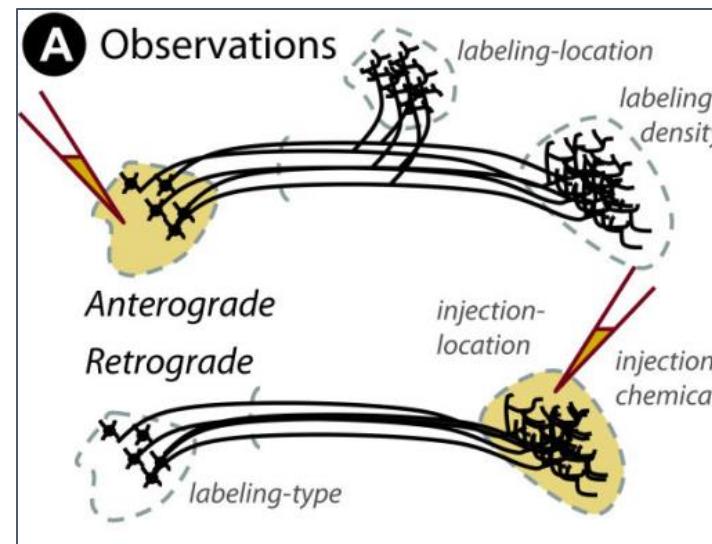
- Cresyl violet
 - Only binds to cell bodies
 - Useful for assessing number of neurons



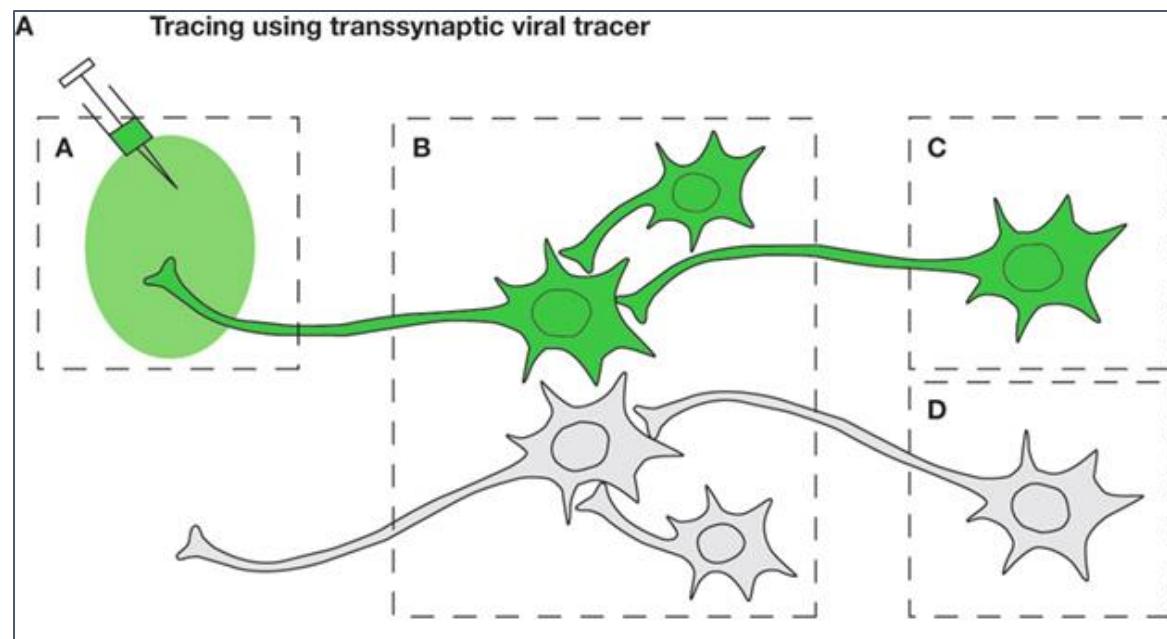
2) Brain/cellular structure

- Neuroanatomical techniques (c'd)
 - **Tracing** techniques (viruses, cholera B toxin, ...)
 - Anterograde tracing
 - Tracing the paths of axons away from an area of interest
 - Retrograde tracing
 - Tracing the paths of axons towards an area of interest
 - Transsynaptic tracing
 - Certain viruses cross synapses, thereby several neurons can be labeled
 - Dual transsynaptic tracing
 - Injections of two viruses with different fluorophores

Intracellular tracing

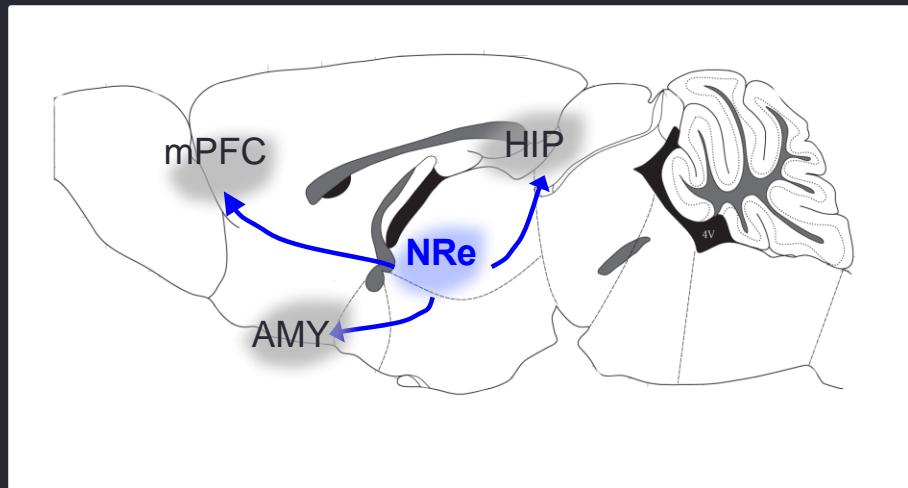


Transsynaptic tracing

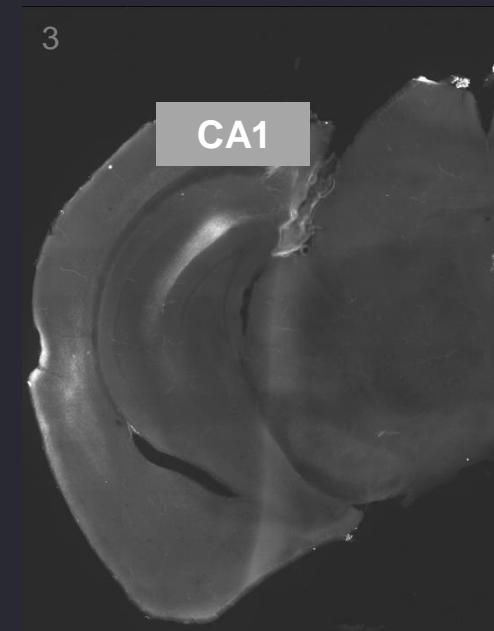
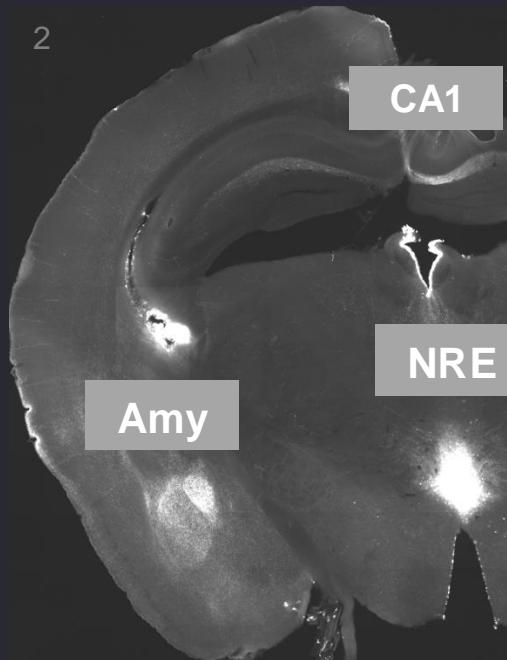
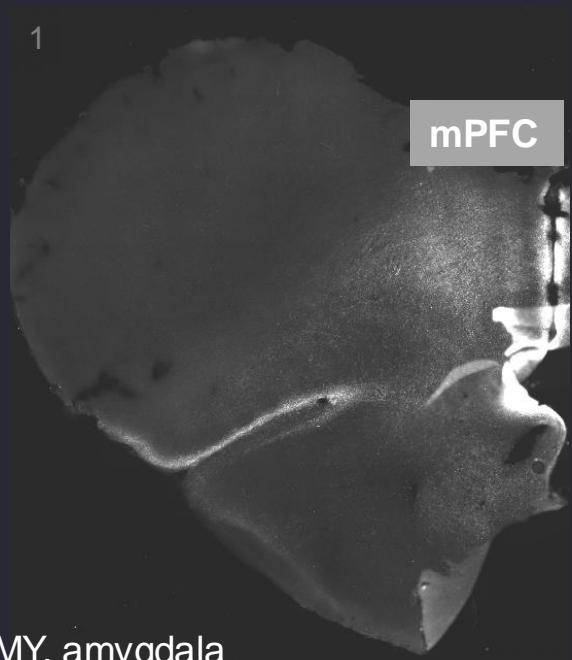


Projections of the **NRe** in the mouse

Transsynaptic tracing – example



AAV6-Cre into Re in R26 FLEX *tdTomato*



AMY, amygdala

HIP, hippocampus

mPFC, medial prefrontal cortex

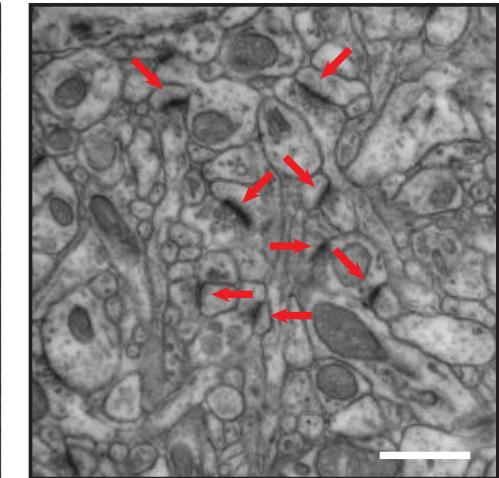
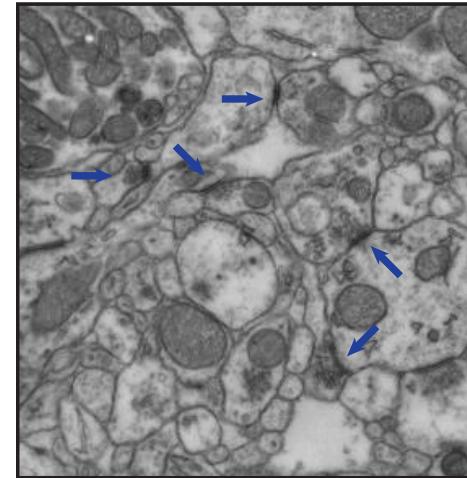
NRe, nucleus reuniens (thalamus)

2) Cellular structure

- Neuroanatomical techniques (c'd)

- **Electron microscopy**

- Transmission



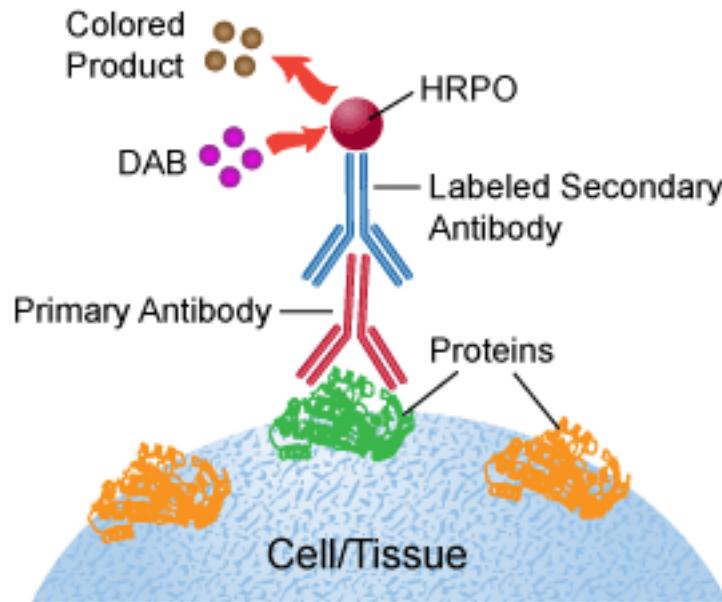
- Scanning



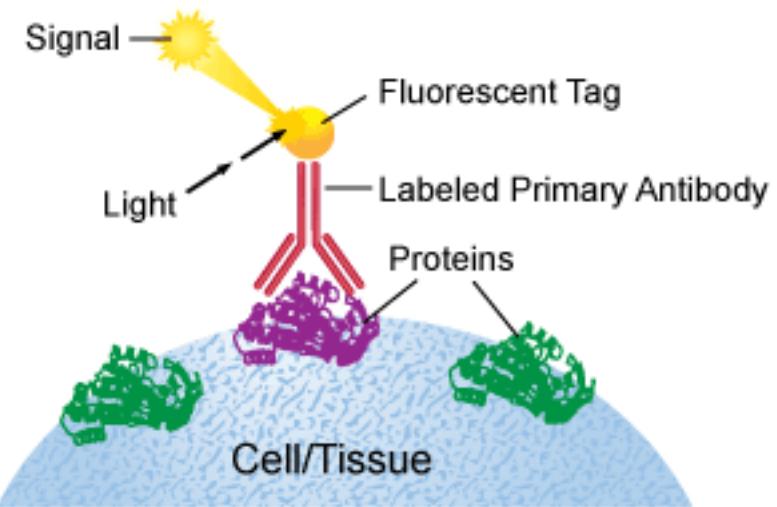
2) Cellular structure

- Neuroanatomical techniques (c'd)
 - **Microscopy combined with immunohistochemistry/immunofluorescence**

Indirect Immunohistochemistry



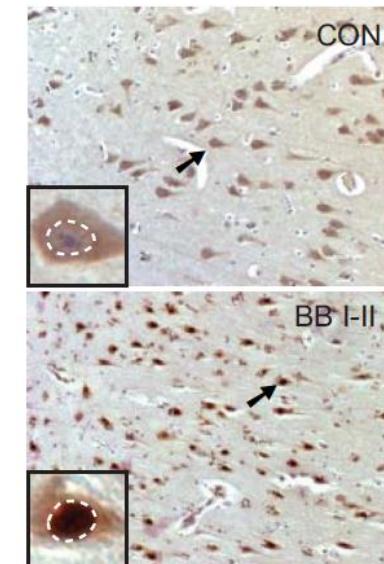
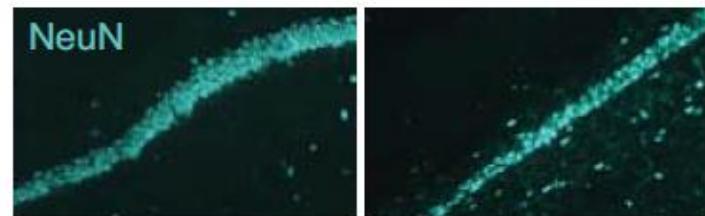
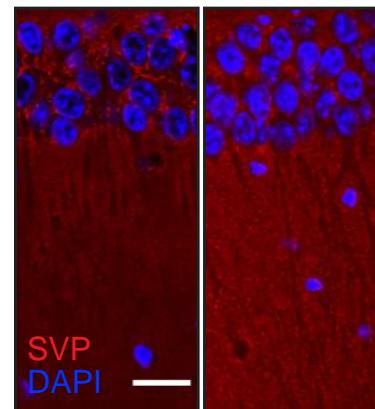
Immunofluorescence



3) Cellular function

- Neuroanatomical techniques (c'd)
 - **Microscopy combined with immunohistochemistry/immunofluorescence**

- Number of cells/neurons/glia
- Protein presence/abundance
- Structural changes
 - synaptic proteins
 - cytoskeletal proteins



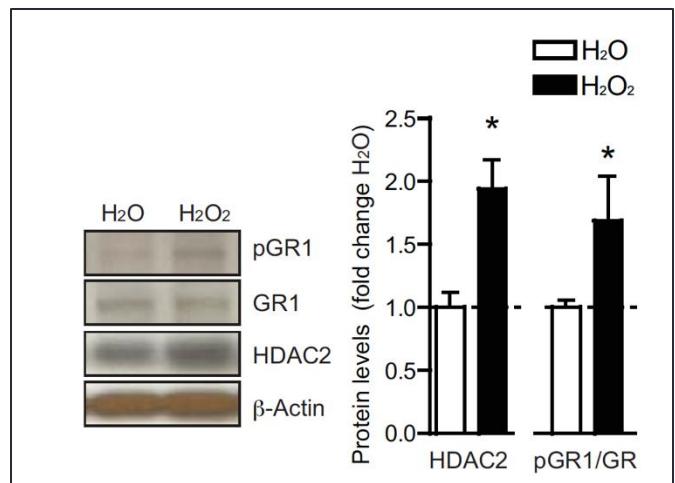
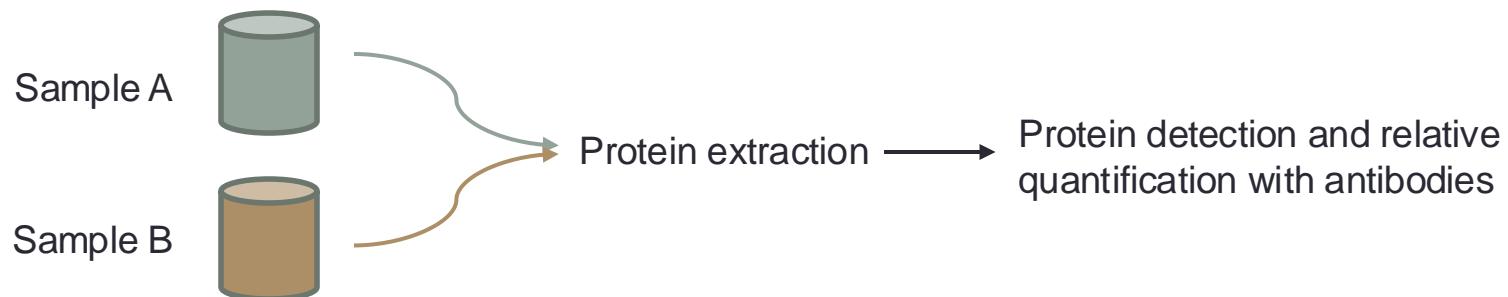
3) Cellular function

- Molecular techniques

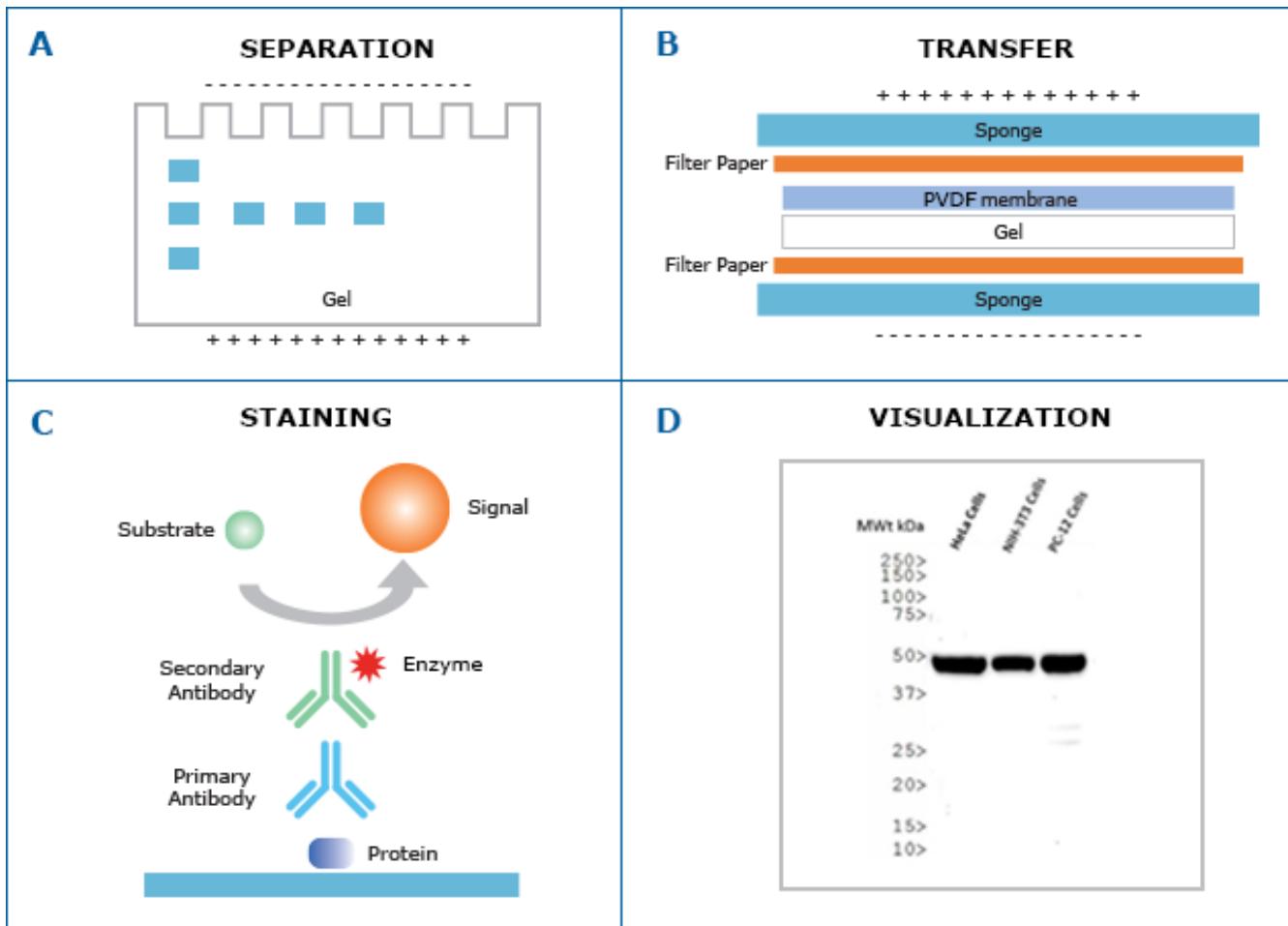
- Western Blotting

- Individual protein presence/abundance

- Principle:



Protein presence/abundance: Western Blotting



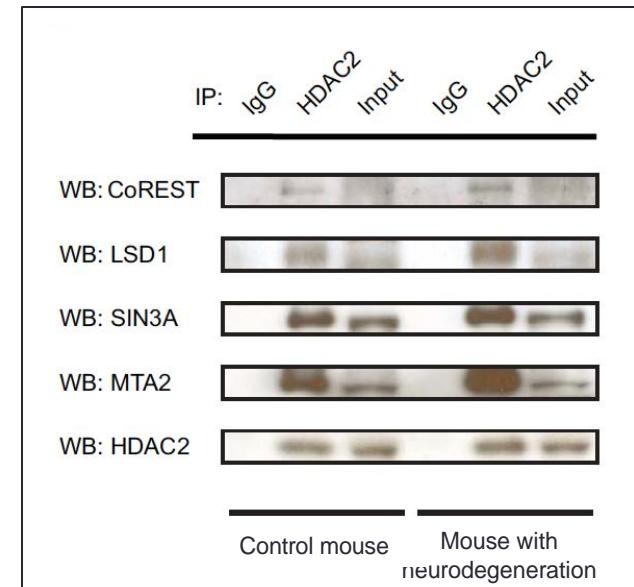
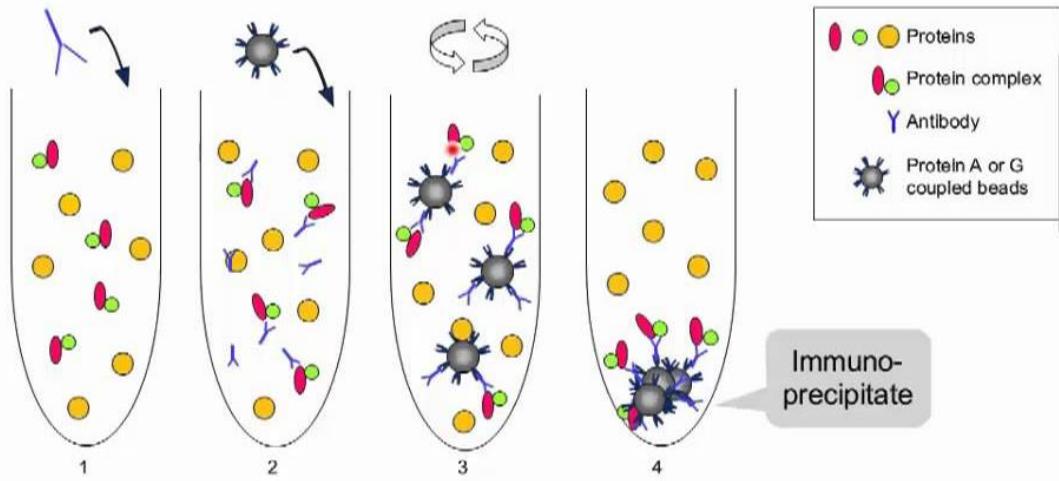
3) Cellular function

- Molecular techniques (c'd)
 - Western Blotting
 - Individual protein presence/abundance
 - **Proteomics**
 - Multiple proteins' presence/abundance
 - ↗ <https://www.nature.com/articles/nature01511>

3) Cellular function

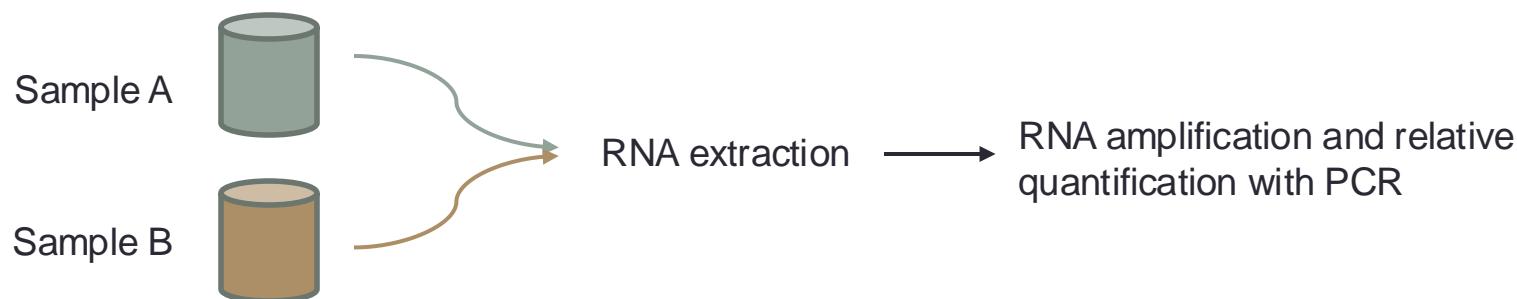
- Molecular techniques (c'd)
 - Co-immunoprecipitation (Co-IP)
 - Protein interaction

A protein complex can be isolated from a protein mixture by using an antibody that is specific for one protein of the complex



3) Cellular function

- Molecular techniques (c'd)
 - qRT-PCR (quantitative reverse transcription polymerase chain reaction)
 - Individual gene transcript (RNA) presence/abundance
 - Principle:



Gene presence/abundance: qRT-PCR

4.8 Reverse transcription polymerase chain reaction (RT-PCR)

In RT-PCR, the RNA population is converted to cDNA by reverse transcription (RT), and then the cDNA is amplified by the polymerase chain reaction. The cDNA amplification step provides opportunities to further study the original RNA species, even when they are limited in amount or expressed in low abundance. Common applications of RT-PCR include detection of expressed genes, examination of transcript variants, and generation of cDNA templates for cloning and sequencing.

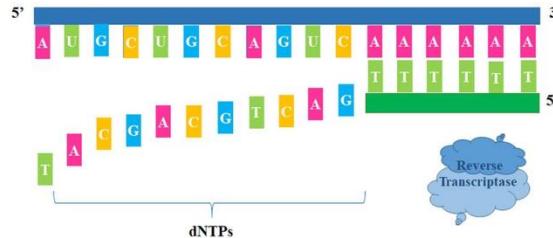
1 a. **RNA**
RNA consist of Start codon AUG and ends with poly A tail



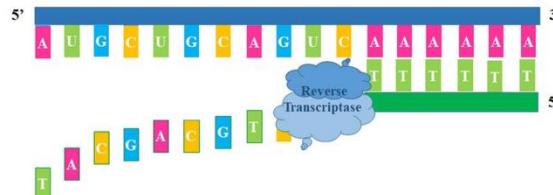
b. **Oligo dT Primer**
Oligo dT Primer is binding to RNA poly A tail



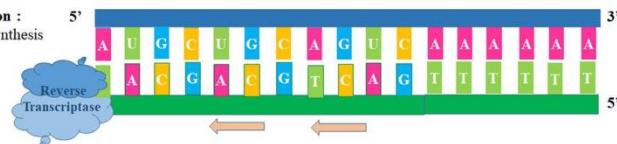
c. **Reverse Transcriptase**
and
dNTPs



d. **Reverse Transcriptase** is an enzymes binds to oligo dT primer and synthesises the cDNA by adding dNTPs



e. **RNA hybrid formation :**
First - strand cDNA synthesis



f. complimentary DNA

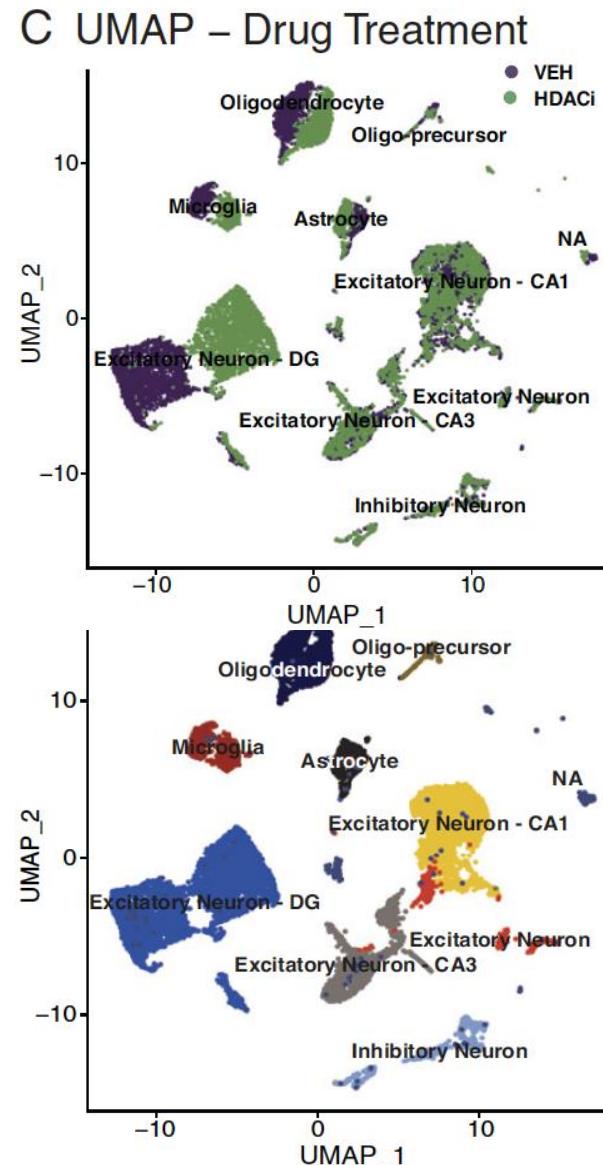


2 a. **Amplification of cDNA with Specific Primers and Tag Polymerase**



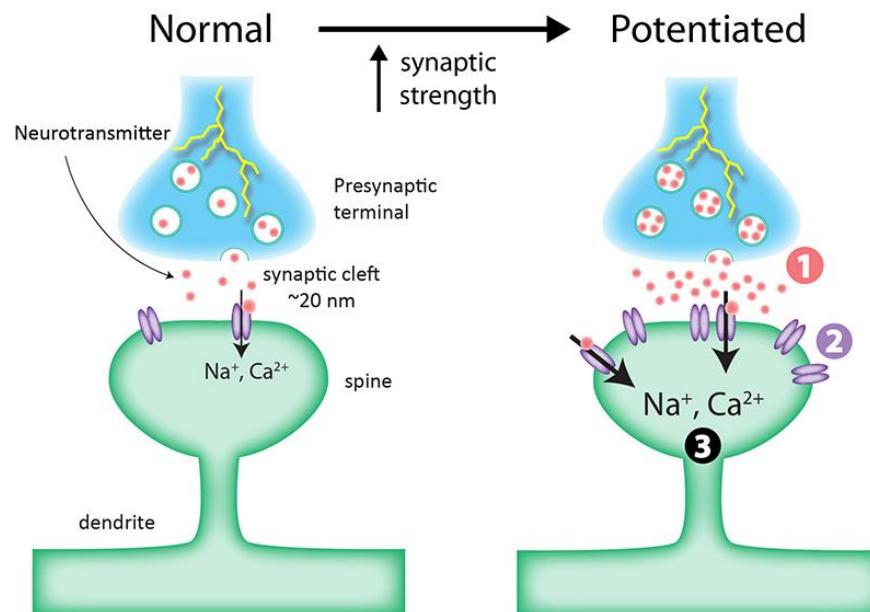
3) Cellular function

- Molecular techniques (c'd)
 - qRT-PCR (quantitative reverse transcription)
 - Individual gene transcript (RNA) presence/abundance
 - Multiple (whole genome) gene transcripts:
 - **RNA sequencing**
 - ↗ <https://www.nature.com/articles/nrg2484>
 - **Single-cell sequencing**
 - ↗ <https://www.nature.com/articles/s41581-018-0021-7>



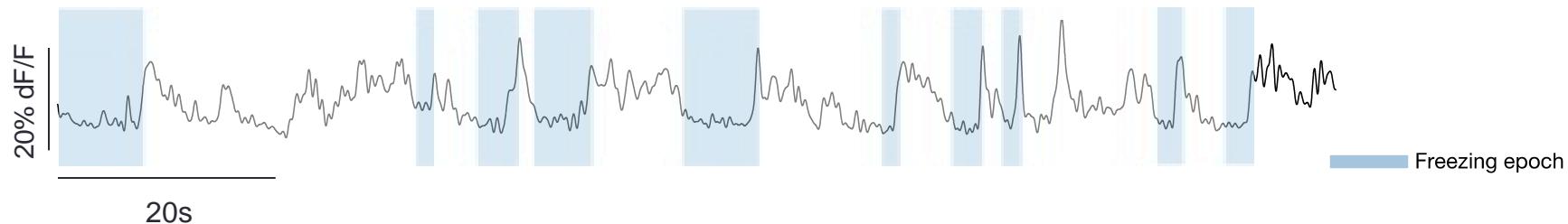
3) Cellular function

- Electrophysiological techniques
 - Long-term potentiation/long-term depression
 - Measures post-stimulus excitation of specific pathways



3) Cellular function

- **Electrophysiological techniques**
 - **Calcium signaling**
 - e.g., in vivo fiber photometry



How to study the nervous system?

- Structure
 - Brain structure
 - Cellular structure
- Function
 - **Brain function**
 - Cellular function

4) Brain function

- Electrophysiological recordings (H&A)
 - Scalp: Electroencephalogram (EEG)
 - Muscle: Electromyogram (EMG)
 - Eye: Electrooculogram (EOG)
 - Skin: Skin Conductance (SCR)

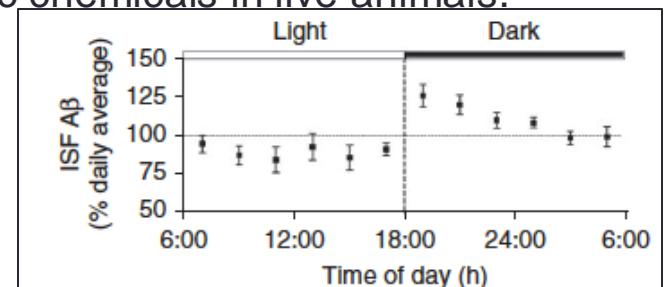
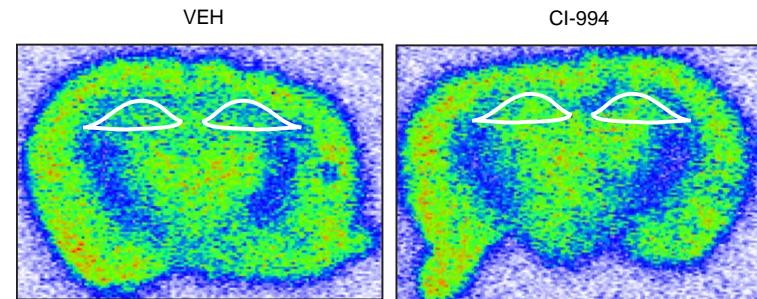


4) Brain function (c'd)

- Measuring chemical activity in the brain

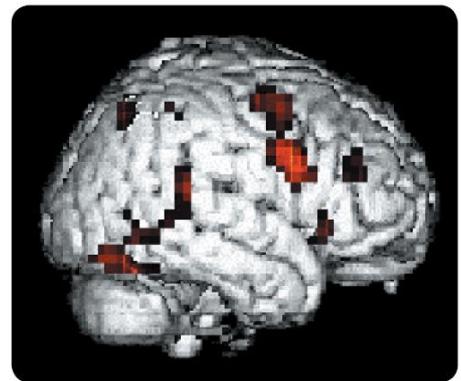
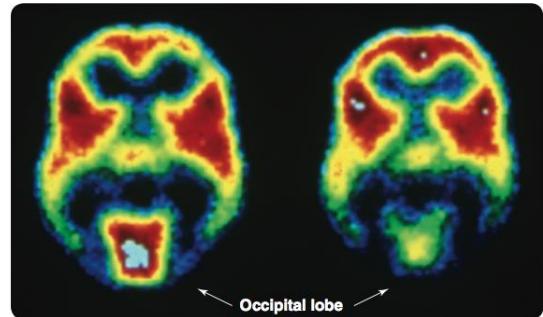
- 2-Deoxyglucose (2-DG) Technique
 - Inject an animal with radioactive 2-DG and allow it to engage in behavior of interest.
 - Use autoradiography to see where radioactivity accumulates in brain slices.

- Cerebral dialysis
 - Measuring extracellular concentration of specific chemicals in live animals.
 - E.g., amyloid- β
 - Fluctuating with circadian rhythm



4) Brain function

- Imaging techniques (H&A)
 - Positron Emission Tomography (PET)
 - Radionucleotide incorporated into active areas
 - functional Magnetic Resonance Imaging (fMRI)
 - Cerebral blood flow correlates with activity
- Lesions
 - Aspiration (H&A)
 - Radio-frequency (A)
 - Knife cuts (H&A)
 - Cryogenic (A)
- Injuries (H)



4) Brain function

- Differences between MRI and fMRI:

MRI studies brain anatomy.



contrasting agent: H_2O

Functional MRI (fMRI) studies brain function.



contrasting agent: O_2

4) Brain function (c'd)

- **Manipulating brain function**

- **Genetic** engineering (A)

- Gene knockout
 - Gene knockin
 - Conditional knockin
 - Transgenic animals
 - RNA interference
 - CRISPR/Cas9

Genetic engineering methods

Transgenic Mouse: Generic term for an engineered mouse that has a normal DNA sequence for a gene replaced by an engineered sequence or a sequence from another organism.

Knockout Mouse: A transgenic mouse in which the normal gene is missing or engineered so that is not transcribed or translated. “Knocks out” that gene.

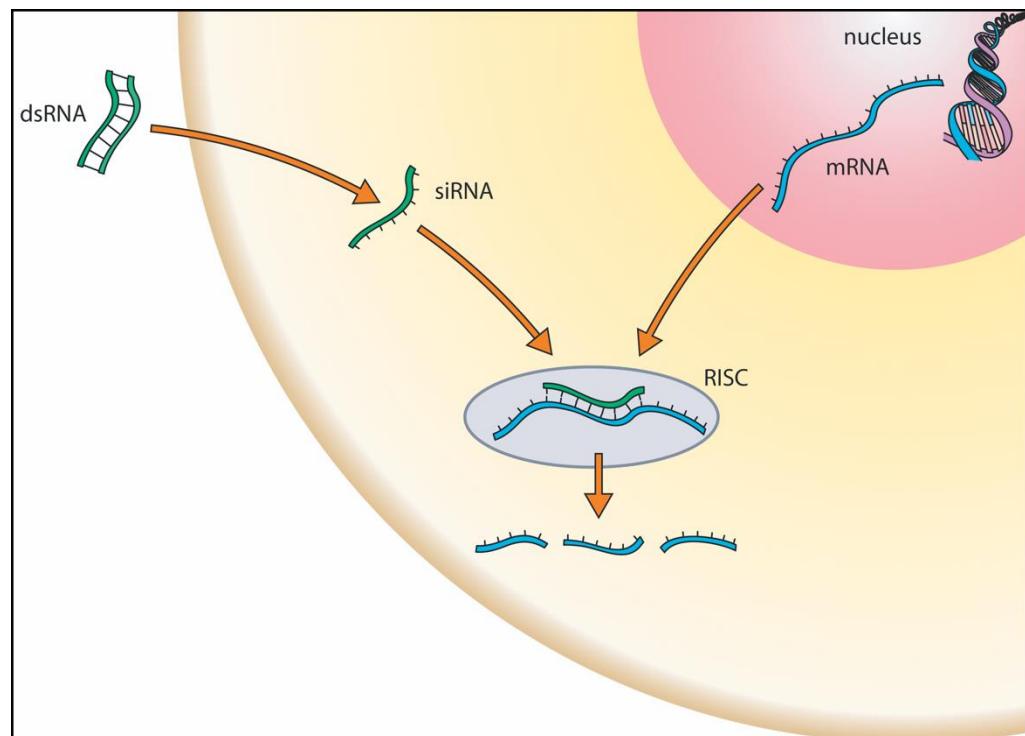
Knockin Mouse: A transgenic mouse in which the engineered gene of interest or “transgene” is subtly manipulated to: (A) alter the function of the gene (e.g., replace one amino acid with another in a site to determine if that site is essential for the protein’s function); (B) change transcription rate to overproduce or underproduce the gene product; or (C) create a fluorescent gene product to map its distribution in tissue.

Conditional Knockout (Knockin) Mouse: A transgenic mouse in which the transgene is knocked out (or in) in specific tissues, at a specific developmental stage, or in response to an exogenous substance (e.g., an antibiotic).

4) Brain function (c'd)

- **RNA interference**

- siRNA, small-interfering RNA
- shRNA, small-hairpin RNA



4) Brain function (c'd)

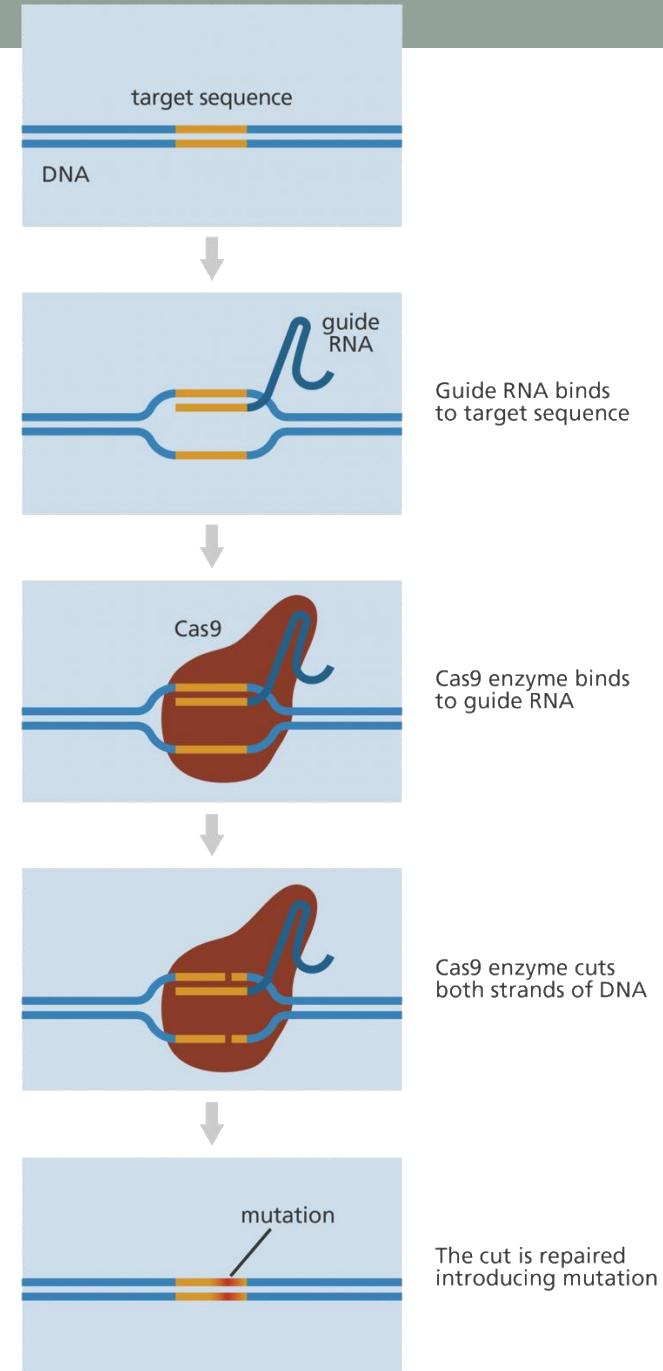
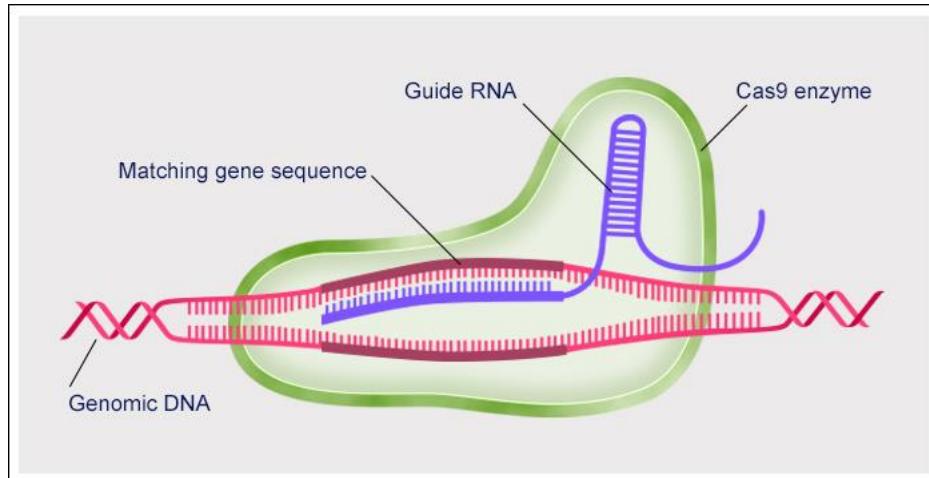
- CRISPR-Cas9



4) Brain function (c'd)

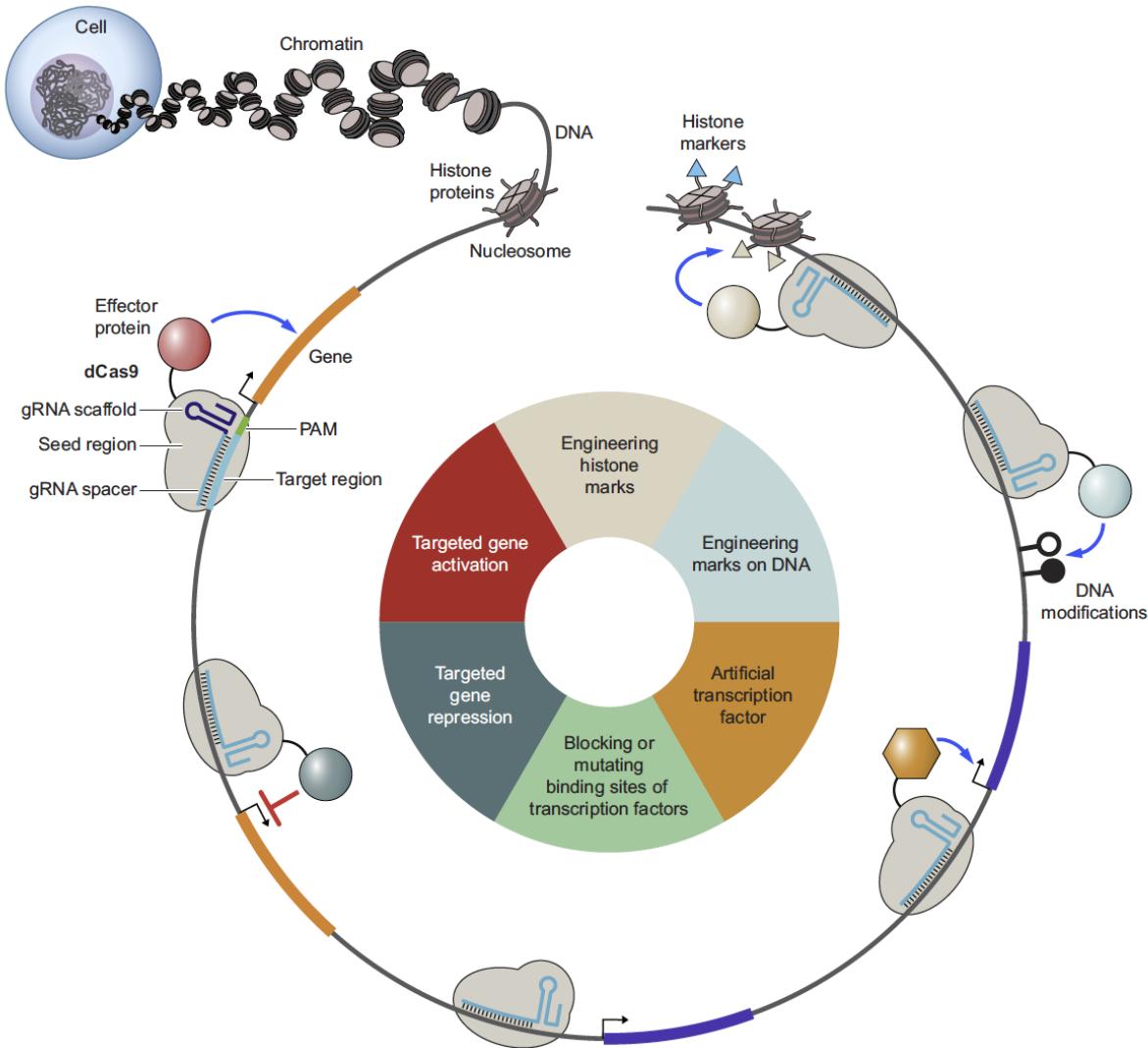
• CRISPR-Cas9

- based on clustered regularly interspaced
short palindromic repeats



4) Brain function (c'd)

- CRISPR-Cas9:
Ramifications

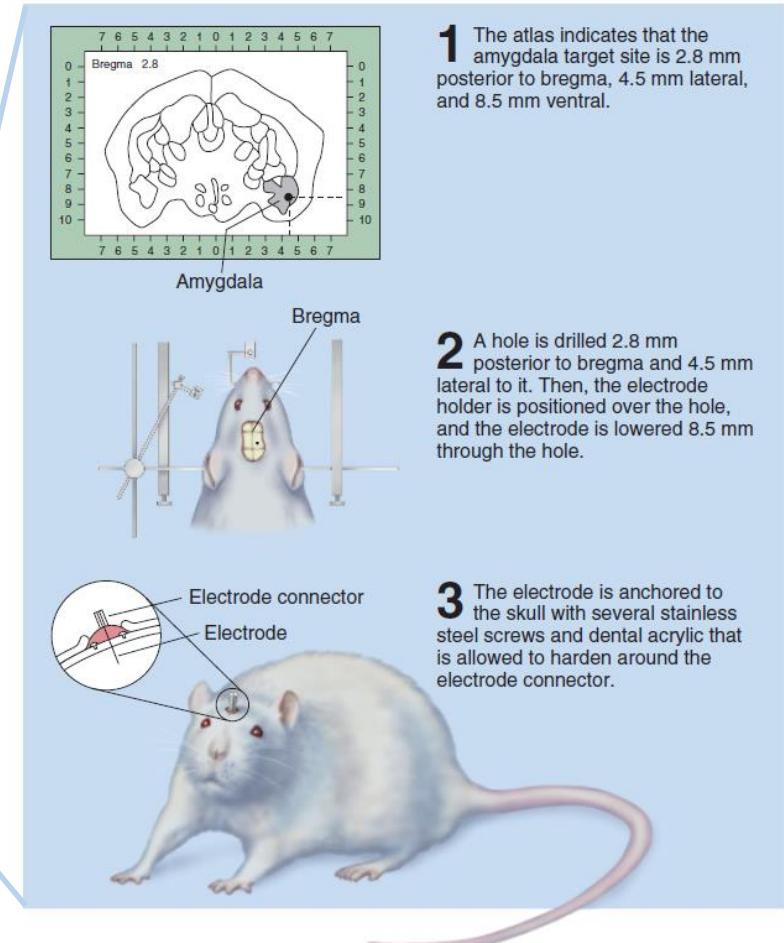


4) Brain function (c'd)

- Manipulating brain function

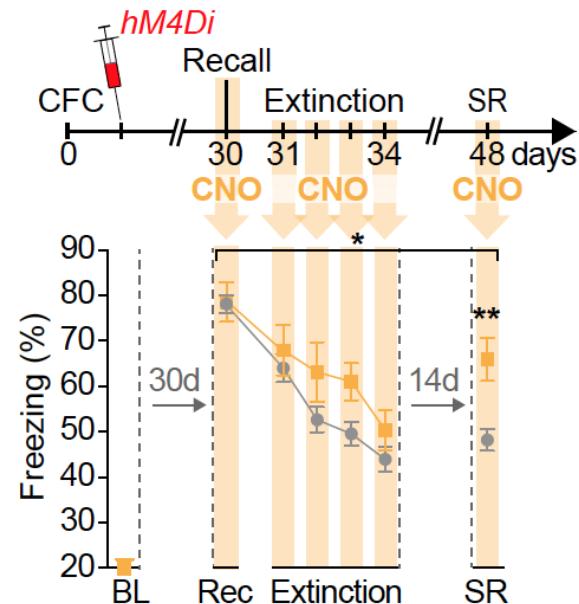
- Drug administration

- oral/food (H&A)
- im – intramuscular (H&A)
- iv – intravenous (H&A)
- sc – subcutaneous (H&A)
- ip – intraperitoneal (H&A)
- icv – intracerebroventricular (A)
- Stereotaxic (H&A)
- Cannulation (A)

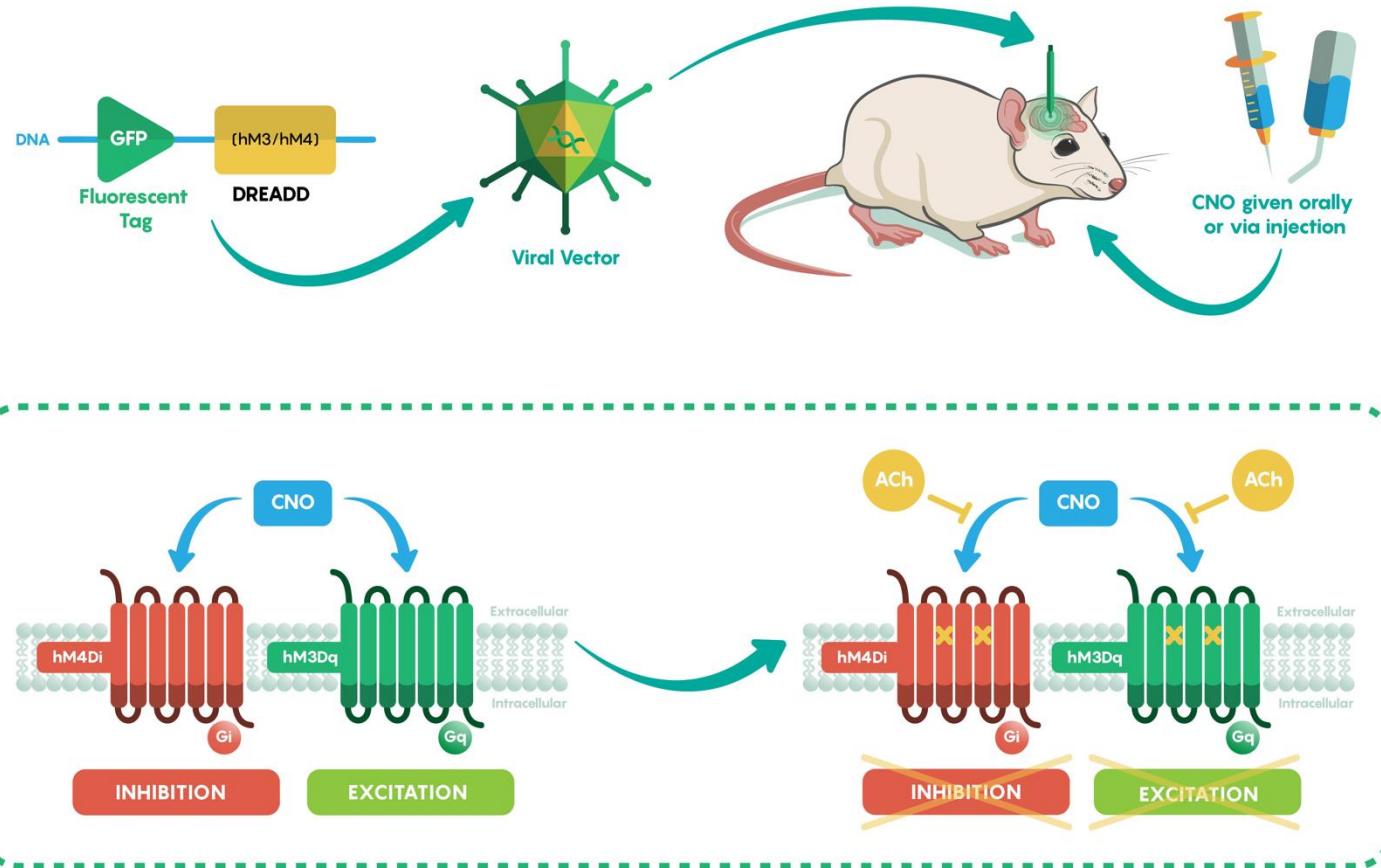


4) Brain function (c'd)

- Manipulating brain function
- Chemogenetics (A)
 - Based on DREADDs (designer receptor exclusively activated by designer drugs)
 - Activated by CNO (clozapine-N-oxide)
 - Inhibitory or activatory



Chemogenetics

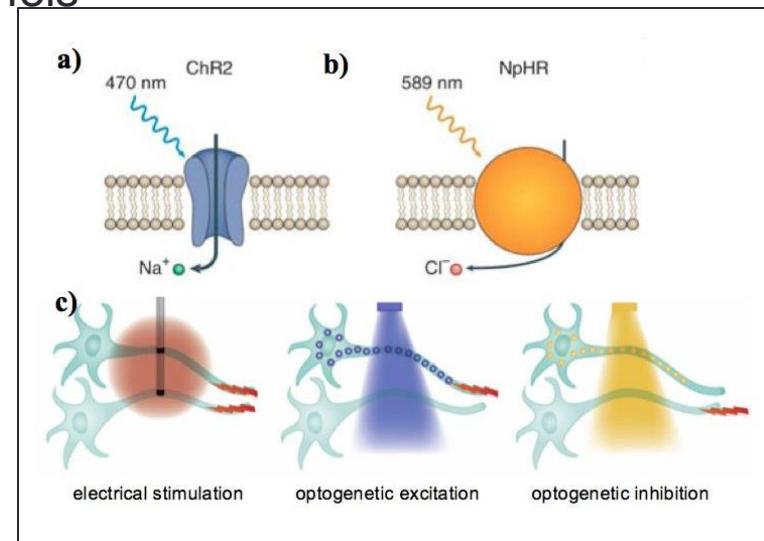


- By combining with cell-type specific promoters, chemogenetics allows for the interrogation of the function of specific neuronal circuits with low-temporal resolution

4) Brain function (c'd)

- Manipulating brain function
- Optogenetics (A)
 - Based on light-sensitive algal channels

- Channelrhodopsin (ChR2)
- Halorhodopsin (NpHR)



- By combining with cell-type specific promoters, optogenetics allows for the interrogation of the function of specific neuronal circuits with high temporal resolution

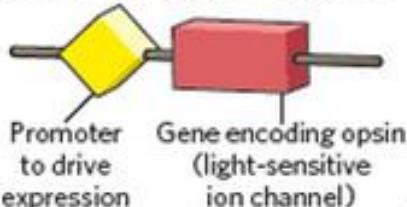
Optogenetics

SIX STEPS TO OPTOGENETICS

With optogenetic techniques, researchers can modulate the activity of targeted neurons using light.

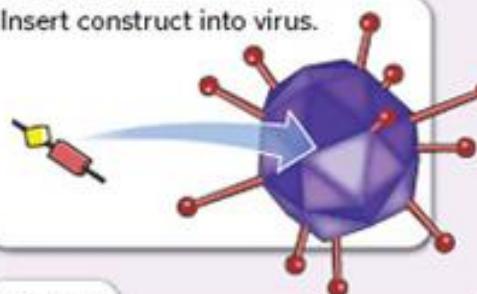
STEP 1

Piece together genetic construct.



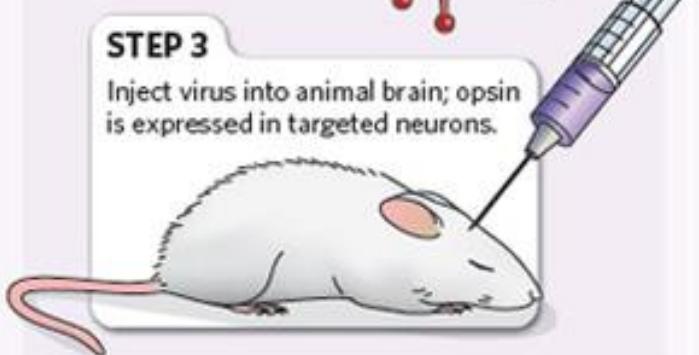
STEP 2

Insert construct into virus.



STEP 3

Inject virus into animal brain; opsin is expressed in targeted neurons.



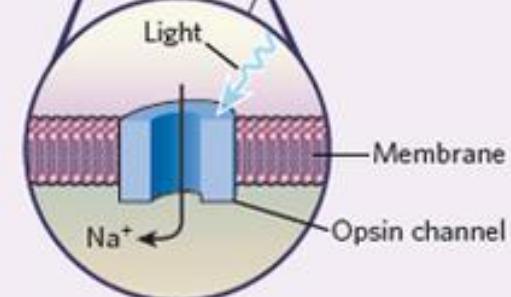
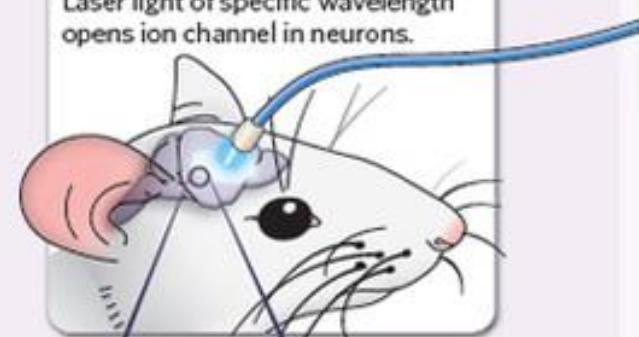
STEP 4

Insert 'optrode', fibre-optic cable plus electrode.

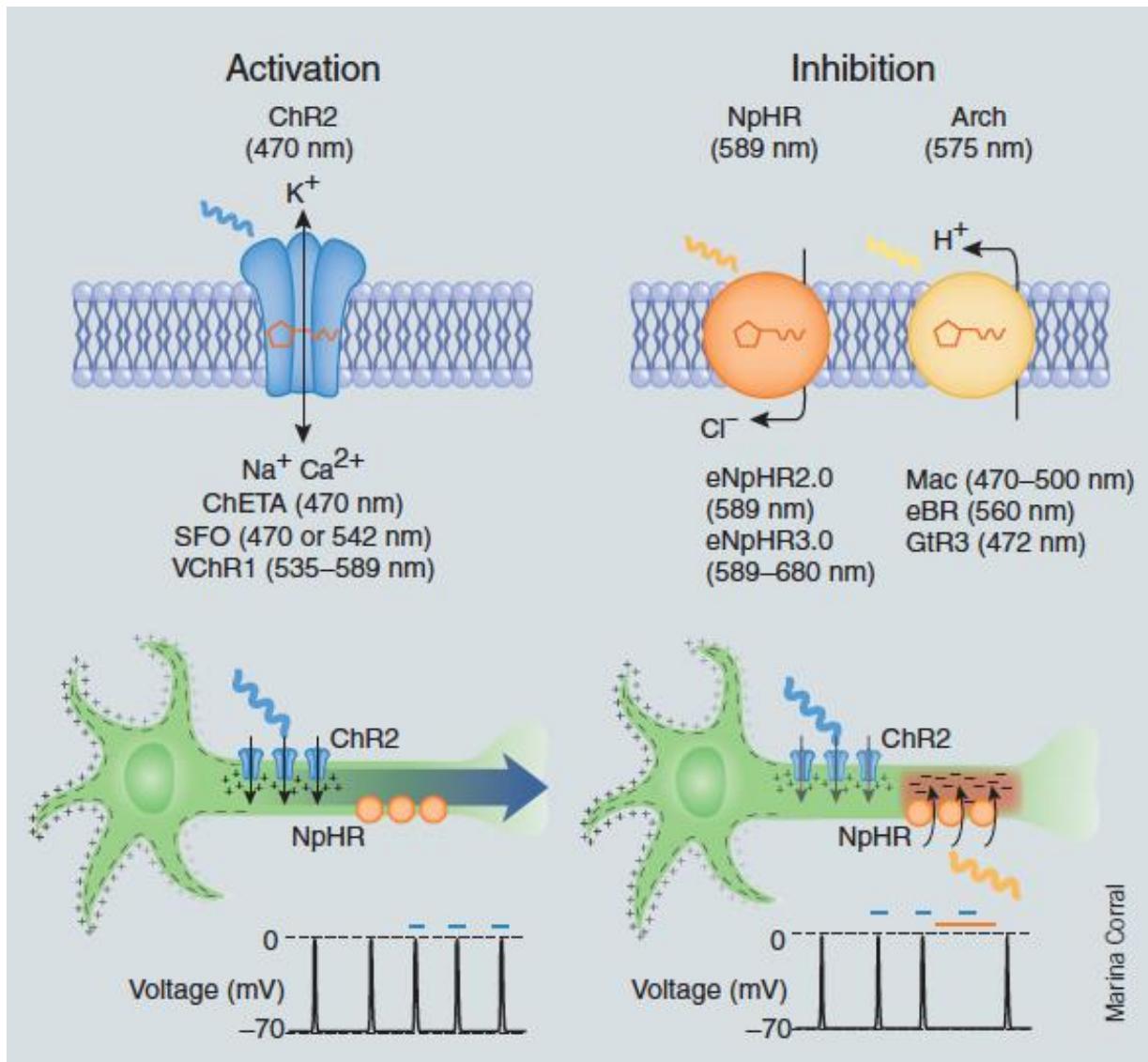


STEP 5

Laser light of specific wavelength opens ion channel in neurons.



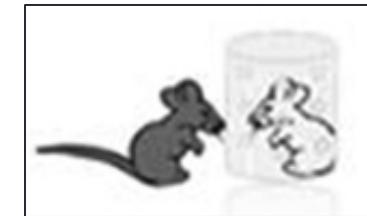
Optogenetics – details



Manipulating brain function and assessing its outcome

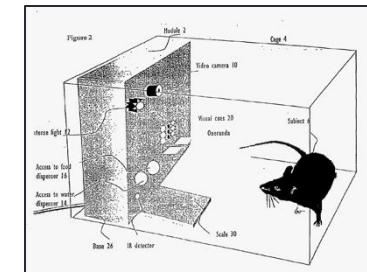
- Paradigms for Assessment of Species-Common Behaviors

- Open-field test
 - Anxiety, activity
- Tests of aggressive and defensive behavior
- Tests of sexual behavior



- Traditional Conditioning Paradigms

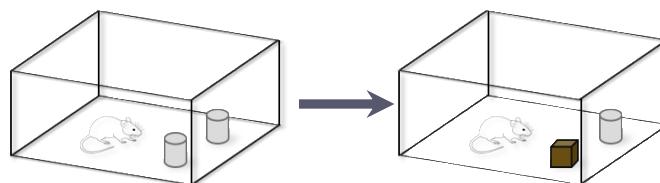
- Operant conditioning
 - Reinforcement and punishment: The animal works for drug administration



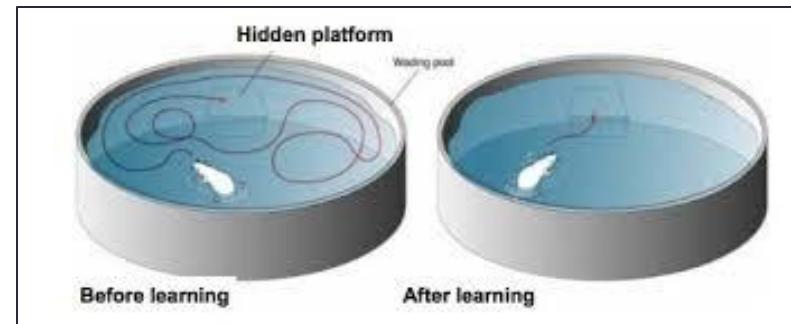
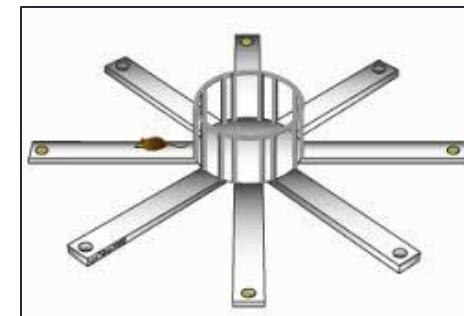
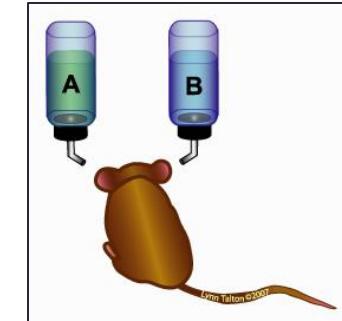
Manipulating brain function and assessing its outcome

- Animal Learning Paradigms

- Conditioned taste aversion
 - Pairing something that makes an animal ill (emetic) with a taste
- Spatial learning
 - Radial arm maze
 - Morris Water maze
- Novel Object Recognition Test



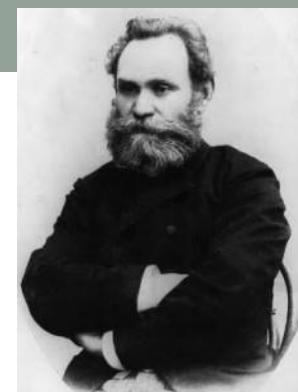
Novel Object Recognition Test



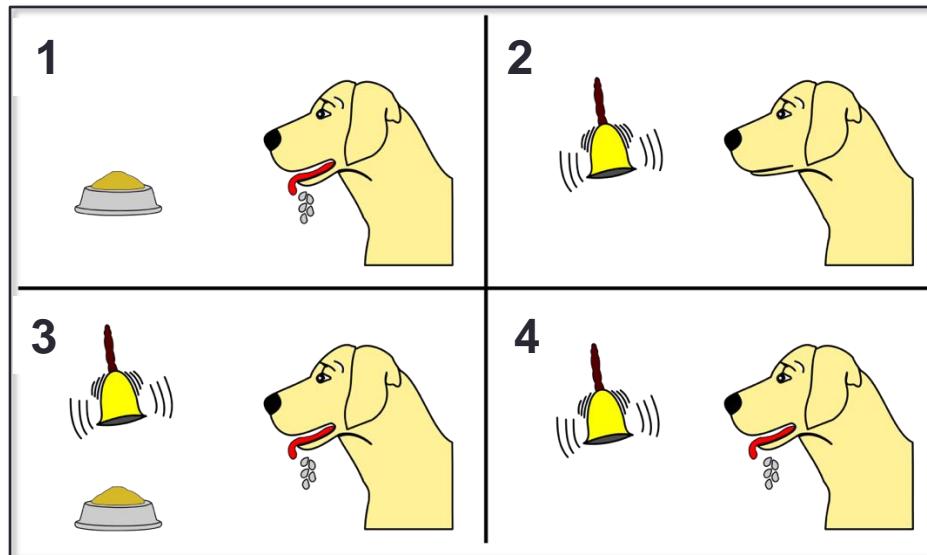
Manipulating brain function and **assessing its outcome**

- Animal Learning Paradigms
 - Pavlovian Conditioning

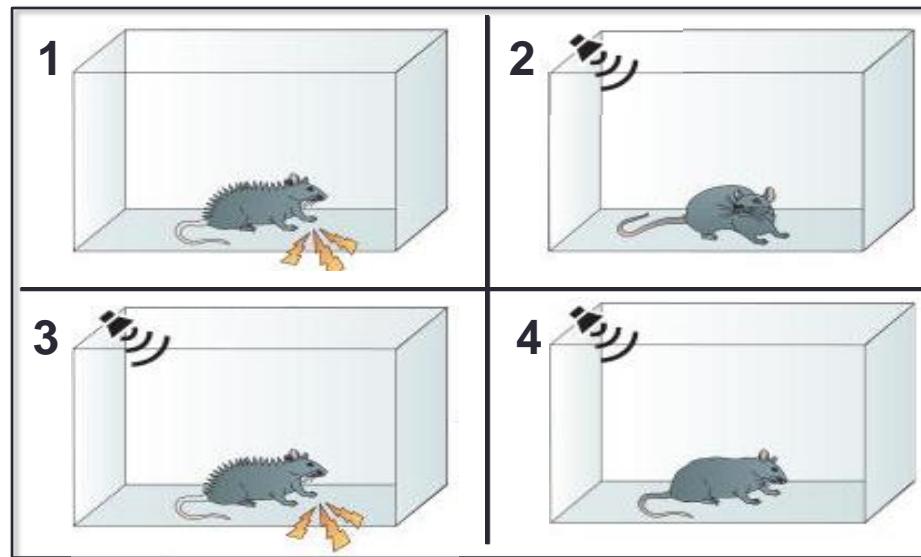
Pavlovian conditioning



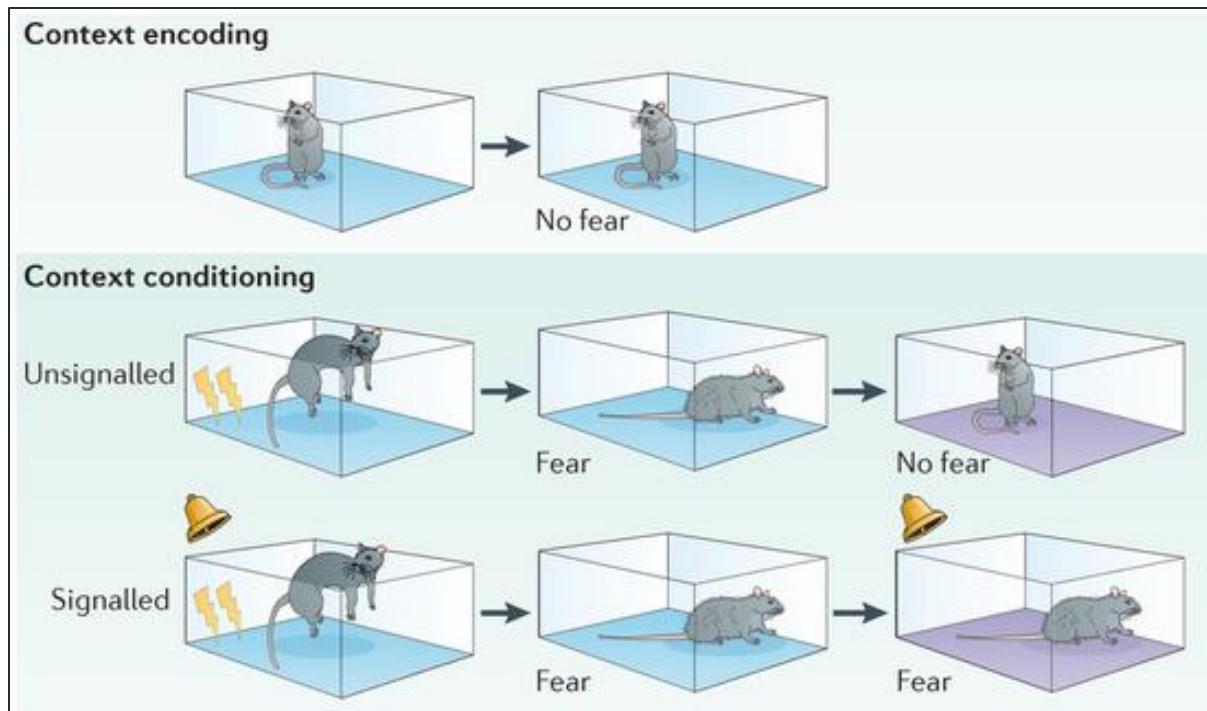
Ivan Pavlov
(1849-1936)



Pavlovian conditioning: Fear conditioning



Pavlovian conditioning: Fear conditioning



- Unconditioned stimulus (US) – electrical foot shock
- Unconditioned response (UR) – fear
- Conditioned stimulus (CS) – context (box) or cue (tone)
- Conditioned response (CR) – fear
 - US-CR->CR

Neuroscience techniques – SUMMARY

