



Techniques to Examine the Brain

Psychology 372

Physiological Psychology

Steven E. Meier, Ph.D.

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Invasive Techniques

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

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Non-Invasive Techniques

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

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The Stereotaxic Instrument

- 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

- 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.

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General Procedure

- 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.

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Techniques You Can Use

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

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Electrical Lesioning

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

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Electrolytic (Use DC current)

- 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.

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Disadvantages

- 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.

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Radio Frequency Techniques.

- 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.

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Advantages and Disadvantages

Advantage:
Avoids metal particles.

Disadvantage:
Still have the electrode tract.

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Chemical Techniques

- 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.

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Neurotoxins

- 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

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Chemical Stimulation Techniques.

- 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.

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General Cannulation Procedures

- 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.

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Disadvantage

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

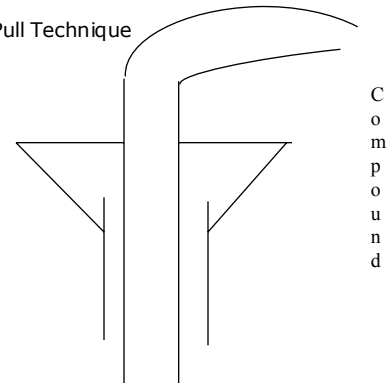
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Push-Pull Technique

- 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.

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Push-Pull Technique



Advantages of Push-Pull

- 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.

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Cannulations

- 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)

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Advantages

- 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.

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Disadvantages

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

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Other Similar Techniques

- 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.

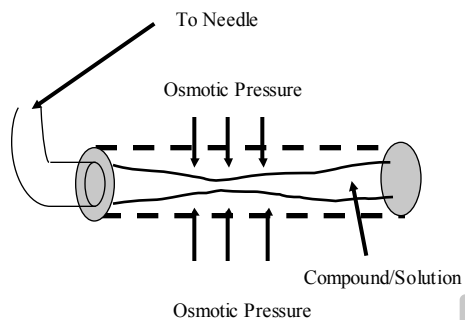
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Osmotic Pumps

- 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.

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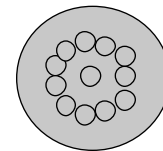
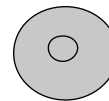
Osmotic Pumps



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Types

- Single Barrel
- Multi Barrel



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Procedure

- 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

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Advantages

- 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

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Autoradiography

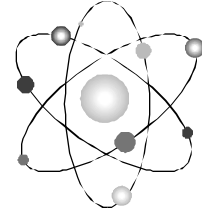
- auto (self-generating) + radiography (using radioisotopes to give you a print on film)
- Uses radioactive isotopes with unstable nuclei that throw off energy that can be recorded.
- Used to locate receptor sites.

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Types of Radioisotopes or Radiolabels

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



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In Vivo Autoradiography Procedure

- 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.

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Steps

- 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.
- Microtome or slice the brain or organ.

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Steps

- 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.

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Advantages

- 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.

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Disadvantages

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

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Invitro Autoradiography

- *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

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Procedure

- 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.

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Procedure

- 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.

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Micropunch Techniques

- Use a microtone 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.

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Micropunch Techniques

- 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.

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Conclusion

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