WEEK 1

HOW TO STUDY THE NERVOUS SYSTEM?

Learning objectives

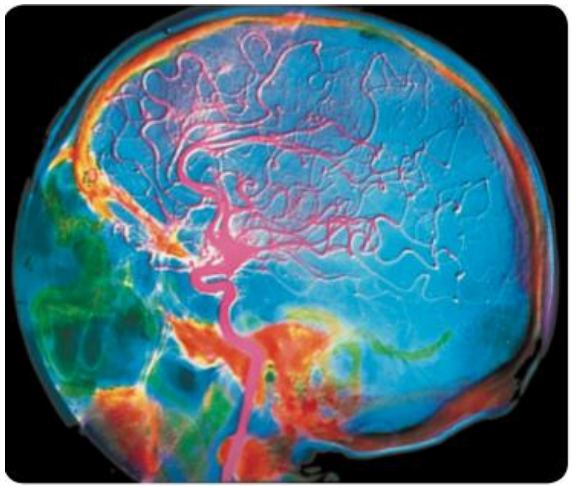
- Know how to study brain structural changes
- Know how to study cellular changes in structure/function
- Know how to manipulate cellular/brain function

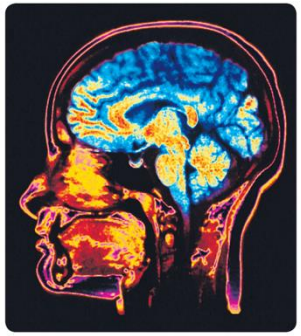
How would **you** study the nervous system?

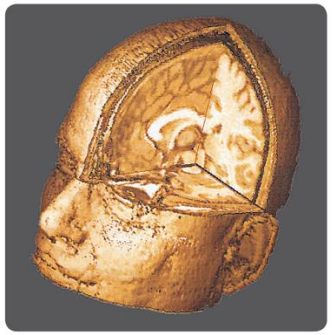
- Structure
- Function

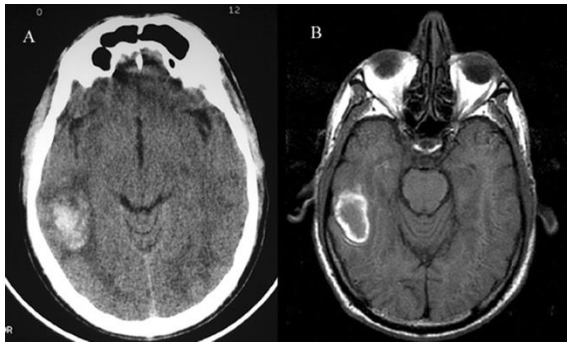
1) Brain structure

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



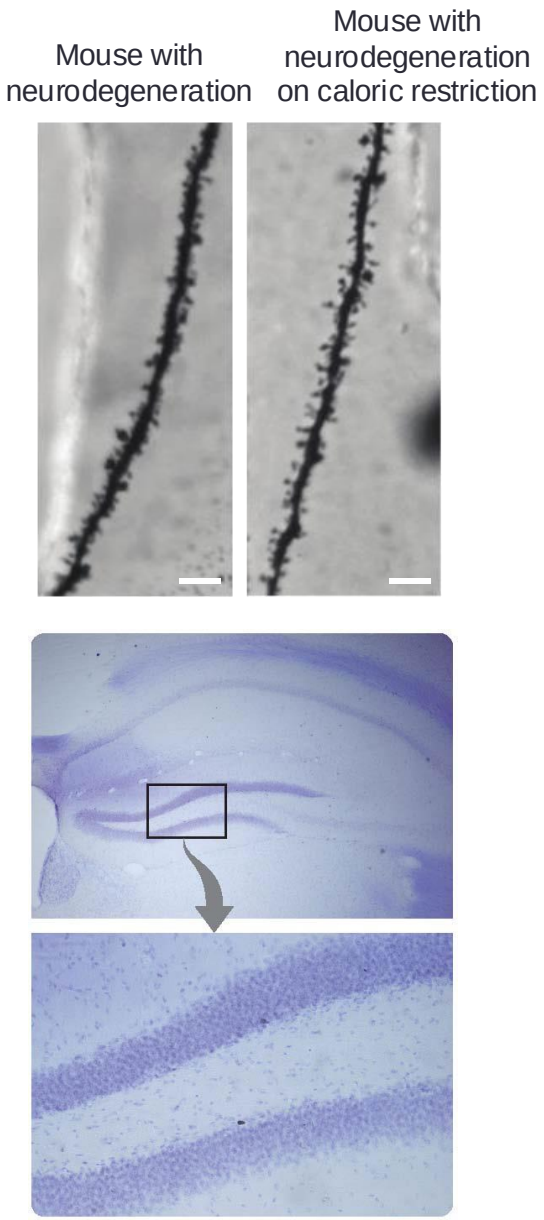






2) Brain/cellular structure

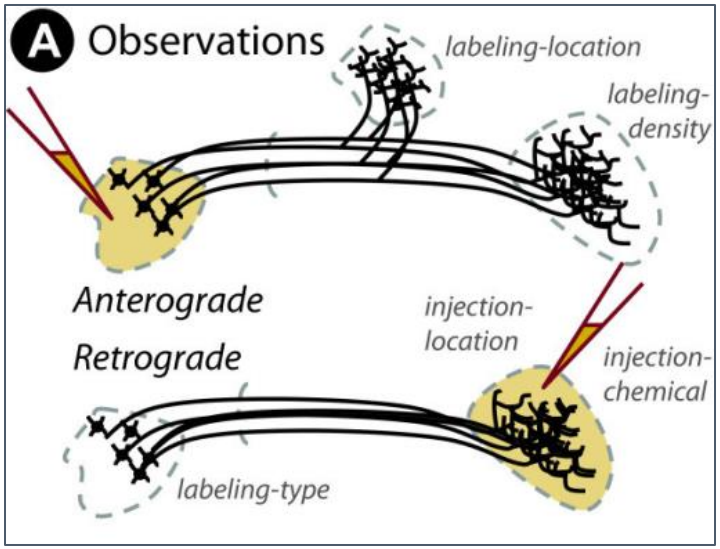
- **Neuroanatomical techniques**
  - **Golgi stain**
    - Potassium dichromate and silver nitrate
    - Stains entire neurons (but not all) black
    - ➢ Useful for structural investigations
  - **Nissl stain**
    - Cresyl violet
    - Only binds to cell bodies
    - ➢ Useful for assessing number of neurons



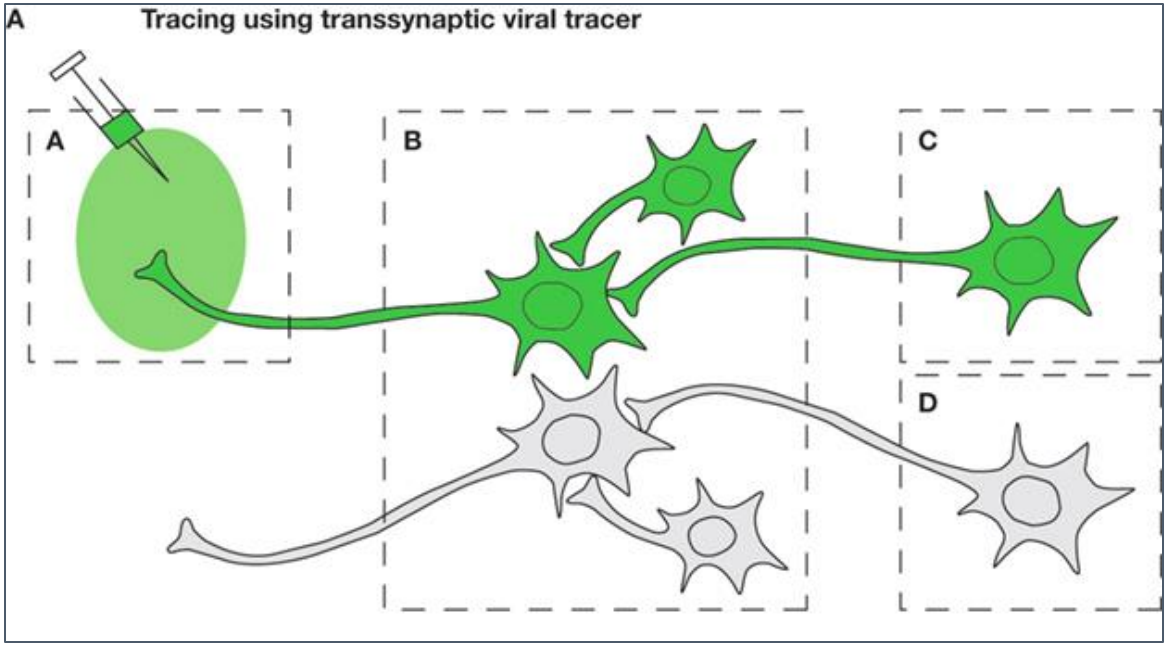
2) Brain/cellular structure

- 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

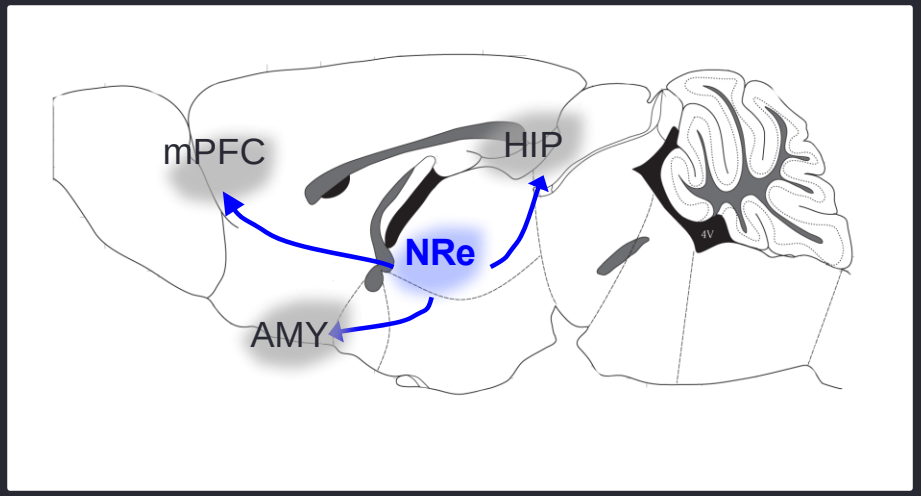


Transsynaptic tracing

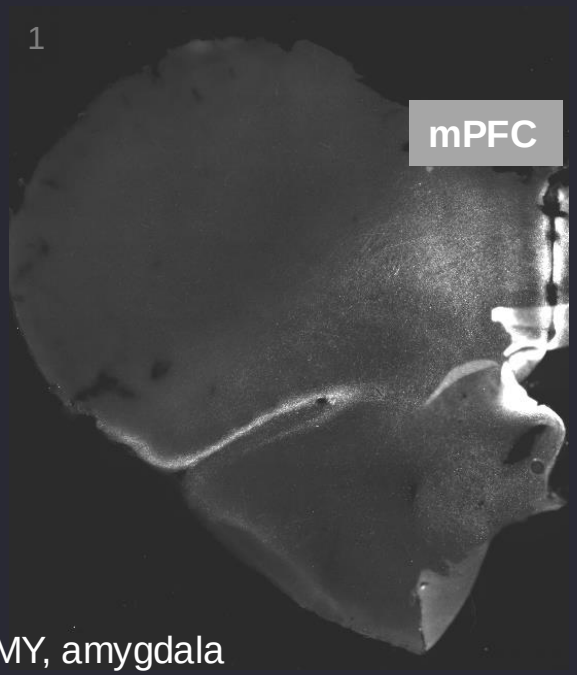


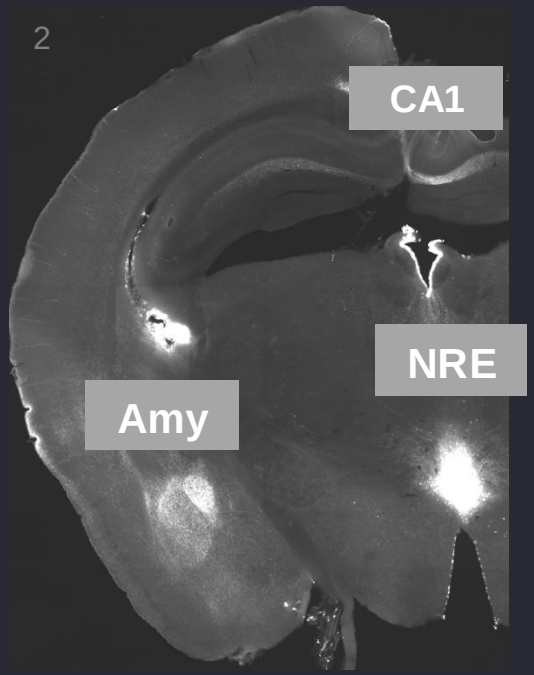
Projections of the **NRe** in the mouse

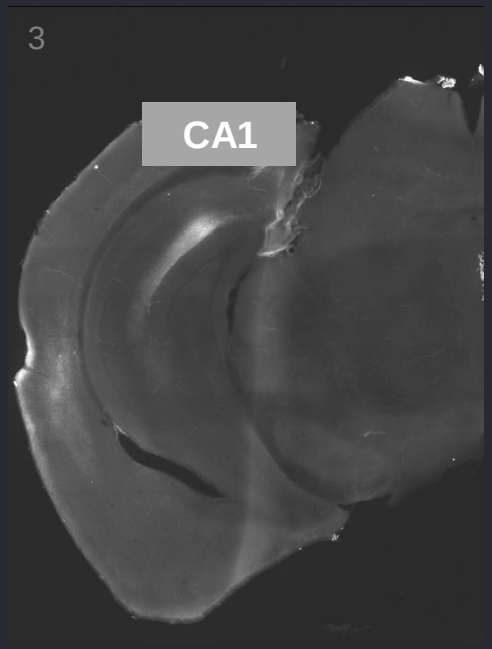
Transsynaptic tracing – example



AAV6-Cre into Re in R26 FLEX tdTomato



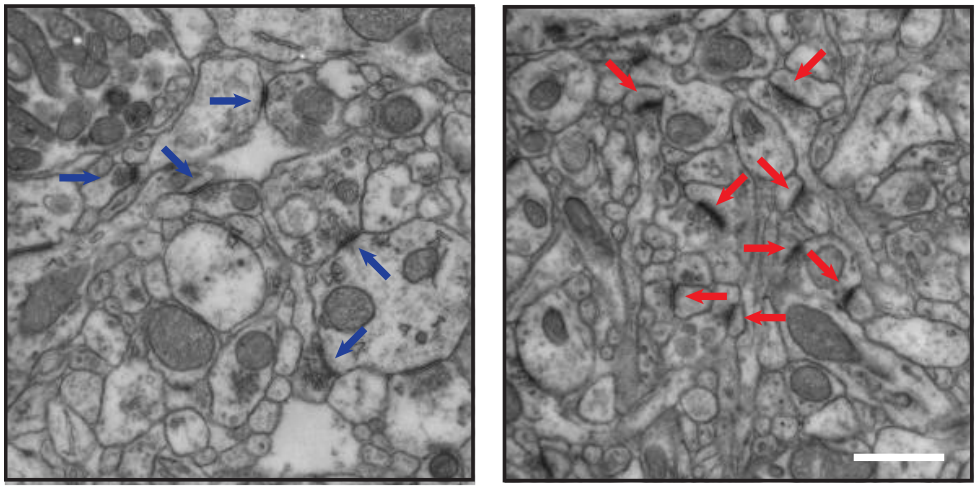




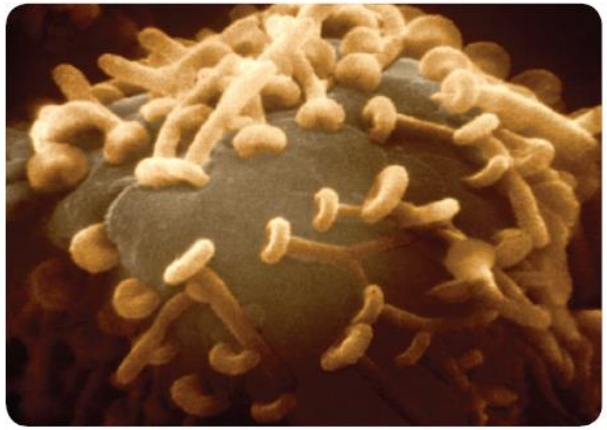
AMY, amygdala
HIP, hippocampus
mPFC, medial prefrontal cortex
NRe, nucleus reuniens (thalamus)

2) Cellular structure

- Neuroanatomical techniques (c'd)
  - **Electron microscopy**
    - Transmission

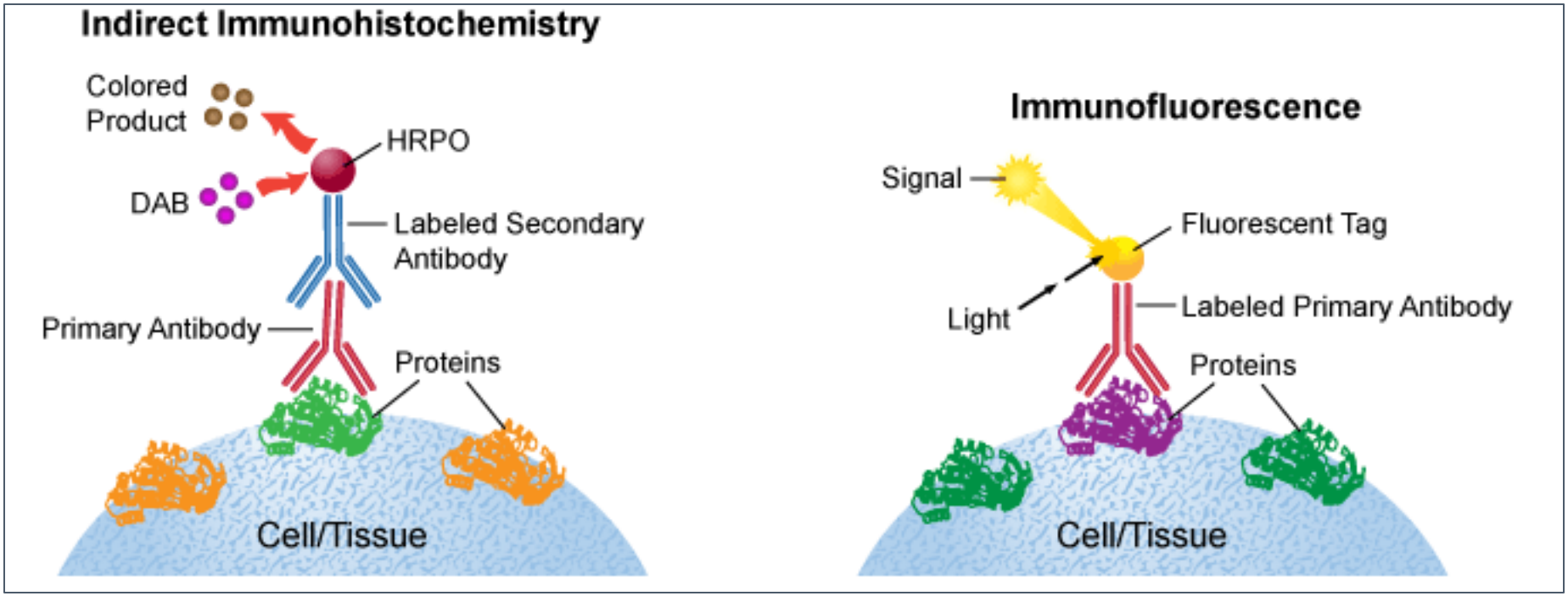


    - Scanning



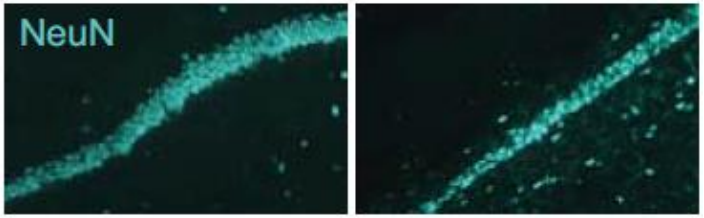
2) Cellular structure

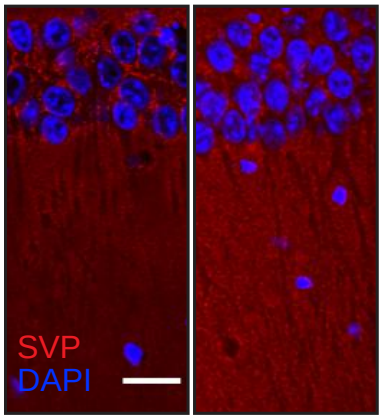
- Neuroanatomical techniques (c'd)
  - **Microscopy combined with immunohistochemistry/immunofluorescence**

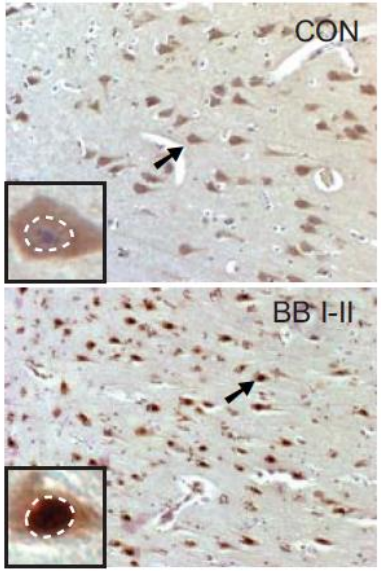


3) Cellular function

- Neuroanatomical techniques (c'd)
  - **Microscopy combined with immunohistochemistry/immunofluorescence**
    - Number of cells/neurons/glia
    - Protein presence/abundance
    - Structural changes
      - synaptic proteins
      - cytoskeletal proteins

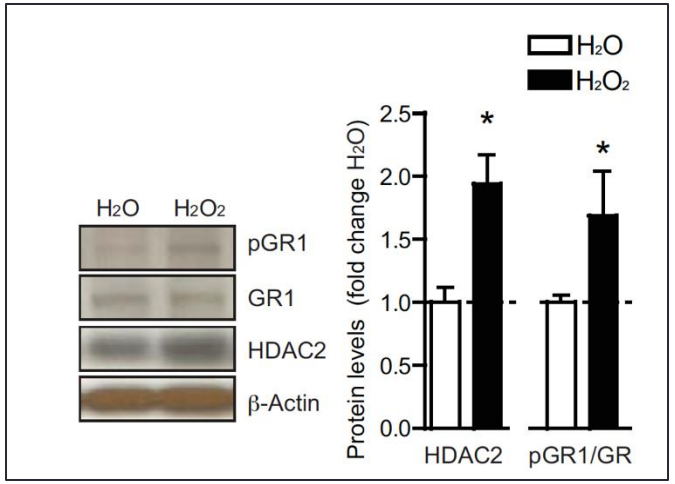


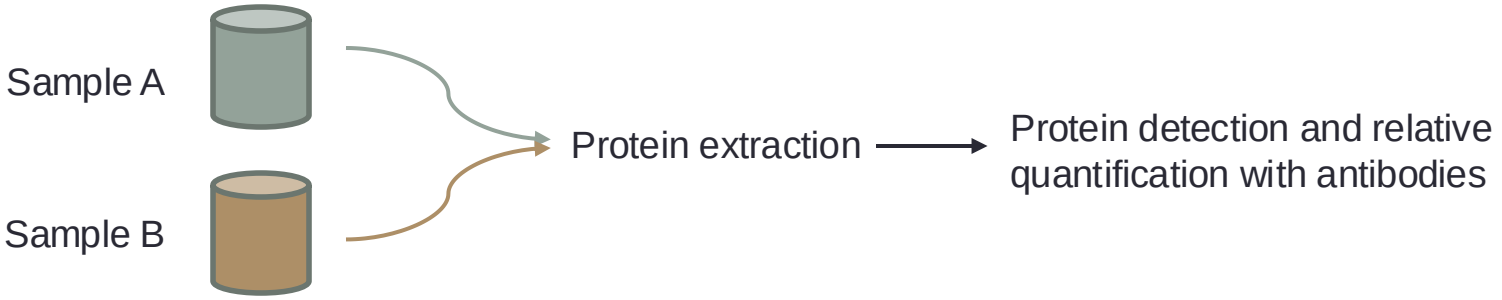




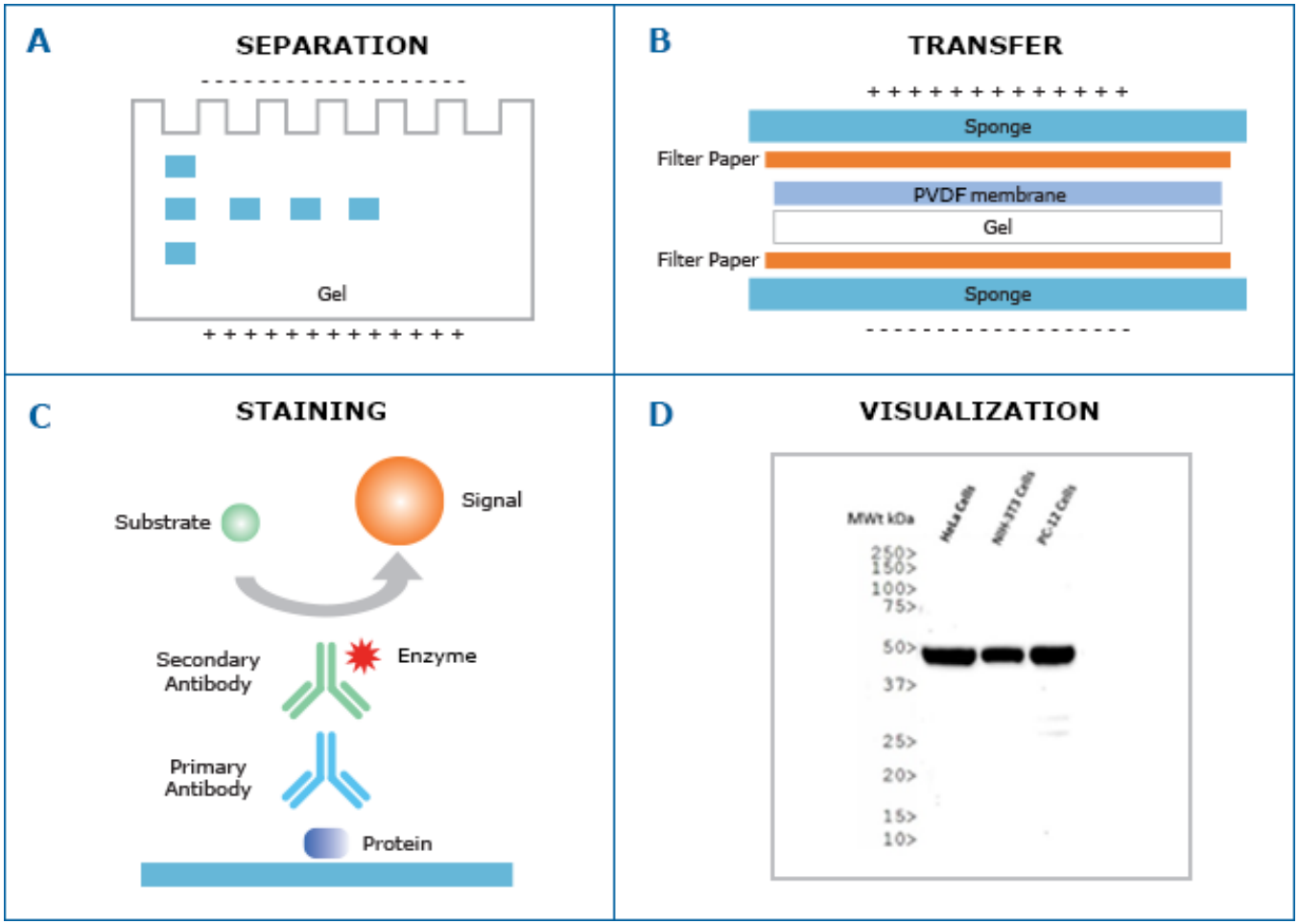
3) Cellular function

- **Molecular techniques**
  - **Western Blotting**
    - Individual protein presence/abundance
    - Principle:





Protein presence/abundance: Western Blotting

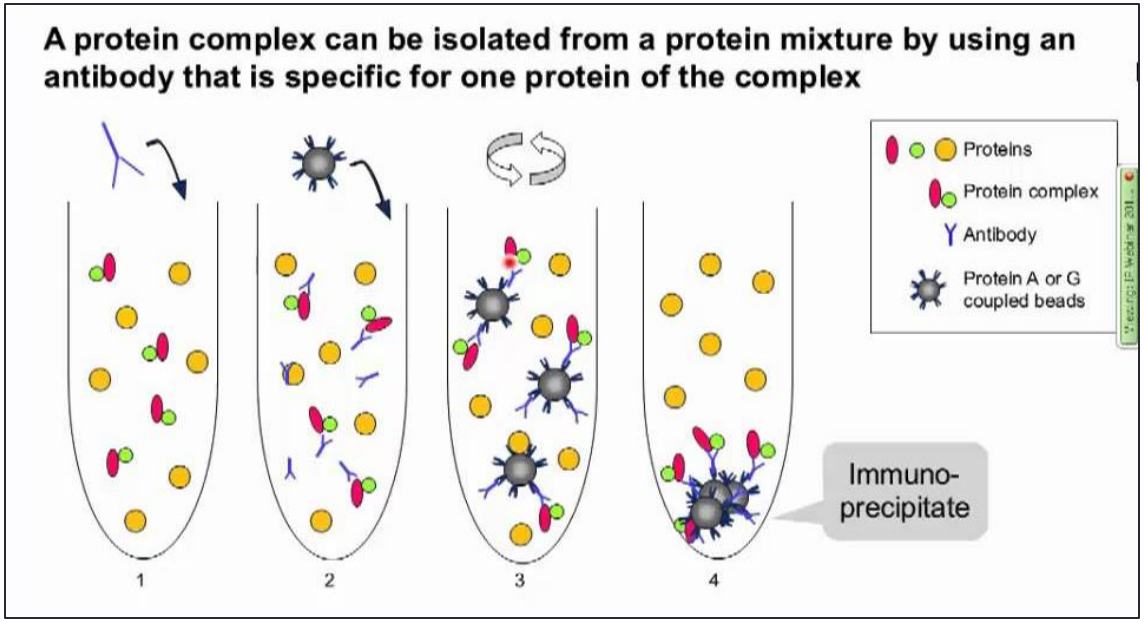


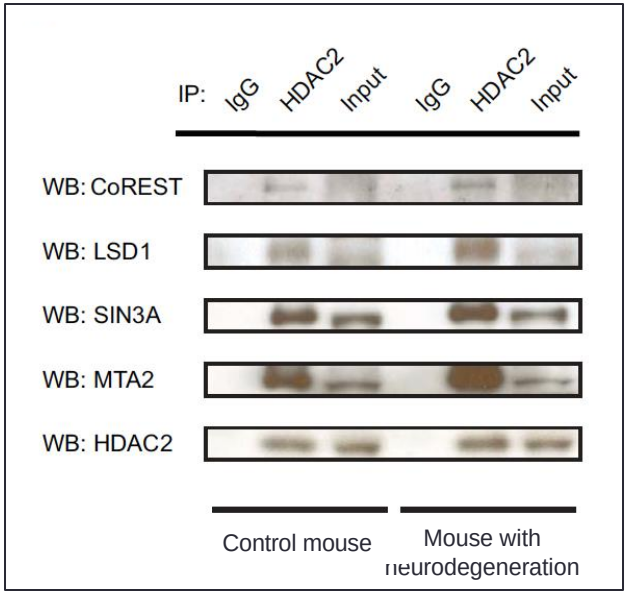
3) Cellular function

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

3) Cellular function

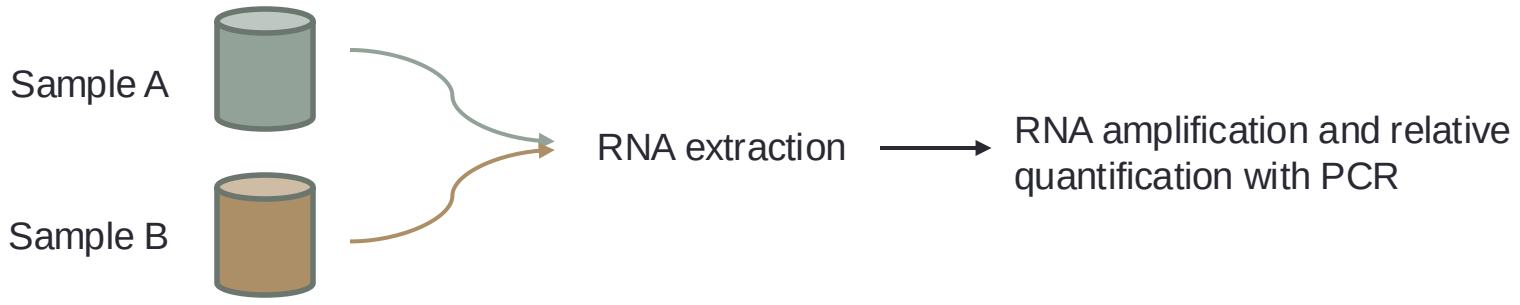
- Molecular techniques (c'd)
  - **Co-immunoprecipitation (Co-IP)**
    - Protein interaction



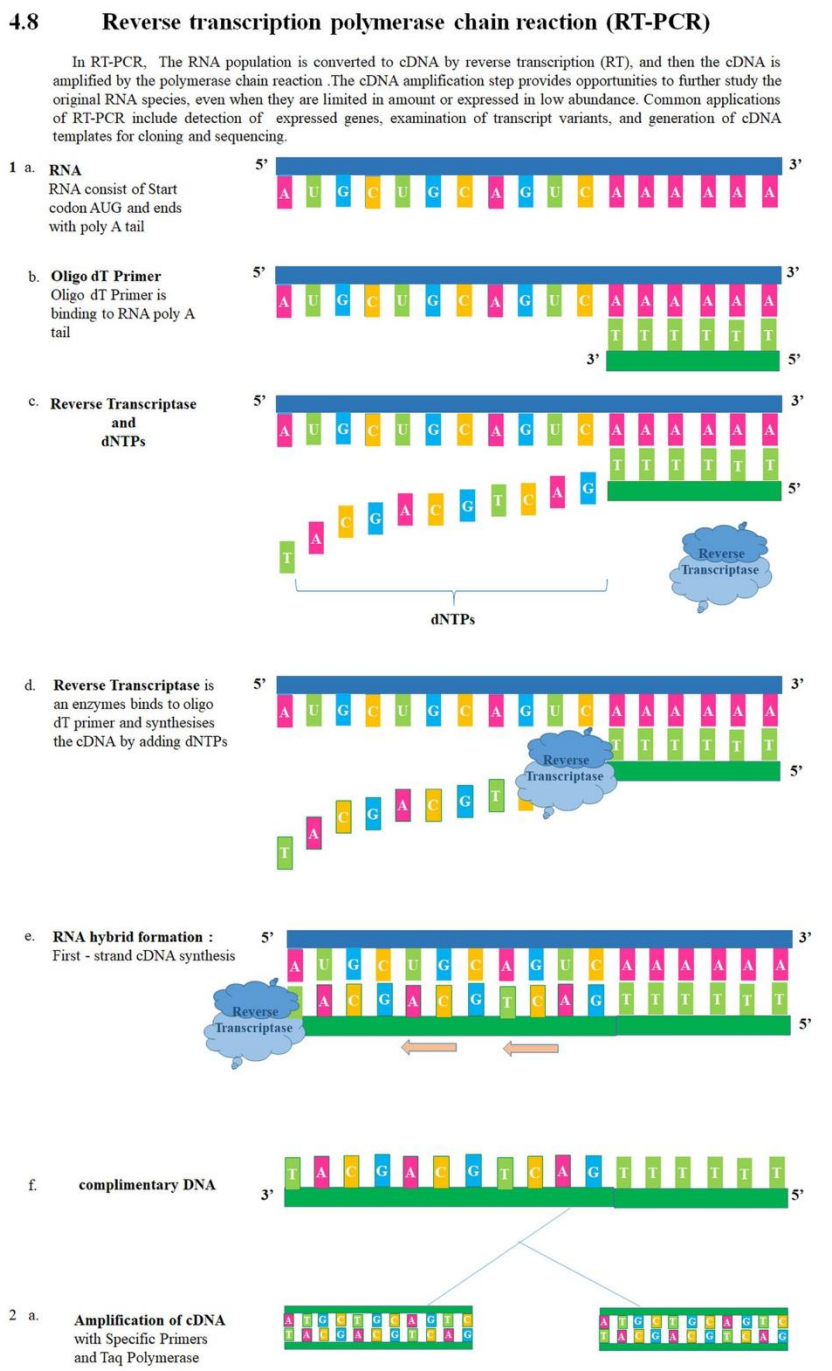


3) Cellular function

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

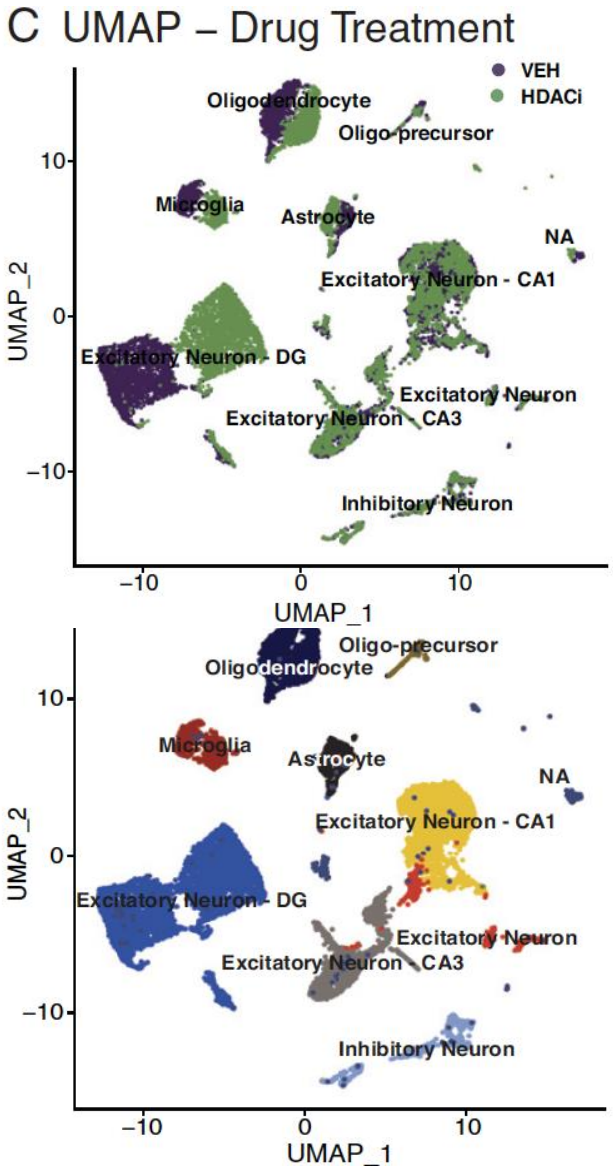


Gene presence/abundance: qRT-PCR



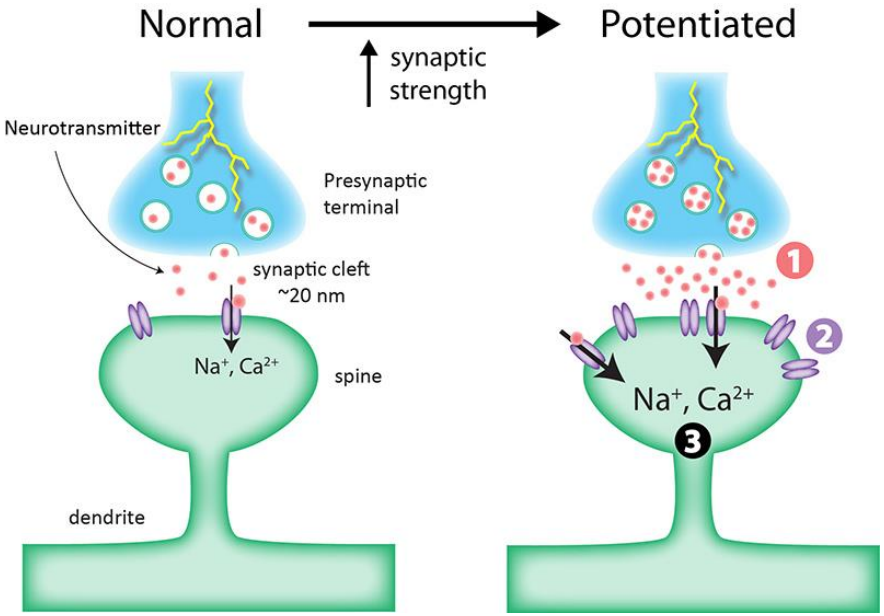
3) Cellular function

- Molecular techniques (c'd)
  - **qRT-PCR** (quantitative reverse transcription
    - Individual gene transcript (RNA) presence/abu
    - 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



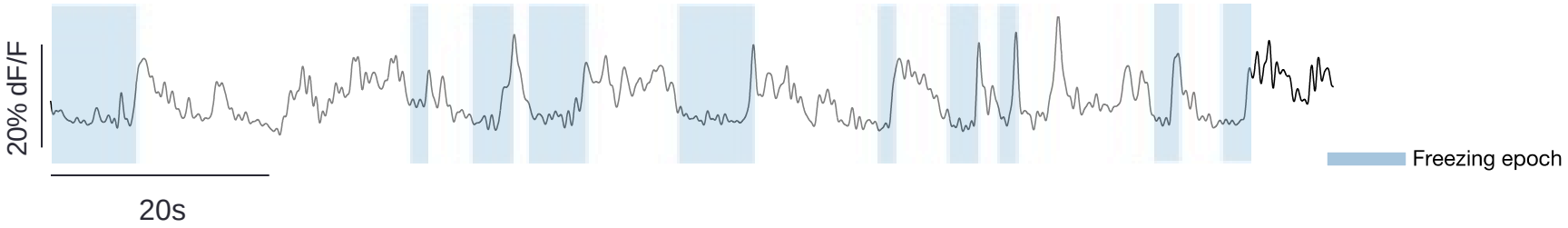
3) Cellular function

- **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



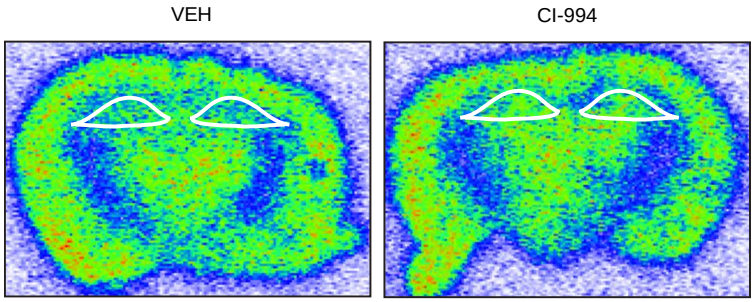
How to study the nervous system?

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

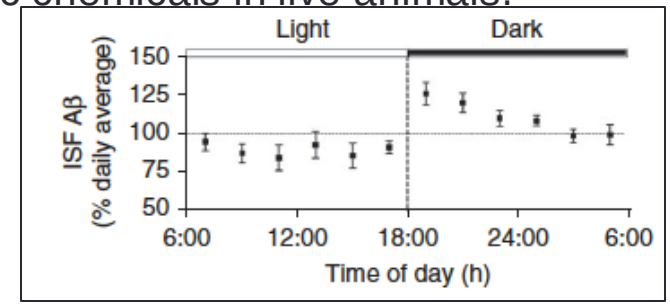
4) Brain function

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

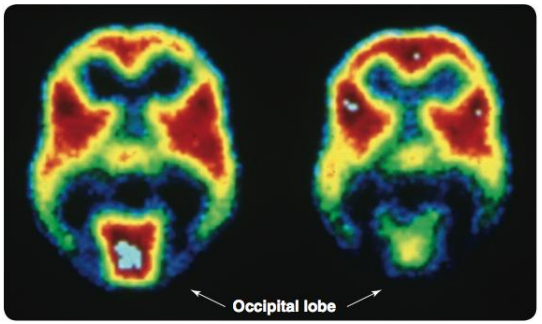
4) Brain function (c'd)

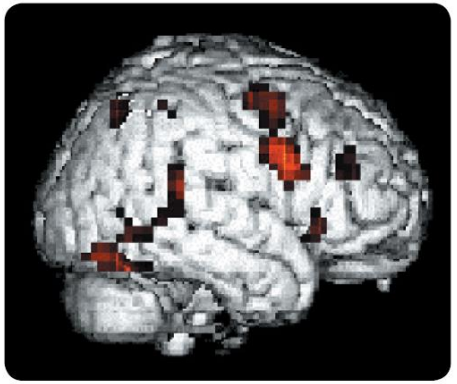


- Measuring chemical activity in the brain
  - 2-Deoxyglucose (2-DG) Technique
    - Inject an animal with radioactive 2-DG and allow it to engage in behavior of interest.
    - Use autoradiography to see where radioactivity accumulates in brain slices.
  - Cerebral dialysis
    - Measuring extracellular concentration of specific chemicals in live animals.
    - E.g., amyloid-β
      - Fluctuating with circadian rhythm



4) Brain function



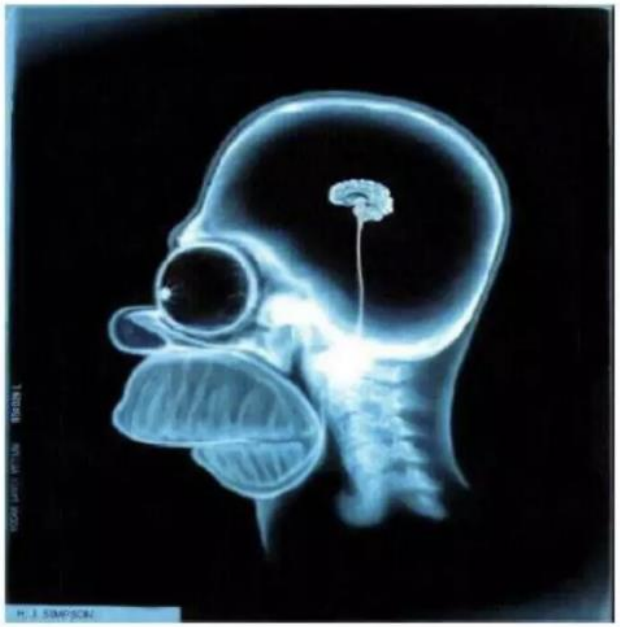


- Imaging techniques (H&A)
  - Positron Emission Tomography (PET)
    - Radionucleotide incorporated into active areas
  - functional Magnetic Resonance Imaging (fMRI)
    - Cerebral blood flow correlates with activity
- Lesions
  - Aspiration (H&A)
  - Radio-frequency (A)
  - Knife cuts (H&A)
  - Cryogenic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

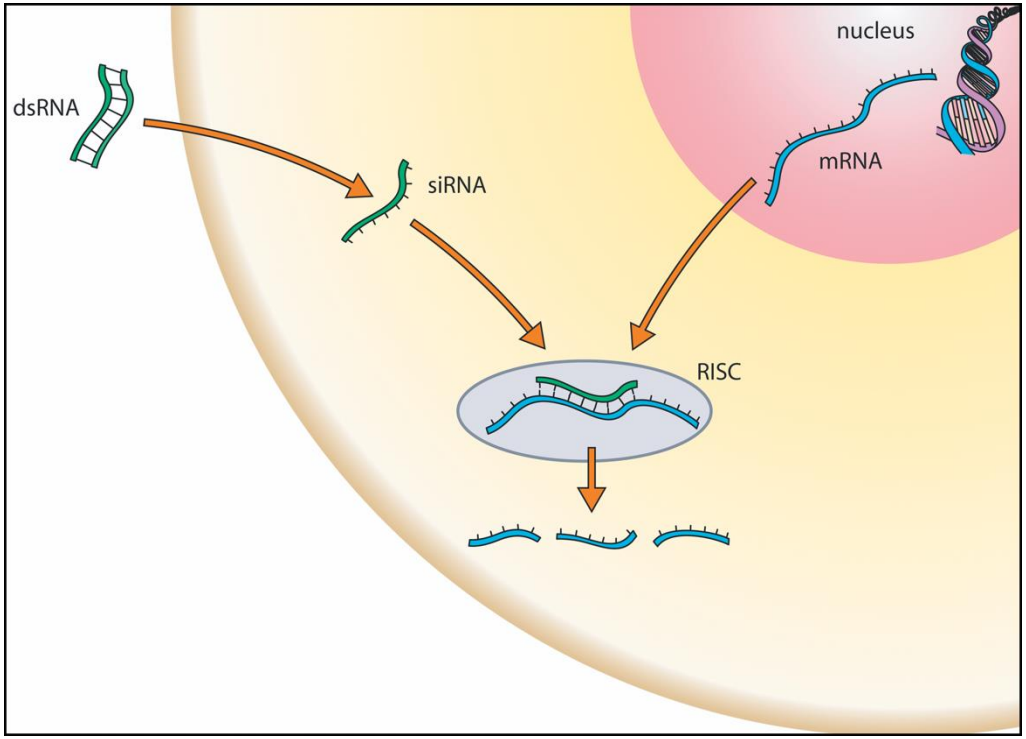
Knockout Mouse: A transgenic mouse in which the normal gene is missing or engineered so that is not transcribed or translated. “Knocks out” that gene.

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

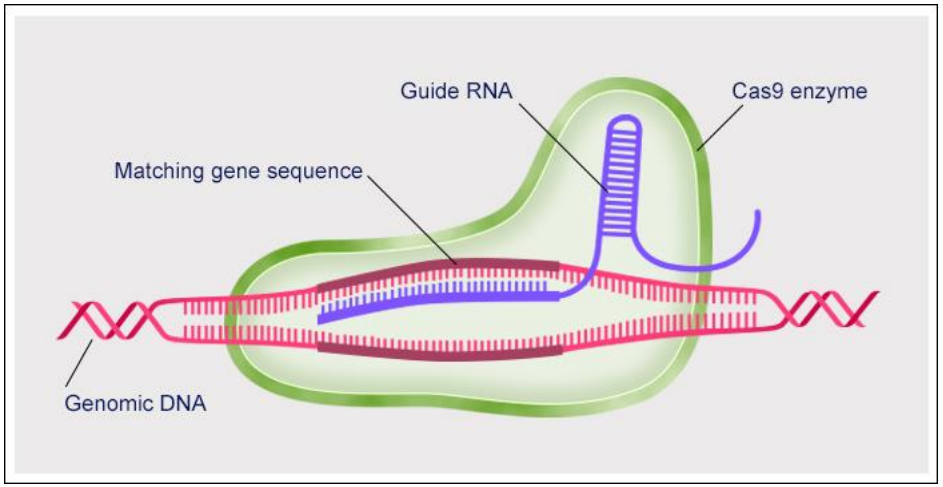


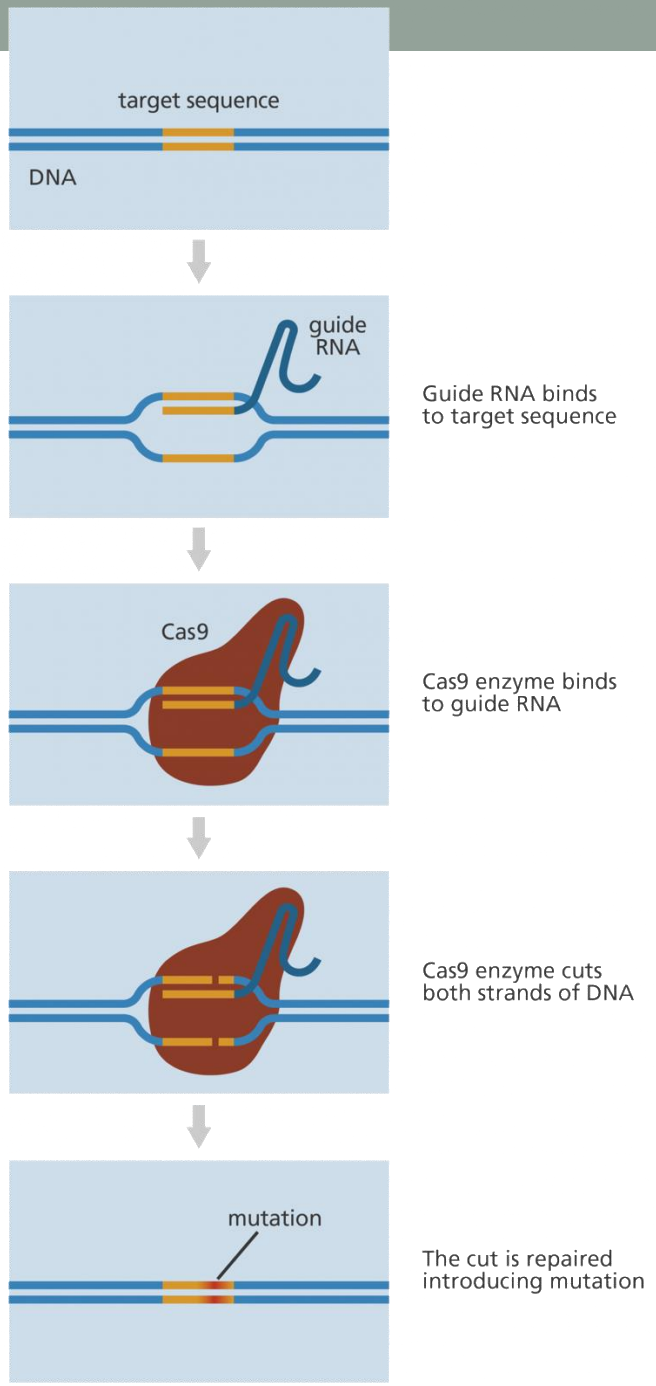
4) Brain function (c’d)

- **CRISPR-Cas9**

4) Brain function (c'd)

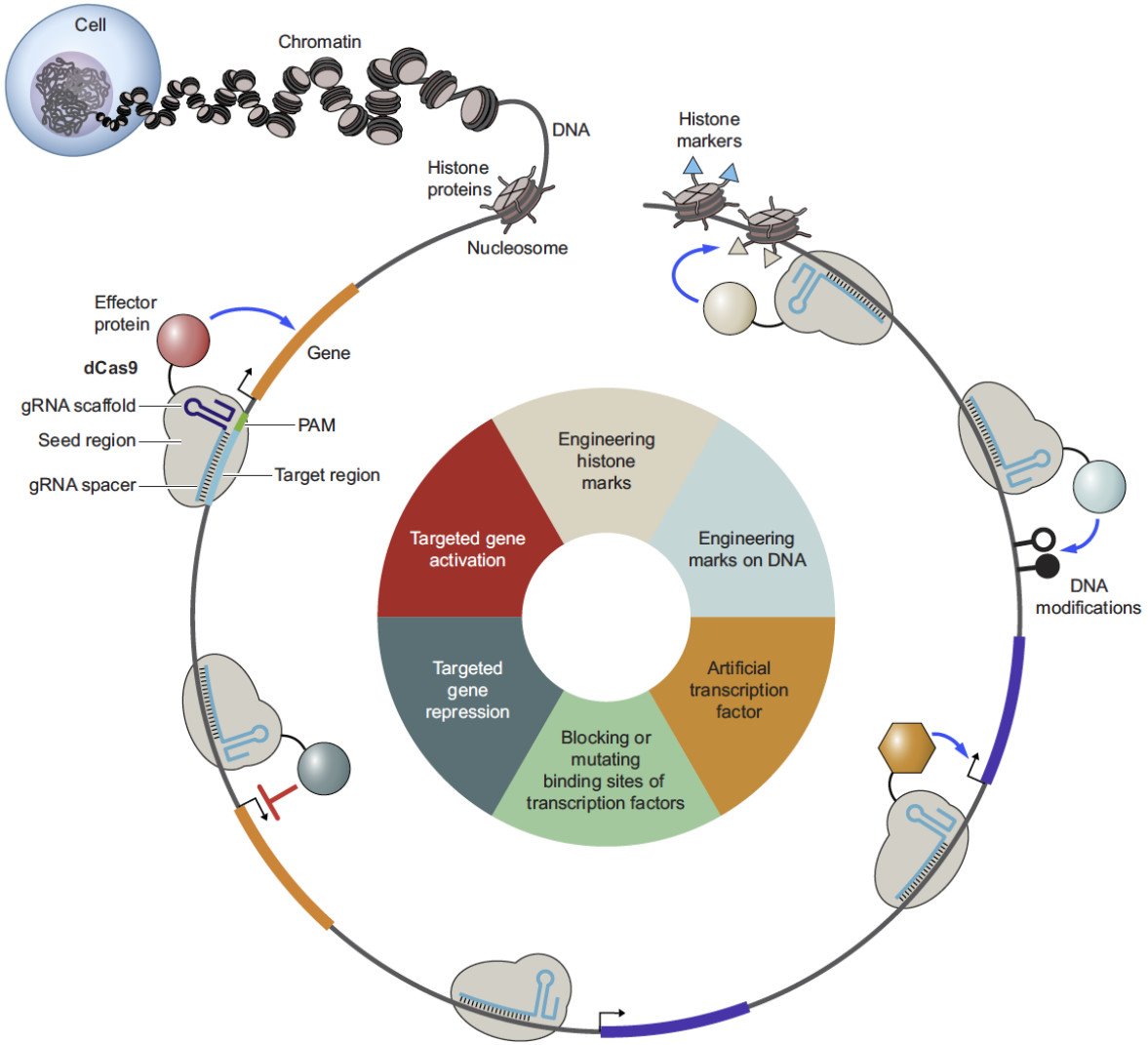
- **CRISPR-Cas9**
  - based on **c**lustered regularly interspaced short palindromic repeats





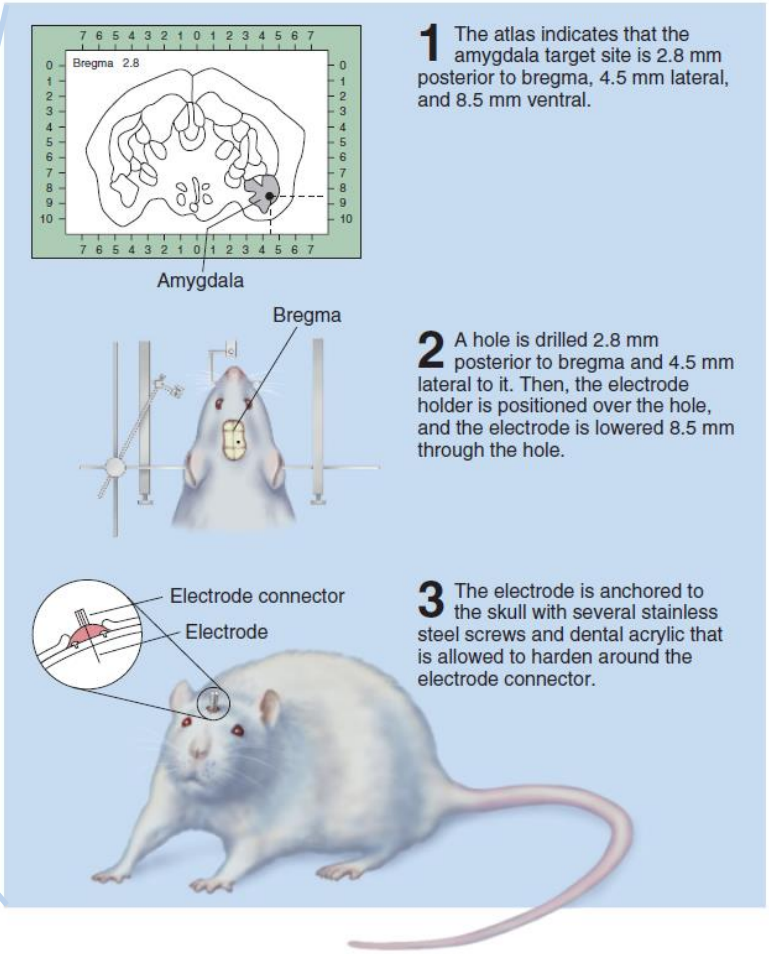
4) Brain function (c'd)

- **CRISPR-Cas9: Ramifications**



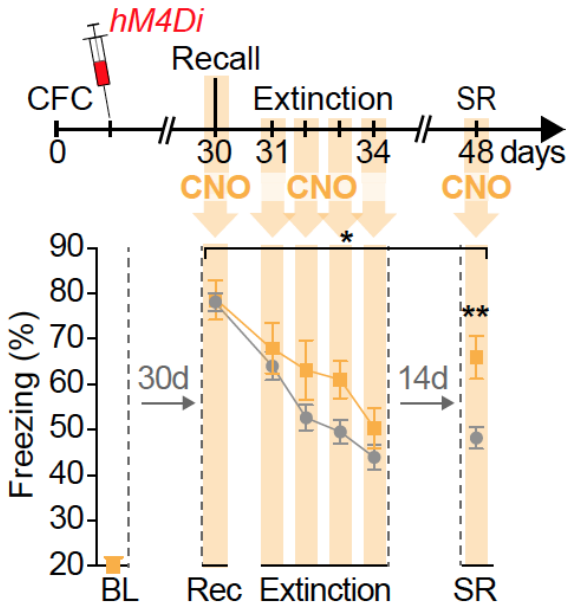
4) Brain function (c'd)

- **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)

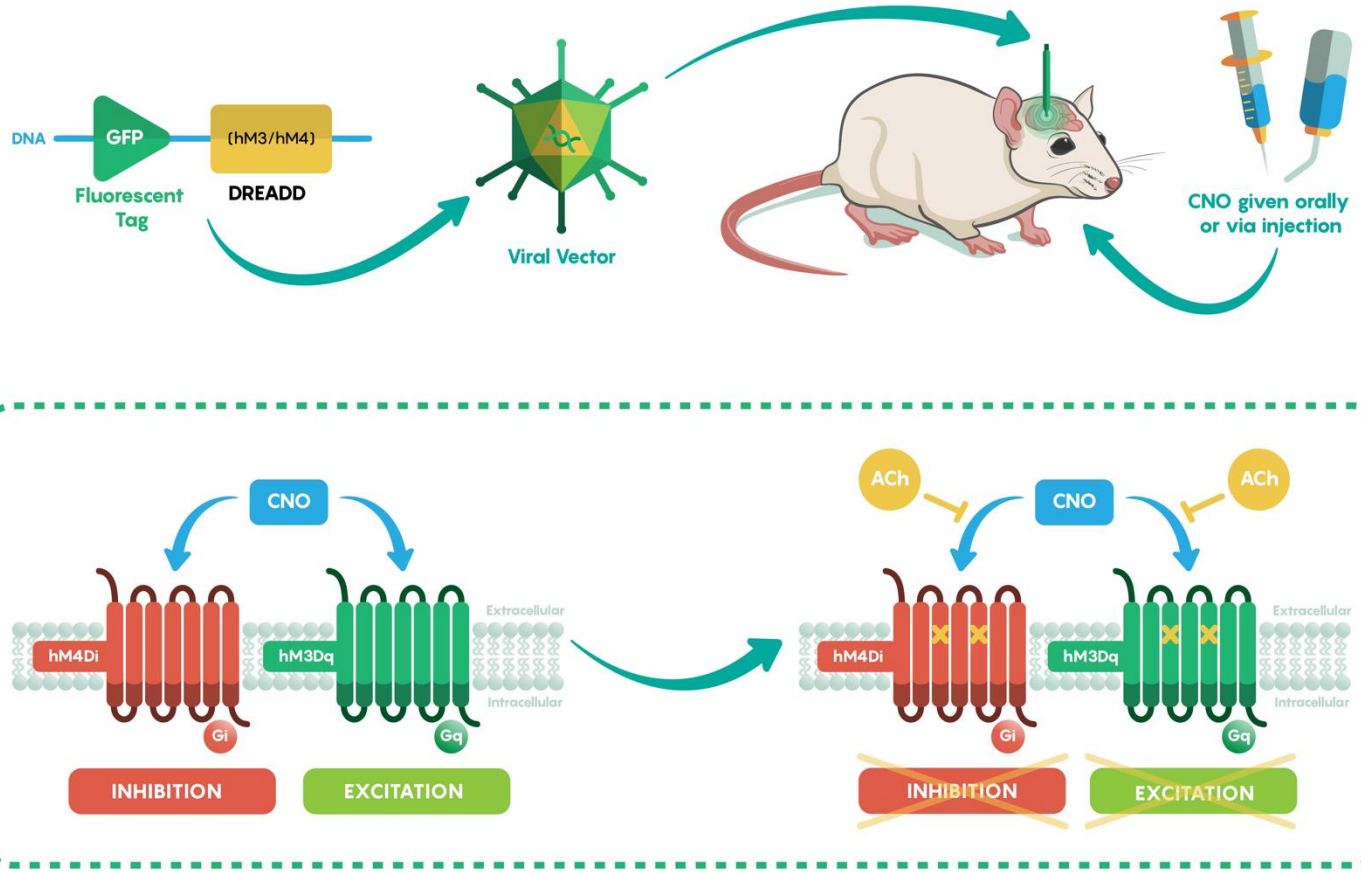


4) Brain function (c'd)

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



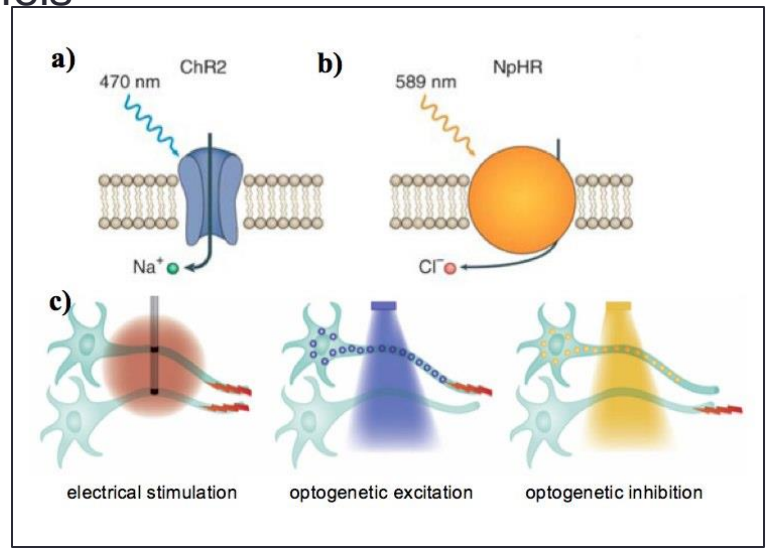
Chemogenetics



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

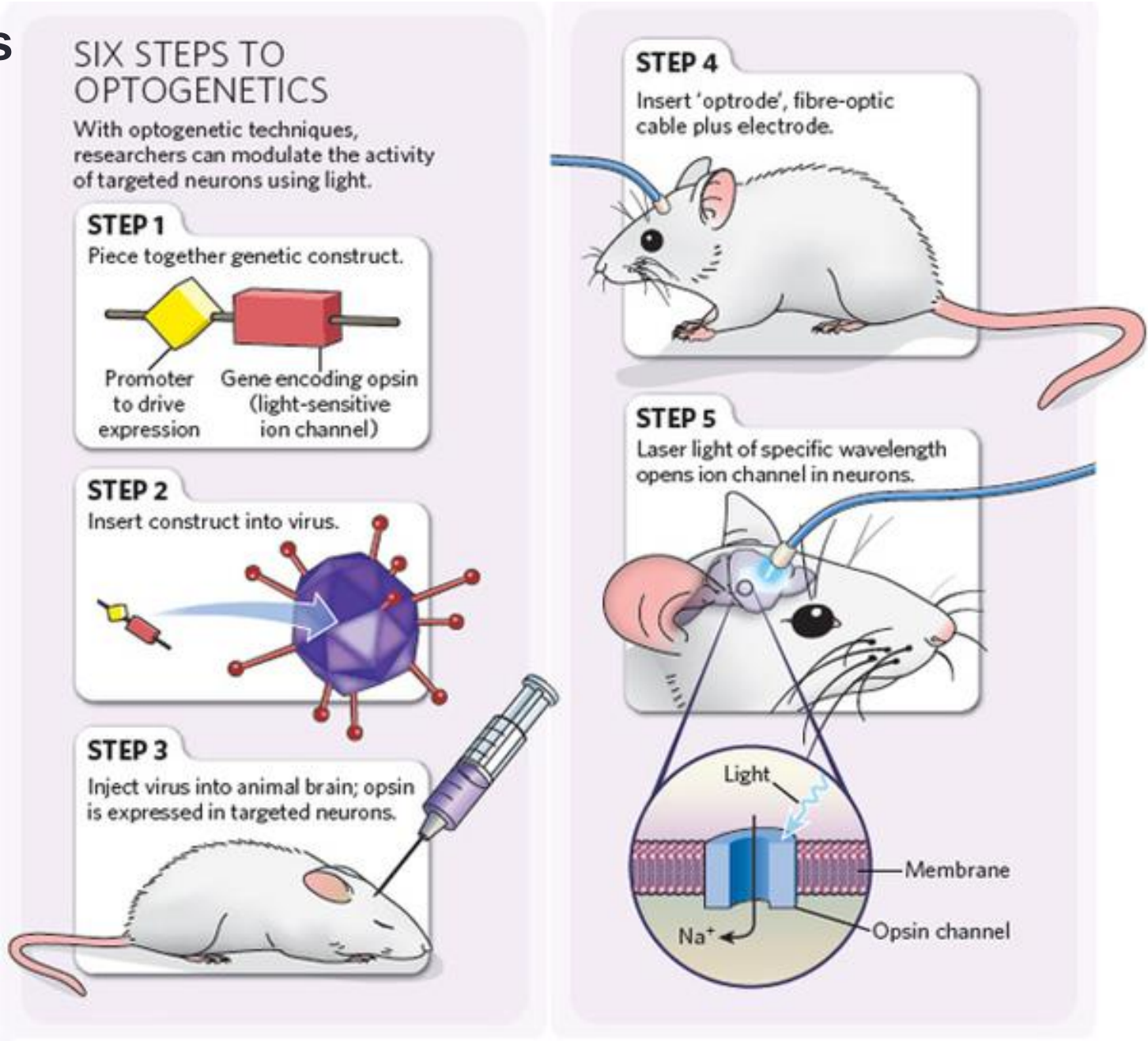
4) Brain function (c'd)

- **Manipulating brain function**
- **Optogenetics** (A)
  - Based on light-sensitive algal channels
    - Channelrhodopsin (ChR2)
    - Halorhodopsin (NpHR)

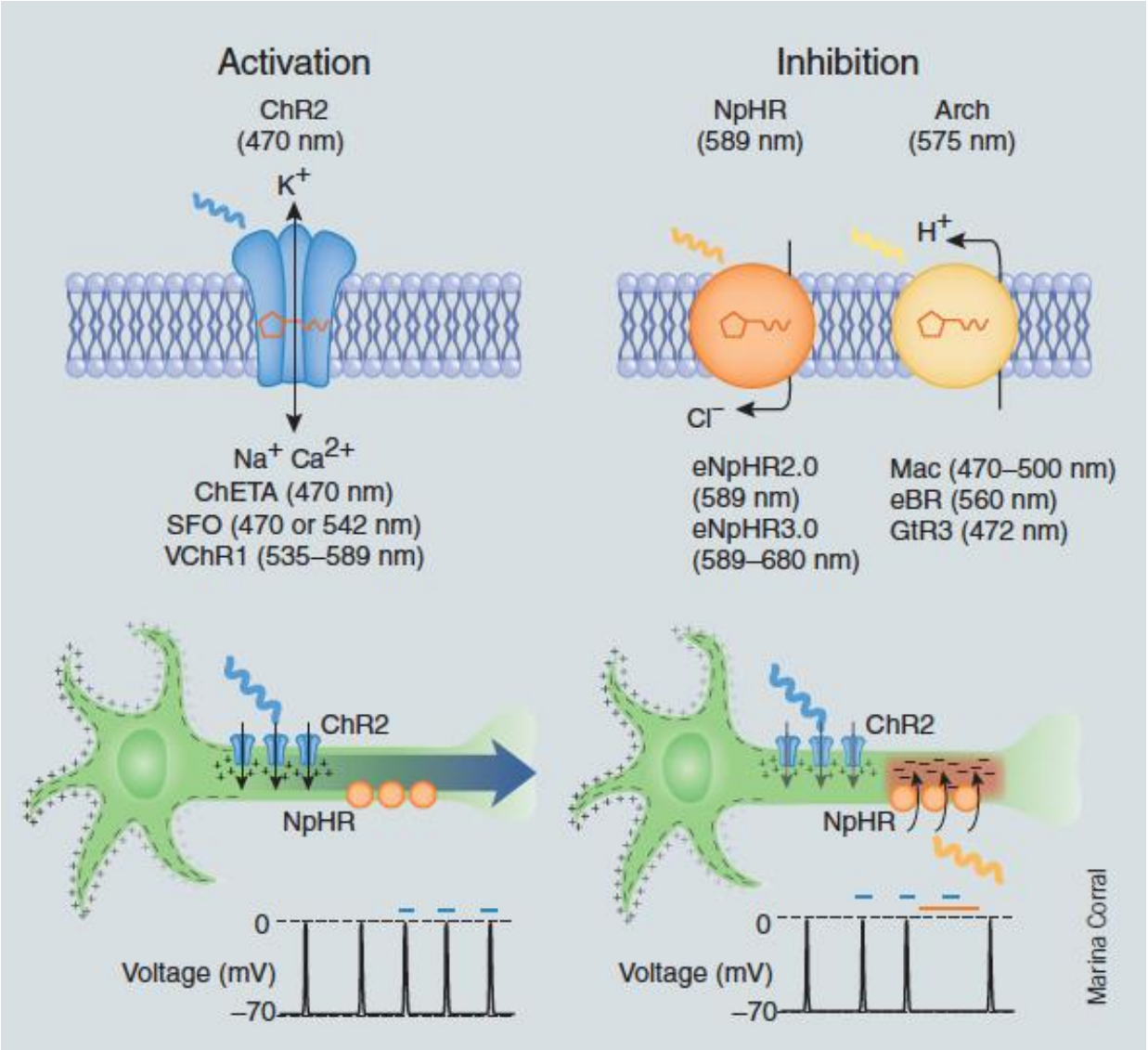


- By combining with cell-type specific promoters, optogenetics allows for the interrogation of the function of specific neuronal circuits with high temporal resolution

Optogenetics



Optogenetics – details

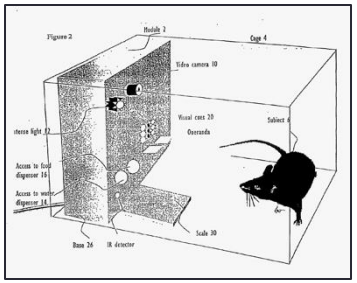


Manipulating brain function and **assessing its outcome**

- Paradigms for Assessment of Species-Common **Behaviors**

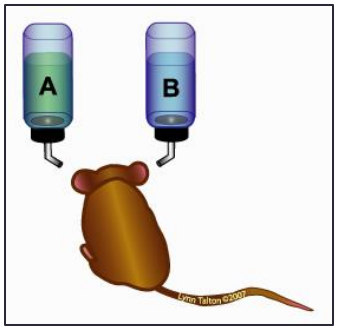
  - Open-field test
    - Anxiety, activity
  - Tests of aggressive and defensive behavior
  - Tests of sexual behavior

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



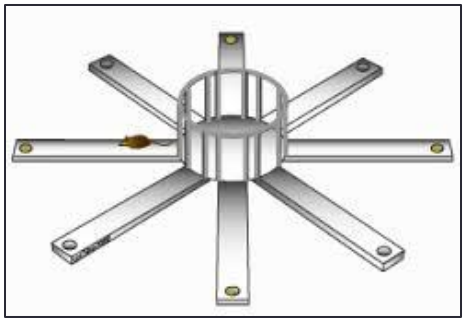
Manipulating brain function and **assessing its outcome**

- Animal Learning Paradigms

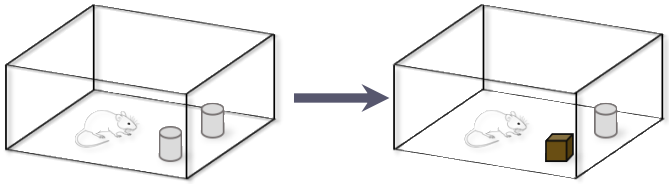


  - Conditioned taste aversion
    - Pairing something that makes an animal ill (emetic) with a taste

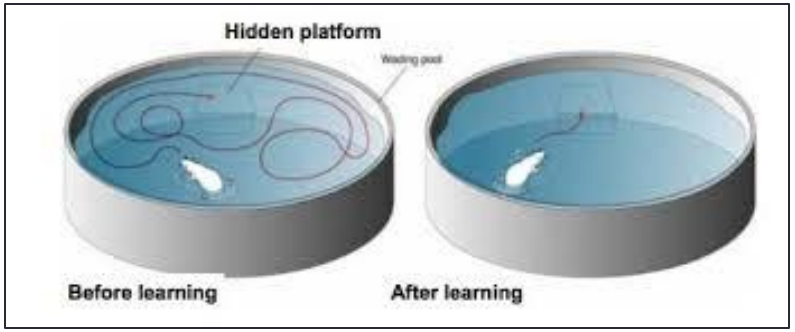
  - Spatial learning
    - Radial arm maze
    - Morris Water maze



  - Novel Object Recognition Test



Novel Object Recognition Test

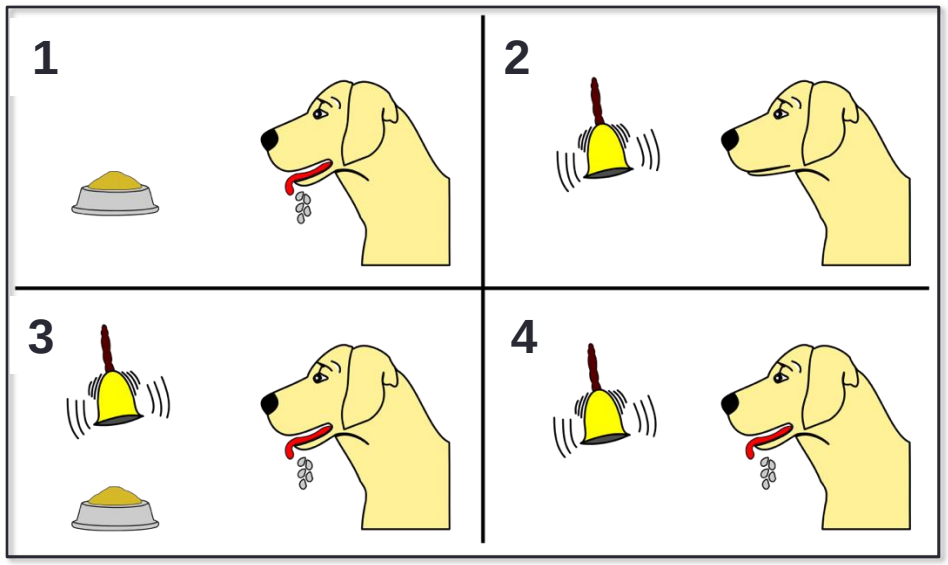


Manipulating brain function and **assessing its outcome**

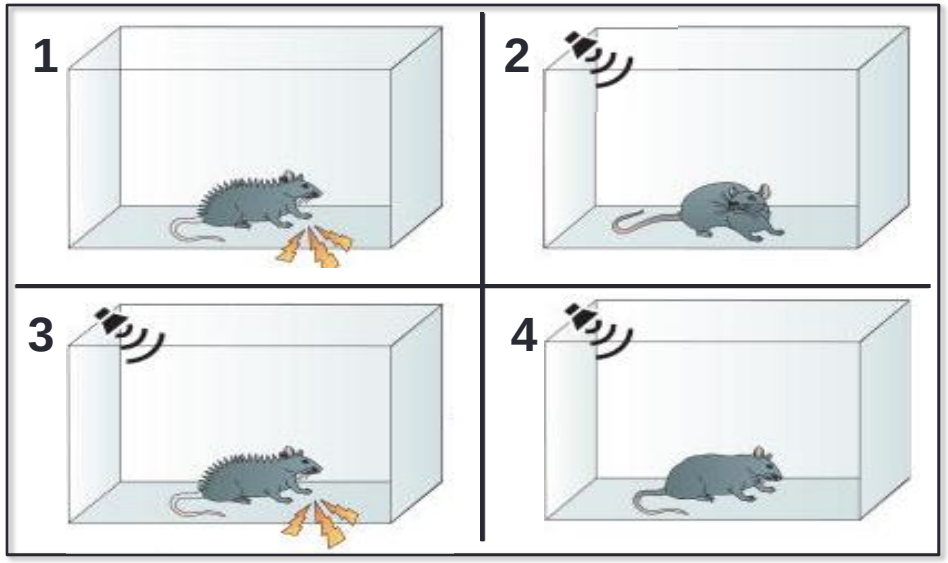
- Animal Learning Paradigms
  - Pavlovian Conditioning

Pavlovian conditioning

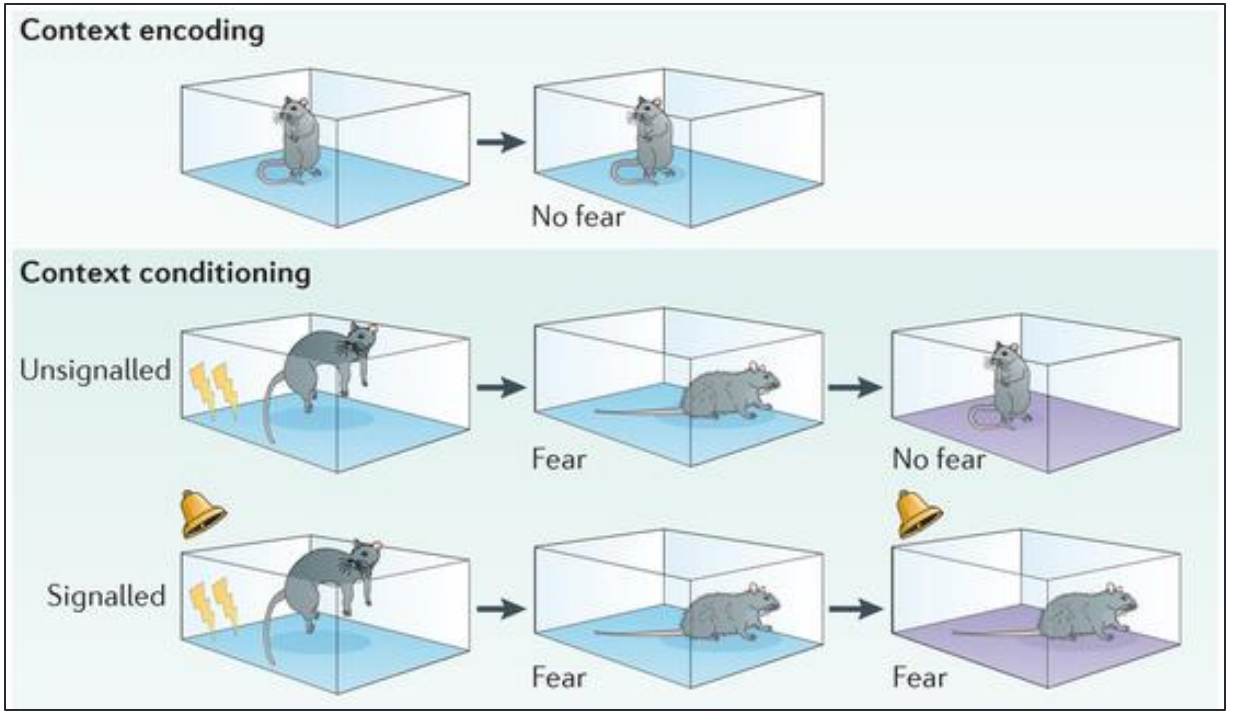
Ivan Pavlov
(1849-1936)



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