



# Techniques to Examine the Brain

Psychology 372

Physiological Psychology

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## Invasive Techniques

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- Lesioning
  - Electrical
  - Chemical
  - Radio Frequency
- Electrical Recording
- Cannulations
- Push Pull
- In vivo Autoradiography
- Others

## Non-Invasive Techniques

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- X-Ray
- CT
- PET
- MRI and fMRI
- Electro Recording (EEG)
- SPECT
- SQUID
- Others

## The Stereotaxic Instrument

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- The holder allows you to swing in all directions.
- A vertical knob allows you to vary the depth of the probe.
- The anterior/posterior knob allows you to go front or back.
- A lateral knob allows you to go from side to side.

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# Animal Stereotaxic Instrument

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## General Procedure

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- Identify and measure the dependent measure in the organism.
- Anesthetize the animal.
- Open the scalp.
- Drill a trephine hole (hole in the skull).
- Place the animal into the stereotaxic instrument.
- Use the brain atlas to see where you want to go.

## General Procedure

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- Find specific brain landmarks.
- Use stereotaxic instrument to insert:
  - a. electrodes.
  - b. cannulas.
  - c. other instruments.
- Perform the technique you want, or you can seal the material in place with a glue-like substance.
- Allow the animal to recover.
- Monitor the dependent measure depending on the technique used.

## Techniques You Can Use

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- Lesioning (Ablations)
- Cannulations
- Push Pull
- Electro recording

## Electrical Lesioning

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- Lesioning destroys particular brain structures.
- You then observe what happens to the animal before and after the lesioning
- There are several ways to lesion.

## Electrolytic (Use DC current)

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- Observe the animal for the particular behavior of interest.
- Insert an insulated needle into the placement point. The needle is insulated except at the tip.
- Apply current and burn the tissue, which ultimately dies.
- Observe the animal for changes in behavior.

## Disadvantages

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- The electrode leaves a tract into the brain. Thus, you are also damaging other brain tissue.
  - Solution: destroy the tissue from several different angles.
- Apply too much current, you will deposit metal particles from the electrode.
  - Metal particles can irritate tissue
  - Can cause focal points which may result in seizures.

## Radio Frequency Techniques.

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- Radio frequency coagulates tissue. (Its action resembles that of a microwave oven.)
- Insert an electrode insulated except at the tip.
- When energy is applied to the electrode, water molecules oscillate inside neurons.
- The oscillation builds up heat and kills the cells in the area.

## Advantages and Disadvantages

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Advantage:

Avoids metal particles.

Disadvantage:

Still have the electrode tract.

## Chemical Techniques

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- Most commonly used technique today.
- Instead of an electrode, use a cannula or tube.
- Allows you to place chemicals in place where they can kill or influence neurons.

## Neurotoxins

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- 6 Hydroxydopamine (6HDA)
  - Destroys dopamine neurons but leaves other neurons alone.
  - Advantage: Allows you to only kill one type of neuron.
- Kanic Acid
  - Destroys somas (cell bodies) of neurons, but leaves axon tracts from other neurons alone.
  - Advantage: Can kill neurons in one area, but does not disturb neuron tracts from other areas

## Chemical Stimulation Techniques.

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- Are the opposite of chemical lesioning
  - Researcher may stimulate neurons by putting in a neurotransmitter.
  - May also put in agonists (analogs of neurotransmitters that behave like them).
  - May put in compounds with unknown effects.

## General Cannulation Procedures

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- Make a trephine hole.
- Insert cannula with stereotaxic device.
- Cement in place with dental cement.
- Allow animal to recover and behave normally.
- Later, deliver compound by injection (usually when the animal is behaving).
- Observe the animal.

## Disadvantage

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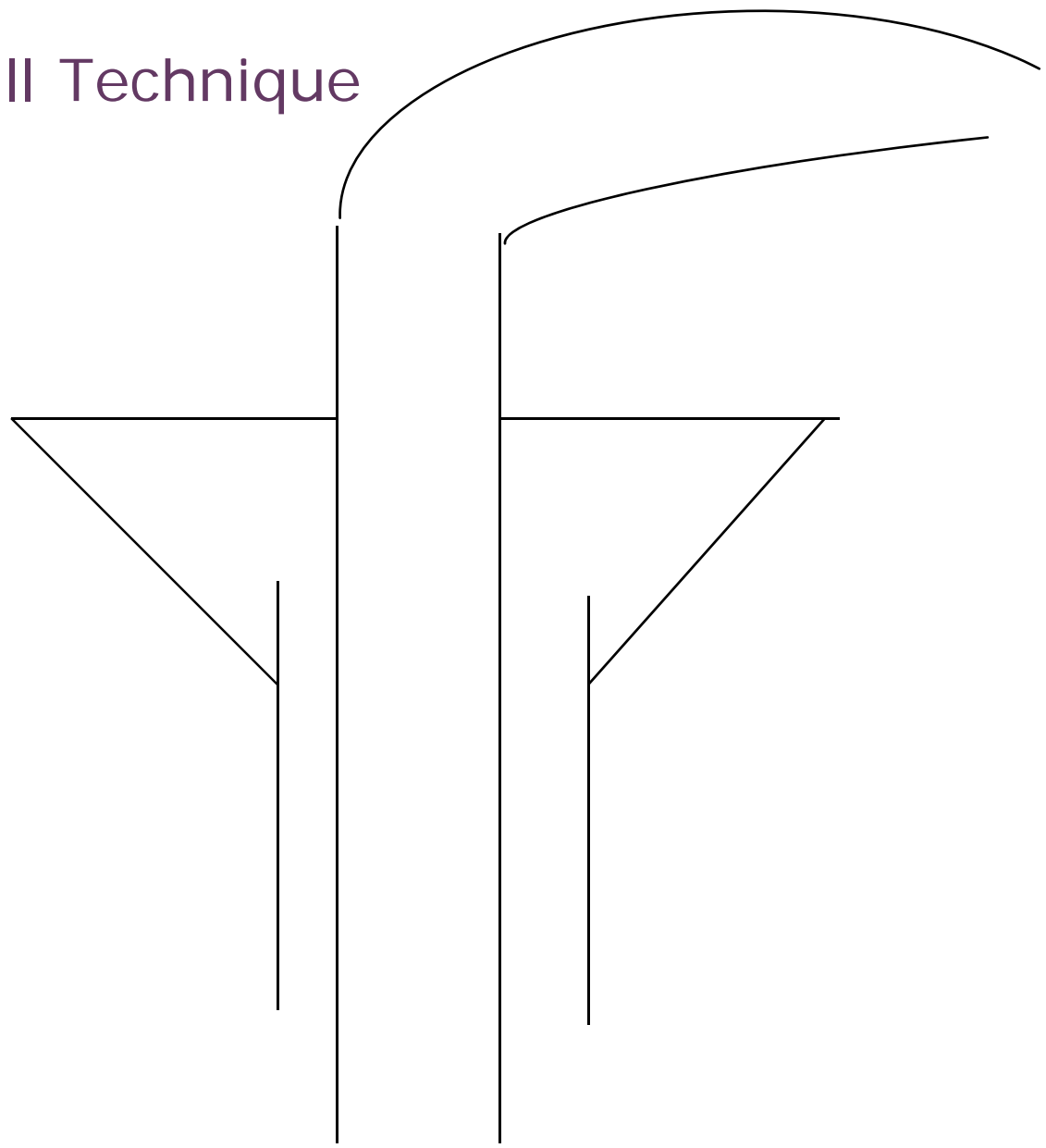
- Over time the area fills up with the chemical.
- Solution: Use a push-pull technique.

## Push-Pull Technique

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- Is similar to other chemical techniques except that the cannula is a little wider and has an extra tube within the main tube.
- Allows you to insert solutions
- Also allows you to withdraw excess compounds or other products for analysis.

# Push-Pull Technique



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## Advantages of Push-Pull

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- Localizes better.
- Can add dyes or radioactive labels
- Can change dosages or concentrations.
- Can change compounds.
- Can analyze materials you withdraw if you get an effect--gives better control.
- Miniaturization is improving the technique further.

## Cannulations

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- Where you place a catheter into the circulatory system of an organism
  - Jugular vein (Neck)
  - Femoral Artery or Vein (leg)
  - Brachial Artery or Vein (arm)
  - Vena Cava (vein that fills the heart)
  - Aorta (artery that leaves the heart)

## Advantages

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- Can put the catheter almost anywhere
- It's a good way to deliver materials in the venous system.
- Some materials are inserted to get past the blood–brain barrier.
- If the molecules of a drug are large, they will stay in the vascular system and not cross the blood-brain barrier.
- You can do the procedure in alert animals.

## Disadvantages

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- Clotting occurs after some time period at the end of the catheter.
  - Femoral 3-10 days
  - Descending Aorta 3-5 weeks

## Other Similar Techniques

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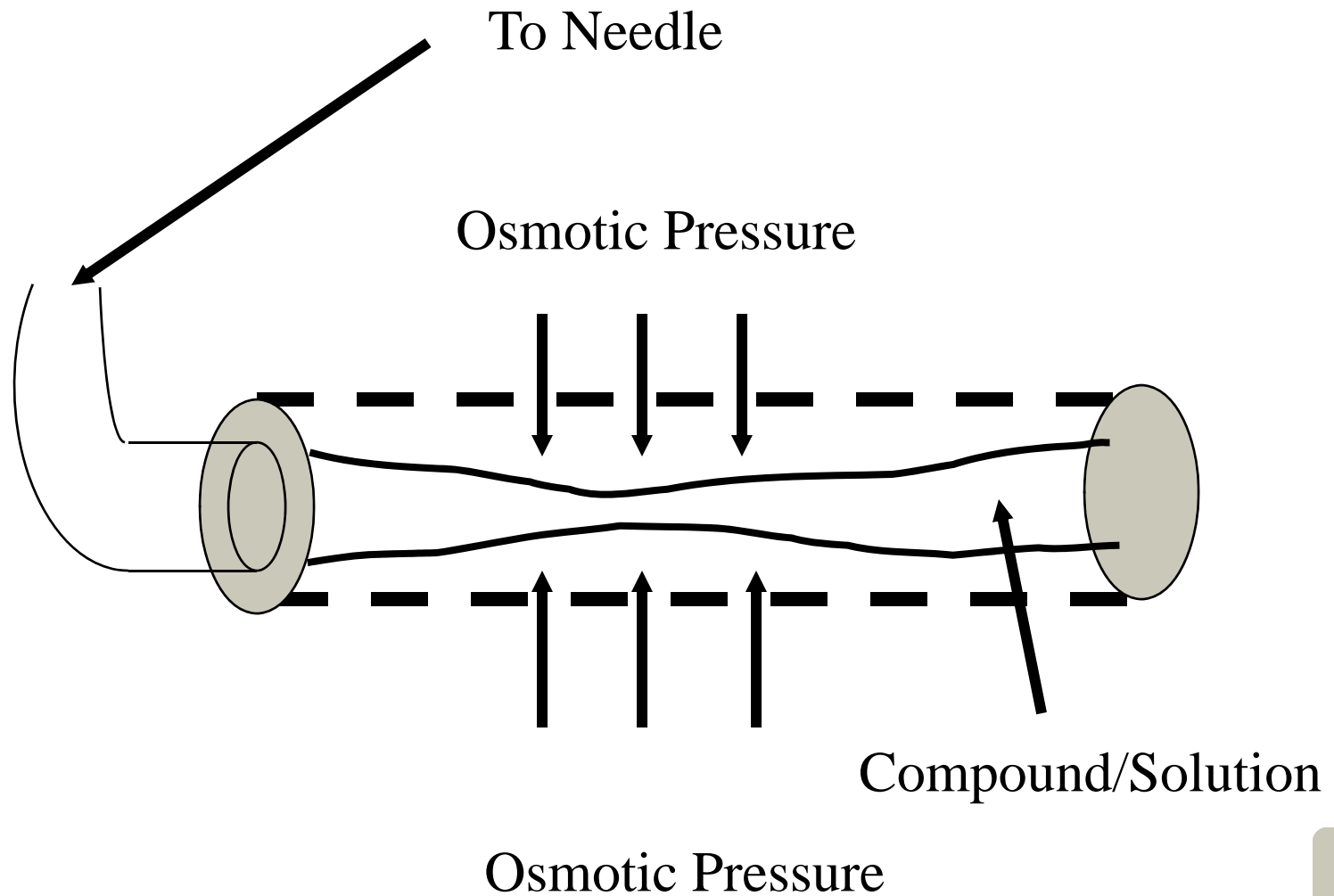
- Can insert cannulas in the mouth.
- Can insert cannulas in the stomach.
- Can insert cannulas in the liver.
- Can insert to withdraw spinal fluid.
- Can also place catheter in the fourth ventricle, substantia nigra, space above the cerebellum,
- Other locations.

## Osmotic Pumps

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- Used to deliver compounds into an organism by osmosis.
- Can deliver one or multiple compounds over a prolonged period.
- Similar devices can be used in humans.

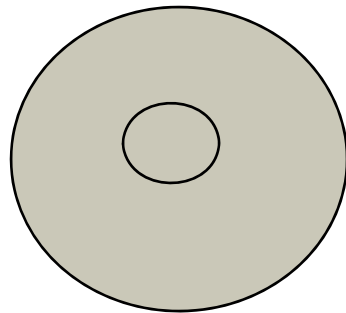
## Osmotic Pumps



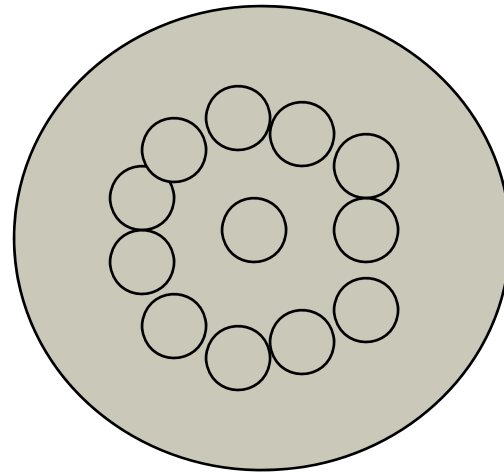
## Types

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- Single Barrel



- Multi Barrel



## Procedure

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- Put a needle where you want it.
  - Can use stereotaxic devices if needed
- Put the pump inside the body (usually the back) and tie off the end with silk until you need to deliver the compound.
- Let animal recover from surgery
- Cut the silk
- Observe the animal

## Advantages

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- Can deliver a substance for prolonged periods
- Can deliver a constant amount of a substance instead of one large amount
  - Decreases the spikes of a drug.
- Easy to do
- Can use computer technology to deliver multiple compounds on some schedule.
- Can use multi-barrel pumps

## Autoradiography

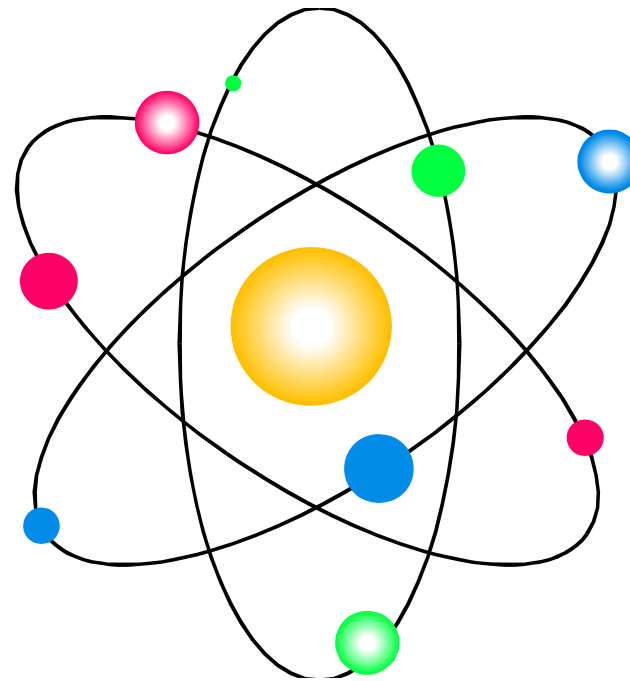
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- auto (self-generating) + radiography (using radioisotopes to give you a print on film)
- Uses radioactive isotopes with unstable nuclei that throw off energy that can be recorded.
- Used to locate receptor sites.

## Types of Radioisotopes or Radiolabels

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- Plutonium 242
- Carbon 14
- Uranium 239
- Tritium
- Calcium 45
- Iodine 125
- Carbon 11
- Others



## Invivo Autoradiography

### Procedure

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- Take an isotope and attach it to the non-working end of the substance you are evaluating (e.g., hormones).
- Resulting isotope is called a tag.

## Steps

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- Radiolabel the substance you want to see.
- Inject the compound into the animal.
- Let it circulate in the blood and go to the receptors.
- Kill (sacrifice) the animal.
- Take out the brain or other organ you are interested in.
- Microtone or slice the brain or organ.

## Steps

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- Put tissue slices on a piece of film. Particles coming off the slice exposes the film (x-ray or 35mm).
- Wait days or weeks.
- Take tissue off the slice.
- Put on a computerized counter screen.
- Look for dark spots.
- If you have any spots, that is where the receptor sites are located for the substance.
- Tells you where the test receptor sites are located.

## Advantages

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- Is a first step in trying to find receptor sites.
- Good procedure if you are not sure where receptor sites are located.
- Good techniques for new substances and you are not sure where they go.
- Is faster than invitro procedures.

## Disadvantages

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- Is more expensive
- Sometimes you don't see anything. If so,
  - There may be procedural errors
  - The assay decayed
  - There may be no receptors

## Invitro Autoradiography

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- *In vivo* means "in life"; *in vitro* means "in a test tube" (literally, "in glass.")
- Used when you have an idea of where the receptor is located.
- Used to determine finer detail

## Procedure

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- Kill (sacrifice) the animal.
- Remove the brain or other tissue.
- Slice the brain.
- Pour the radioactive hormone or other substance over the tissue
- Allow to incubate. The radio labeled compound will bind to the receptors.
- Allow the label to bind.

## Procedure

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- Pour off the excess compound.
- Put the slice into a computer image analysis scan system to detect/count the particles.
  - If the slice gives off activity, the substance must be binding with receptors.
  - If no activity, there are no particles binding to receptors. Thus, you can conclude there are no receptors.
- You can put the tissue under a scanning computer and count the number of receptor sites.

## Micropunch Techniques

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- Use a microtome to slice the tissue.
- Use a specialized hypodermic needle sharpened at the end to punch out a piece of tissue.
  - Microsample could be a nucleus.
  - Sample size usually ranges between 10-50 micrograms or micrometers.
- Take a core sample.
- Blow material from the needle into a test tube.

## Micropunch Techniques

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- Break up the Tissue.
  - Use a Polytron.
  - Radial blades vibrate cells, mild enzymes separate ligands.
  - Destroys ligands but not cell bodies.
  - Exposes the cell membrane so all the receptor sites are exposed.
- Incubate tissue in solution.
- Wash off liquid from cells with a buffer.
- Put tissue in a counter.
  - High count, you have lots of receptors.
  - Low count means low receptors.

## Conclusion

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- Many invasive techniques
- Used for lots of reasons.
- Allows for fine level of analysis.