Remaining classes

- Thursday
  - Lecture on social behavior (no reading)
  - Vote on which target articles
  - Sign up for presentations
  - Demonstration presentation

- Next Tuesday (target article 1 presentations; ~10 people)

- Next Thursday (target article 2)

If you suspect “X” influences behavior “Y”, what do you measure?

Studying Behavior

- Describing behavior
- Asking how and why

- In many ways, far more complicated than physical methods

Abstractions

- Who is stronger -- men or women?
- Which sex has bigger brains?

- What conditions do you choose?
- Do lab tests mirror real behavior?

Why do birds sing?

- Immediate causation
- Development
- Evolution
- Adaptive function

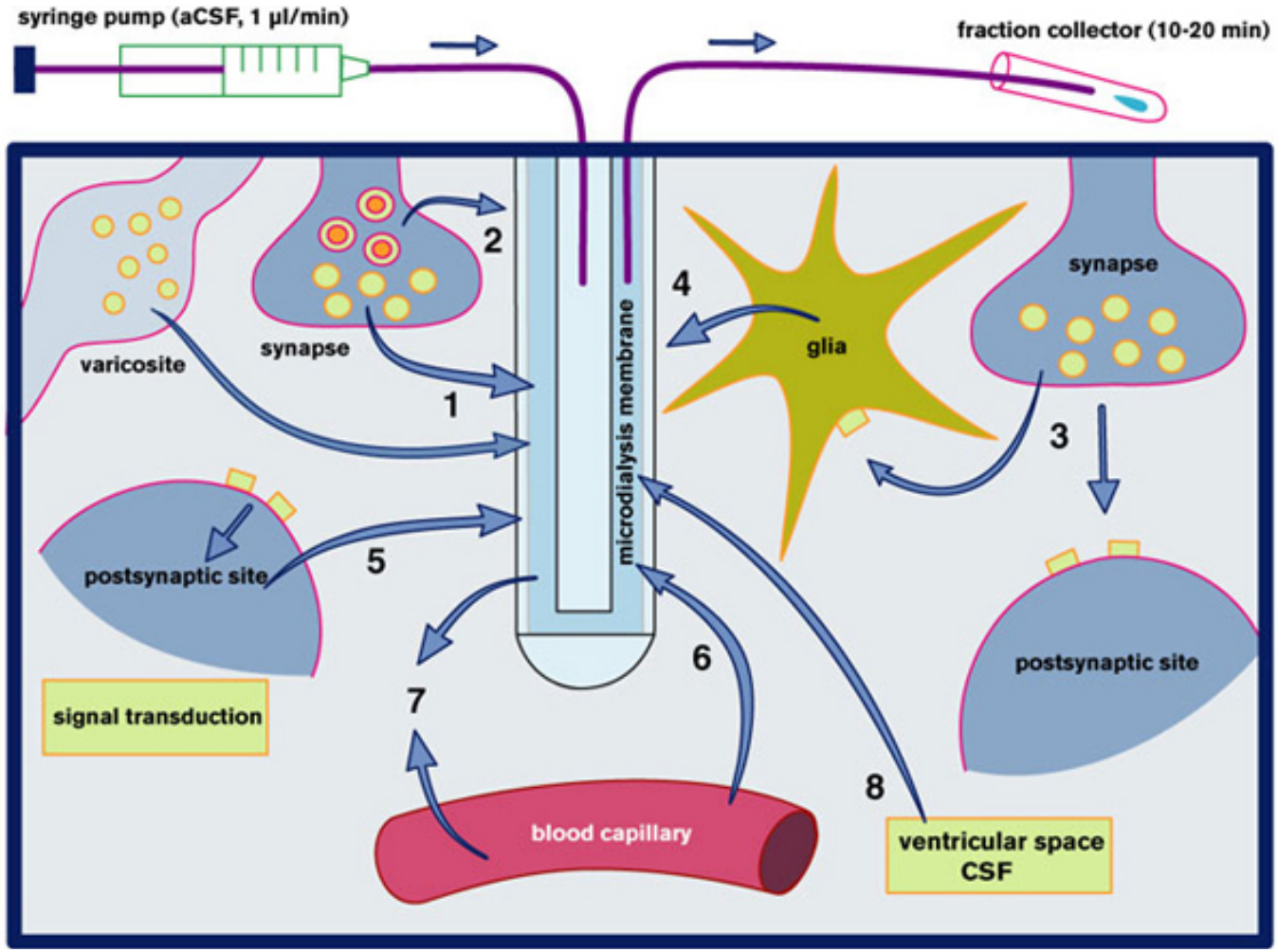
- **Measuring hormones and receptors**

Tissue collection

- Blood — easy, somewhat invasive
- Urine — easy, non-invasive
- Saliva — easy, non-invasive
- Cerebrospinal fluid (CSF) — invasive
- In situ (e.g., brain nucleus) — terminal only

- Trend is towards more specific, localized measurements

Probes in particular brain regions



Probes in particular brain regions

Can measure

- firing – electrodes
- chemicals released – microdialysis

Can deliver

- electrical stimulation (lesion or stimulating)
- drugs/hormones

Quantifying Hs and Rs-make something stick to them

Hormones stick to receptors
can stick labeled H to measure R

Antibodies (Ab) stick to antigens
can stick labeled Abs to H or to R

cDNA sticks to DNA, RNA
can stick labeled cDNA to RNA

Make a sticky thing visible

Radioactive

Coloring Enzymes

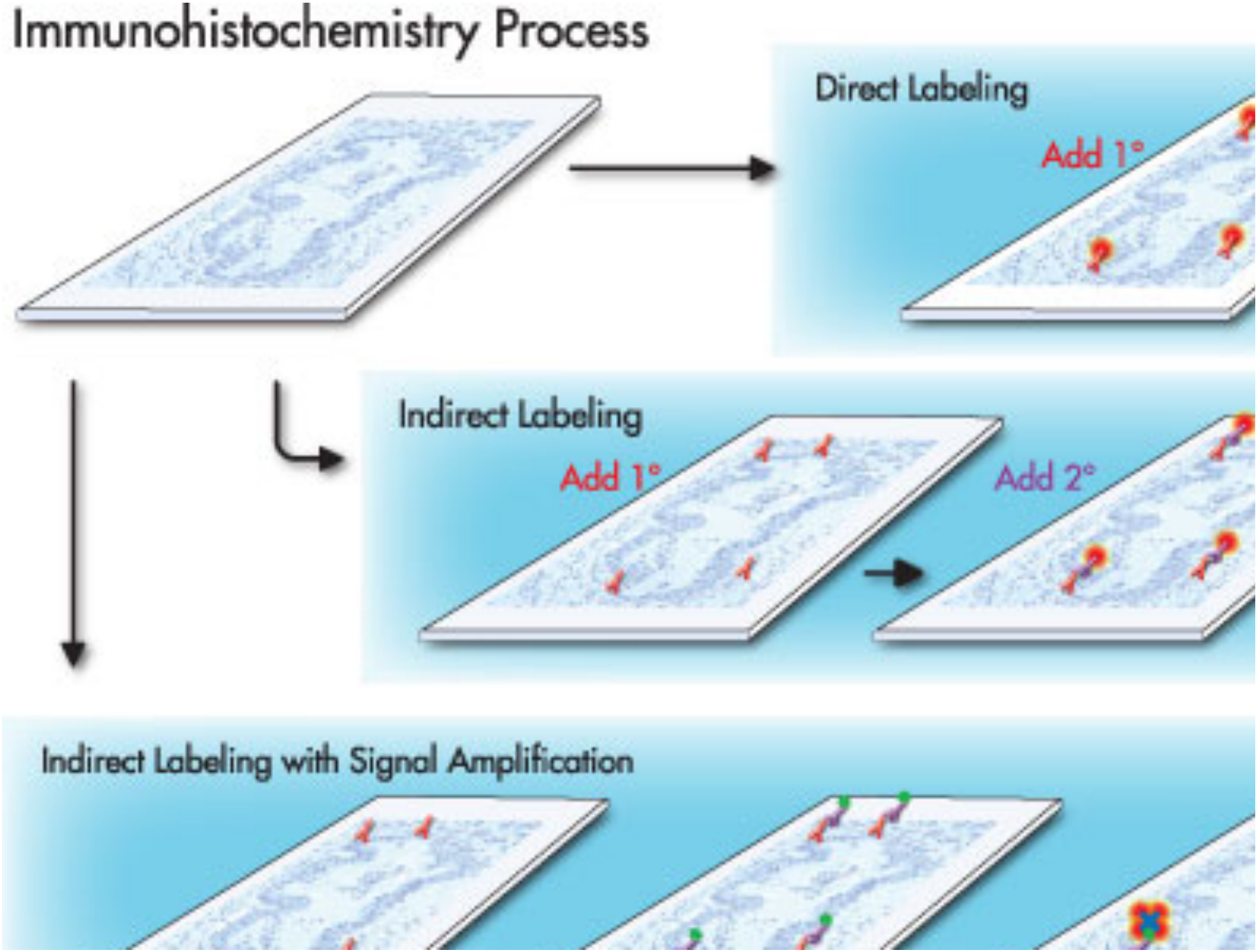
Fluorescence

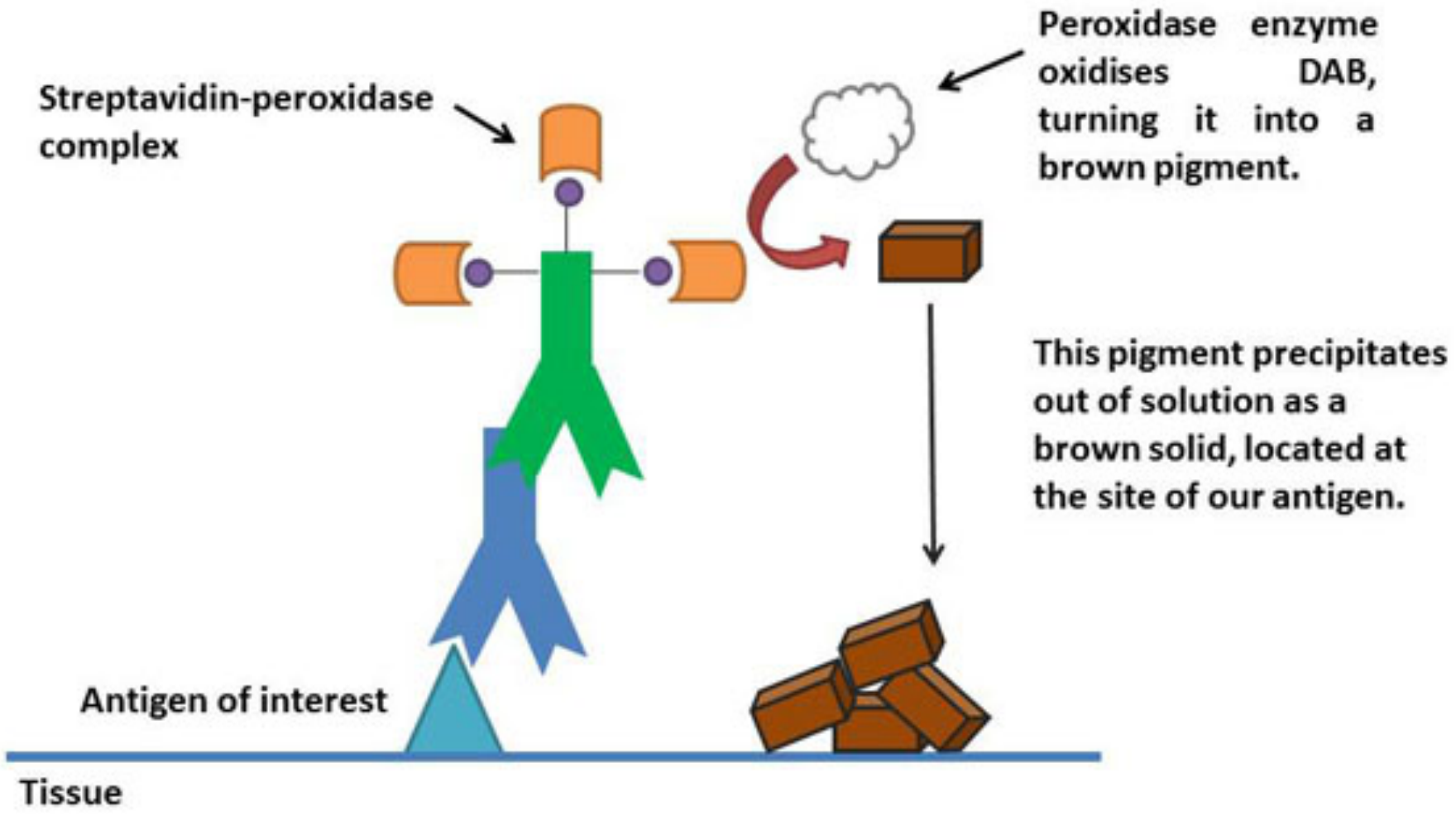
Preparing brains

- Freeze or fix brain
- Slice it thinly

- Non-specific staining allows structure to be seen

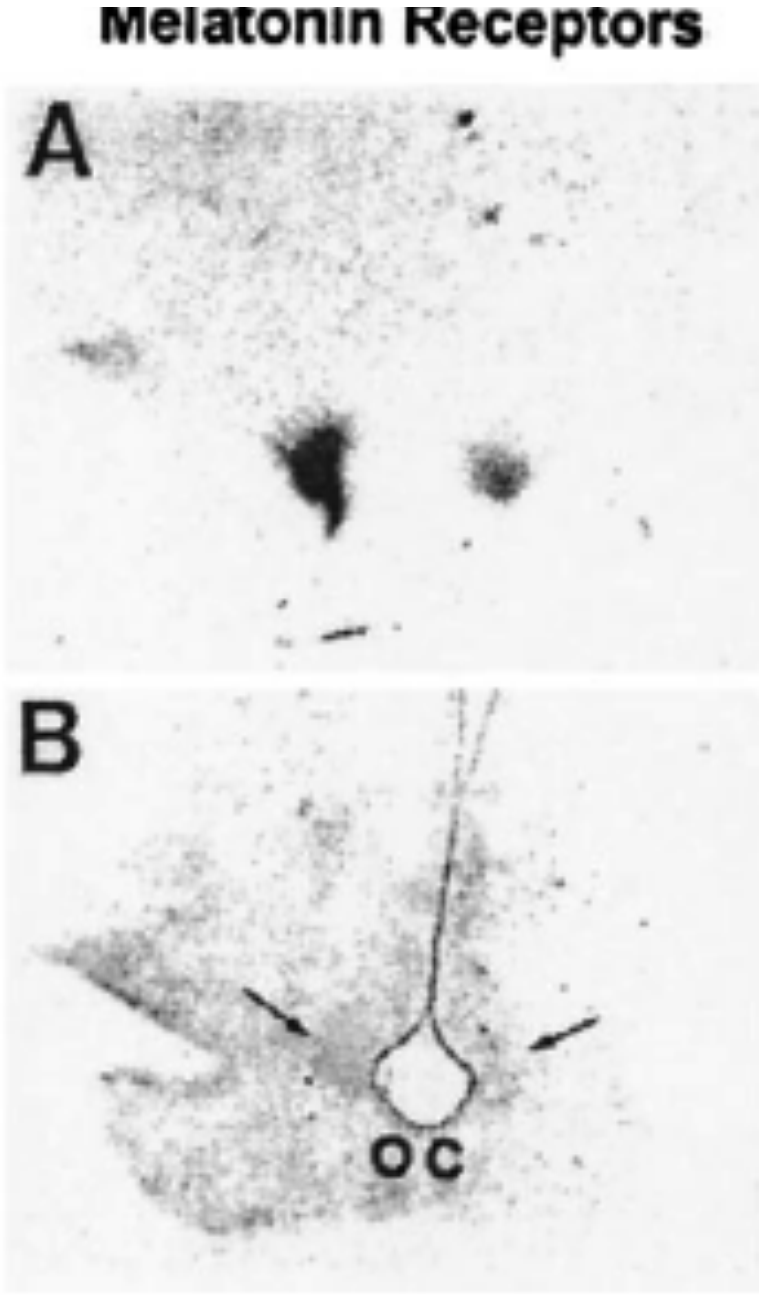
- Expose it to sticking stuff
- Develop it
- Look under microscope


Immunohistochemistry Process
Direct Labeling
Add 1°
Indirect Labeling
Add 1°
Add 2°
Indirect Labeling with Signal Amplification


Streptavidin-peroxidase complex
Peroxidase enzyme oxidises DAB, turning it into a brown pigment.
This pigment precipitates out of solution as a brown solid, located at the site of our antigen.
Antigen of interest
Tissue

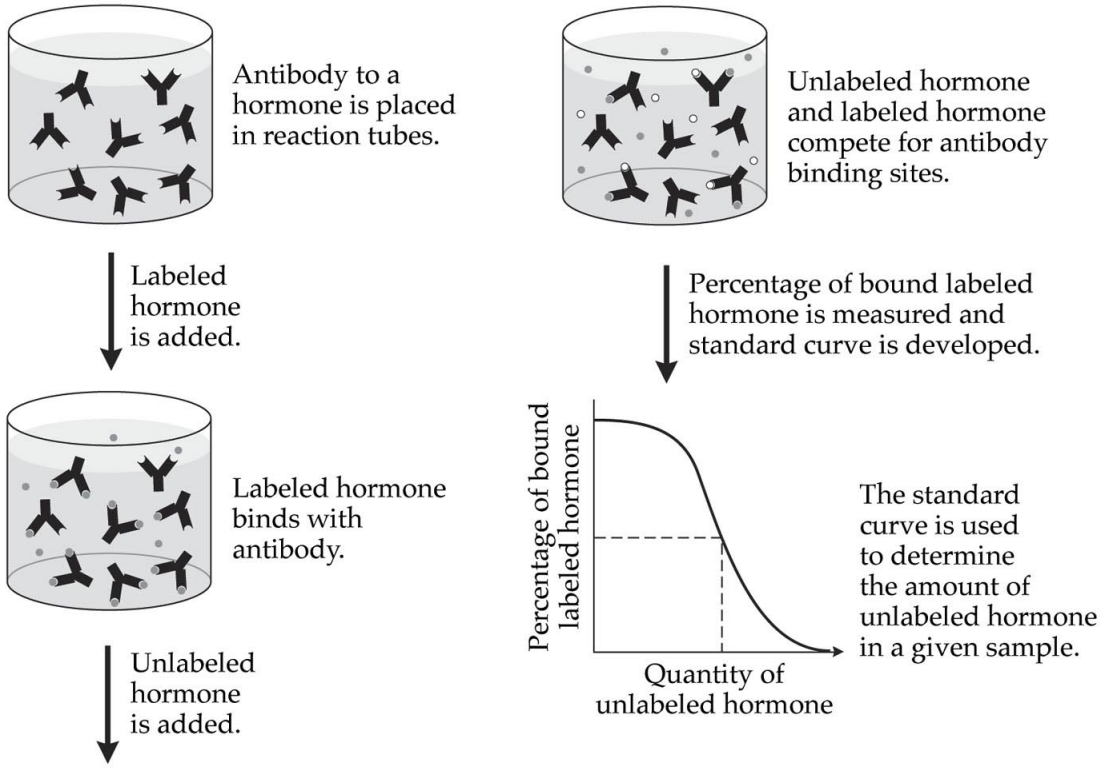
Example: Autoradiography

Fig 4. A, Localization of [^{125}I]melatonin binding to the SCN of an 18-week gestation human fetus. Specific labeling is shown in black. B, The stained section used to generate the autoradiograph in A. Reproduced with permission from Reppert et al.[36] C, Localization of [^{125}I]SKF38393 binding to D1 dopamine receptors in the SCN of a 20-week postconceptional human infant. Specific labeling is shown in black. D, Nonspecific labeling. Reproduced with permission from Rivkees et al.[37] OC, optic chiasm; ST, striatum. Arrows identify the SCN.



Example: Radioimmunoassay (RIA)

Common method for measuring hormones – uses Abs that recognize only the hormone of interest. Can be TRICKY method



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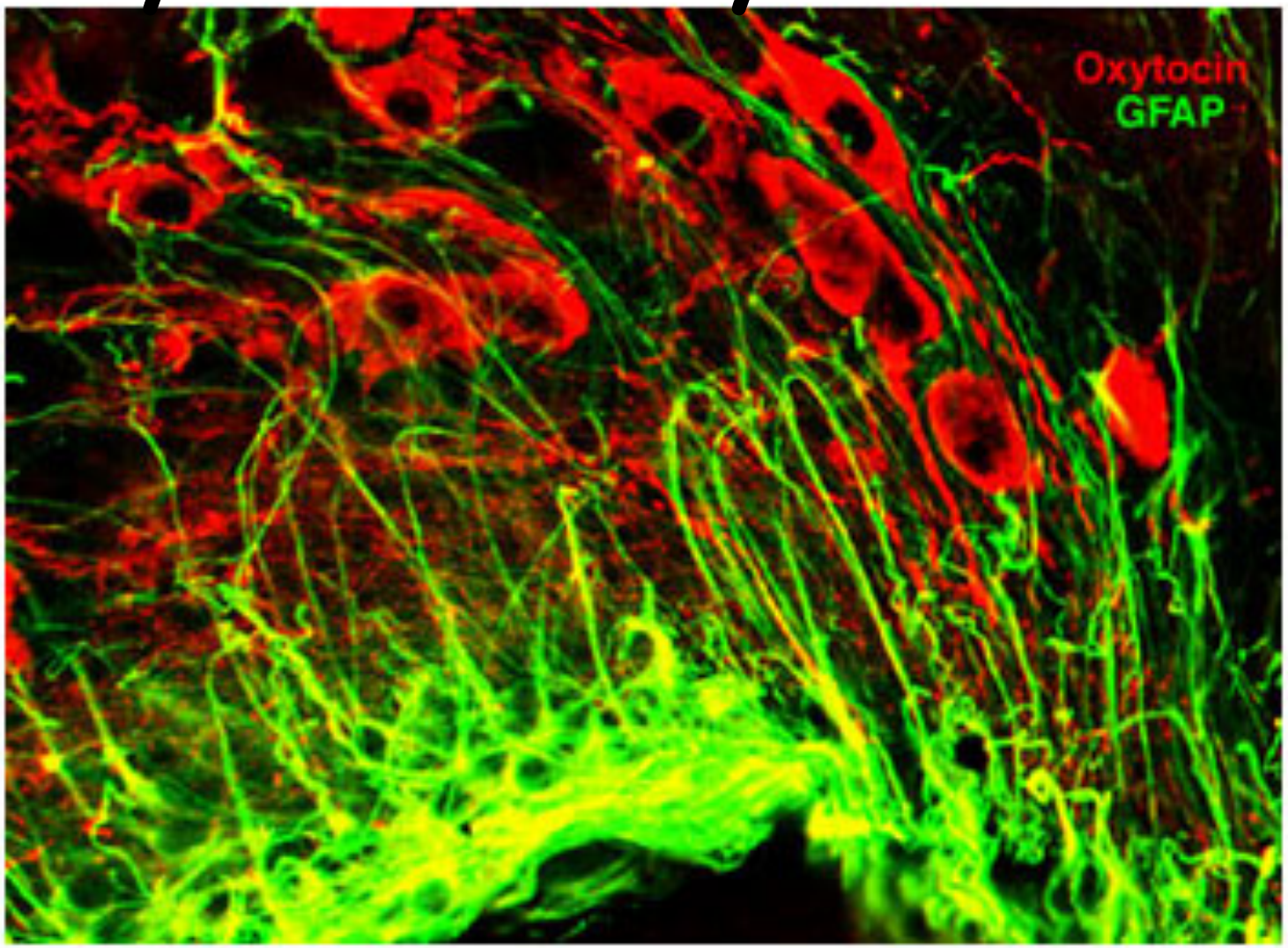
Example: Immunocytochemistry



Oxytocin and GFAP (acid glial filament protein) immunoreactivities in the SON.

Measuring activity/activation

- Blood flow (humans and animals)
  - fMRI
- Electrical activity (very invasive)
  - Insert electrode in brain
- Signature of activity (terminal)
  - C-fos gene/c-Fos protein
    - Usually use immunocytochemistry to quantify

Example: Activated cells

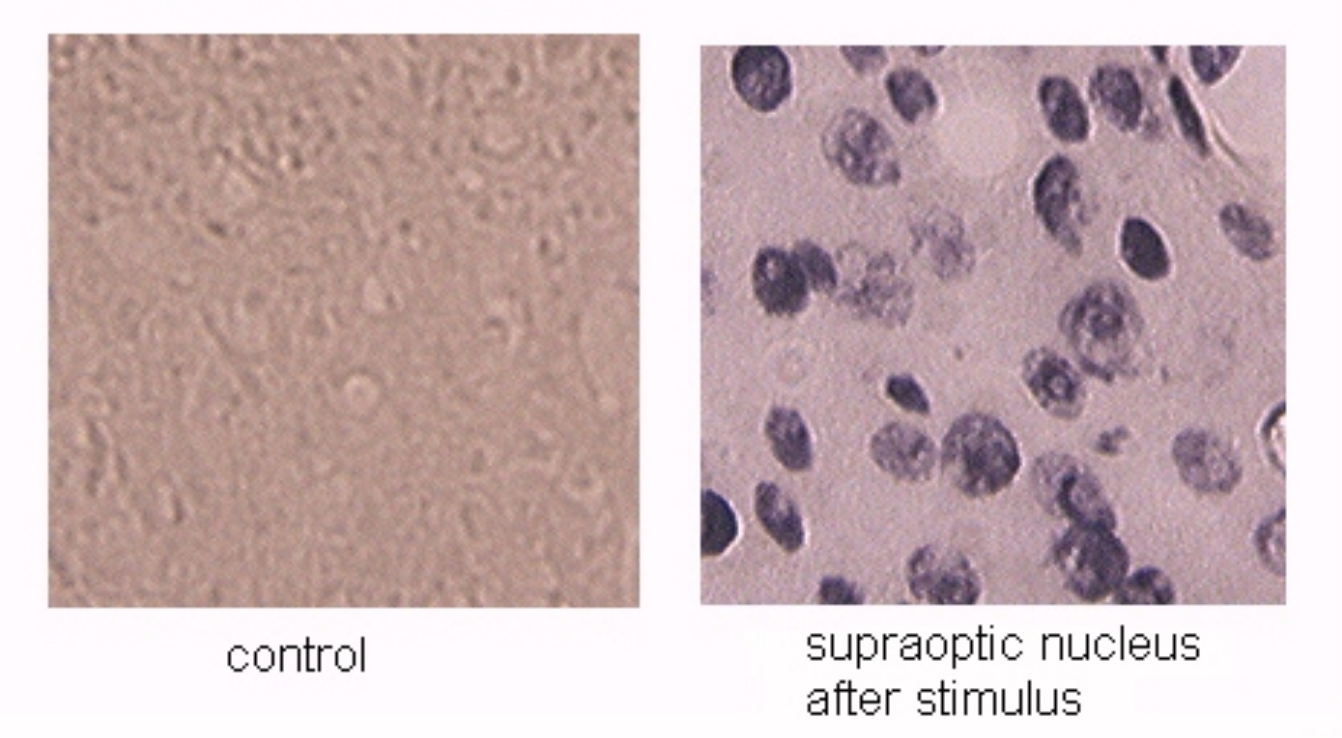
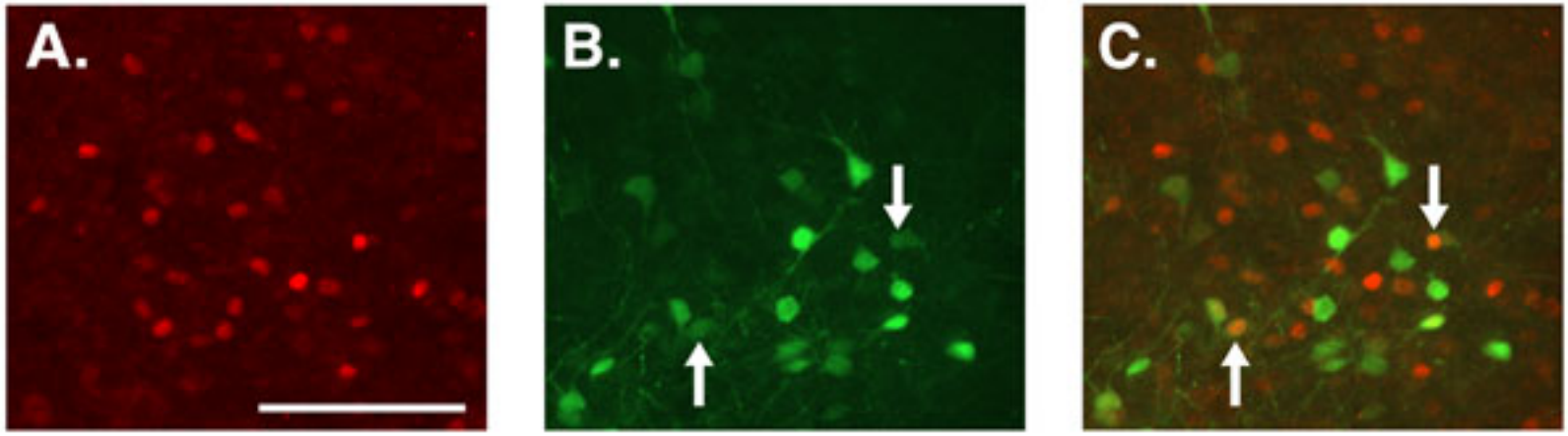
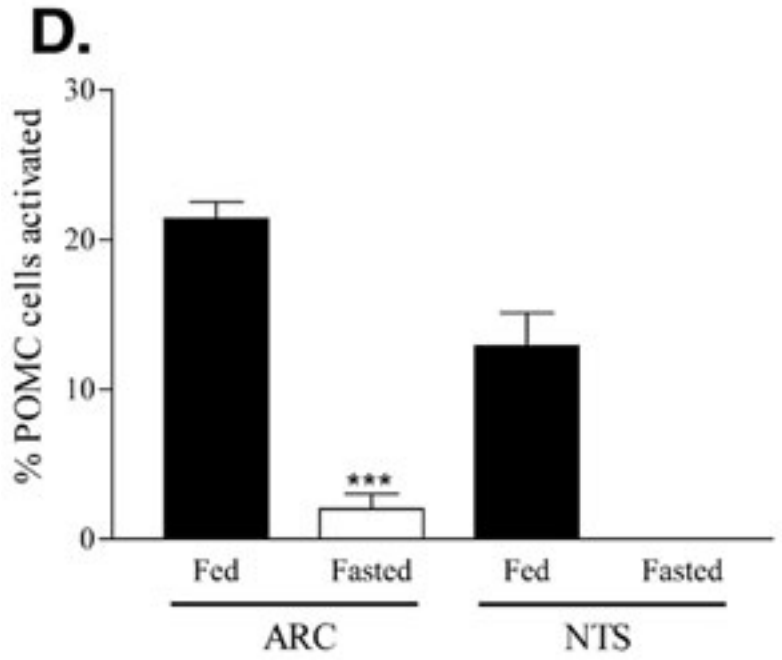


Figure 1 – Above are 2 micrographs of a group of neurosecretory neurones which have been immunocytochemically stained to demonstrate FOS. The cells in the right hand picture received an excitatory stimulus 2 hours before the section was taken for histology. A non – stimulated section (left hand picture) is shown for comparison. Above is shown a high power micrograph comparing sections of the supraoptic nuclei from a control rat and a rat which had been drinking a hypertonic (high osmotic pressure) solution. The supraoptic nucleus which contains only oxytocin and vasopressin cells is located in the hypothalamus.

Example: identifying which activated cells





E.

Feeding activates NTS POMC neurons. (a) c-Fos-IR neurons in the NTS 60 minutes after food intake (b) POMC-EGFP-IR cells in the NTS 60 minutes after food intake. (c) Colocalization of c-Fos and POMC in NTS neurons 60 minutes after food intake. (d) The percent of NTS POMC neurons at 1100h expressing c-Fos in ARC and NTS in control (fasted) animals, or animals receiving food (fed) at 9000-1000h. (e) Percent of c-Fos-IR neurons in ARC or NTS expressing POMC-EGFP in animals in the fed (n=8) or fasted state (n=3). ***P<0.001. Size bar (a) = 100M. Arrows indicate cells expressing both EGFP-IR and c-Fos-IR.

Genotyping

- What version of a gene does a person have?

- Sequencing (getting cheaper)
  - Done for breast cancer

- Single nucleotide polymorphisms (SNPs)

Measuring gene expression

- Genes are expressed (turned on) and mRNA is produced
- Design probe for mRNA you are interested in
- PCR (polymerase chain reaction) allows you to cheaply measure amount of specific mRNA
- Not available for many species

Measuring all genes expressed

- Tiny chips with cDNA to all genes (micro-arrays)
- Stick sample onto chip
- Machine reads how much cDNA attached to EACH gene
- Can assay 30,000 genes for ~$500

Drugs (pharmacology) to manipulate …

Synthesis (e.g., aromatase blocker)

Receptor activation

- antagonists (e.g., anti-androgens CPA)
- agonists (mimic steroid)

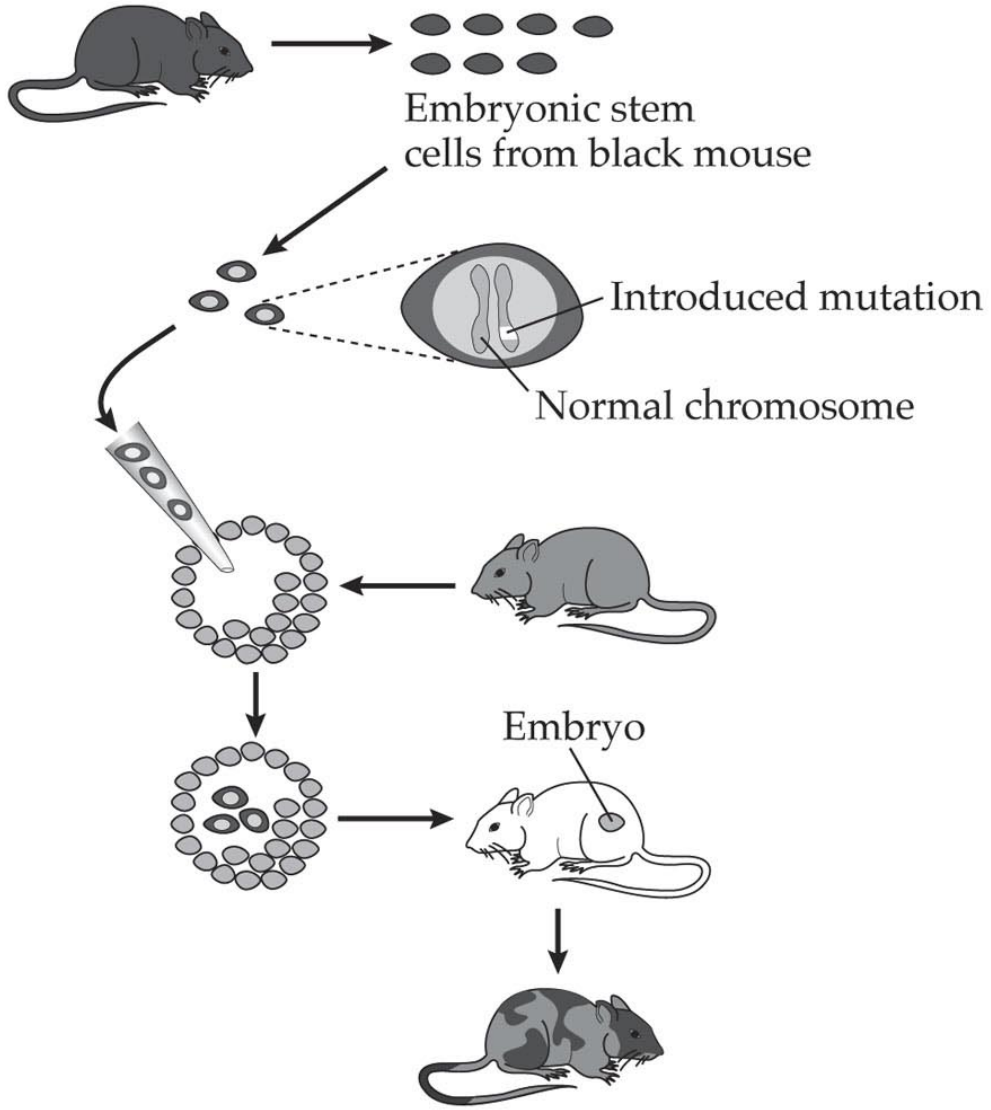
Given systemically (globally into blood) or locally (implanted in a brain region)

Genetically engineered animals

Lacking H or R

Overexpressing H or R

Ideal: ability to turn
H or R on or off



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Engineering knockout mice has challenges

- Global knockouts
  - Effects everywhere
  - Compensation
- Tissue-specific knockouts/knockins
  - Delivered by virus
  - OR only affected in certain cell types
- Conditional knockouts
  - Turned on or off (avoids compensation)

Genetic engineering

- Alters gene of interest
- Often alters nearby genes
- Typically alters 100s to 1000s of others
- Developmental compensation

Summary

- Many approaches to physical characterization of hormone/ behavior systems
- Multiple research questions about behavior
- These issues at forefront in hormone-behavior *interactions*