Carlson (7e)

PowerPoint Lecture Outline

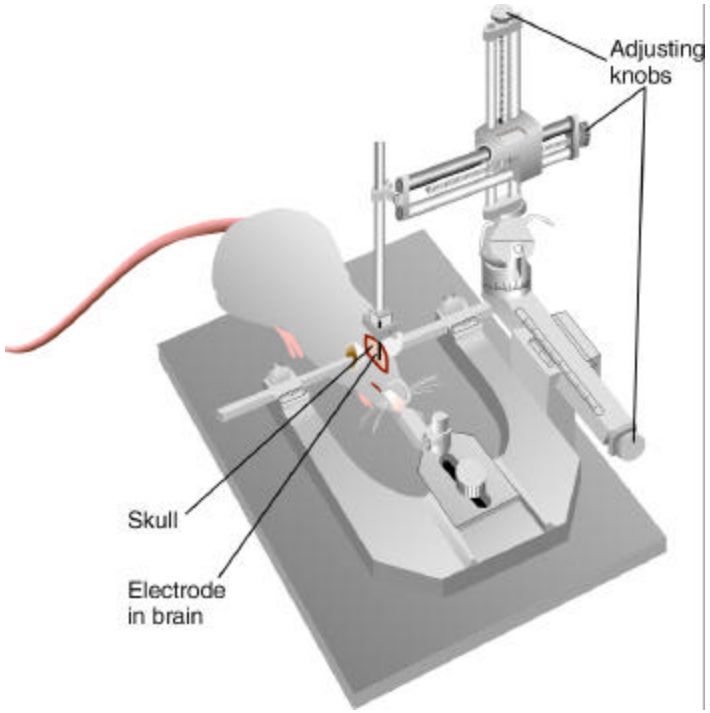
Chapter 5: Methods and Strategies of Research

Experimental Ablation

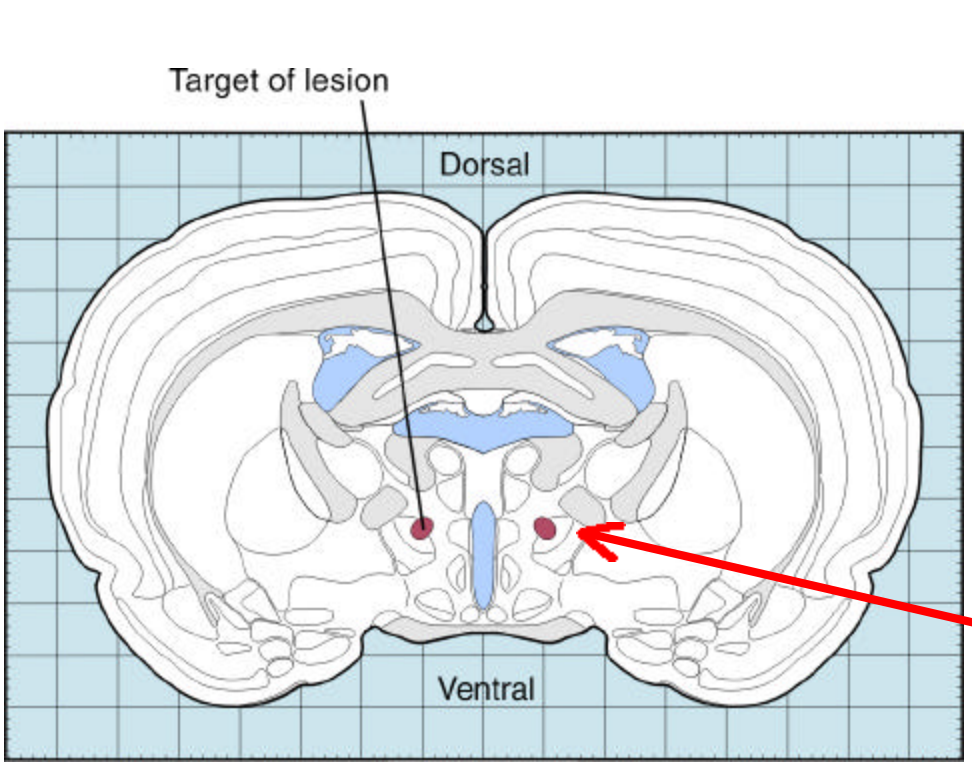
- Ablation involves the destruction of brain tissue followed by an assessment of subsequent changes in behavior
- Ablation techniques include
  - Electrolytic lesions/Radio Frequency lesions
  - Excitotoxic lesions (kainic acid)
  - Neurochemical lesions (6-OHDA)
  - Aspiration
  - Knife cuts
- Distinction between *functions* and *behaviors*
- Brain lesion studies are complicated by the fact that all regions of the brain are interconnected

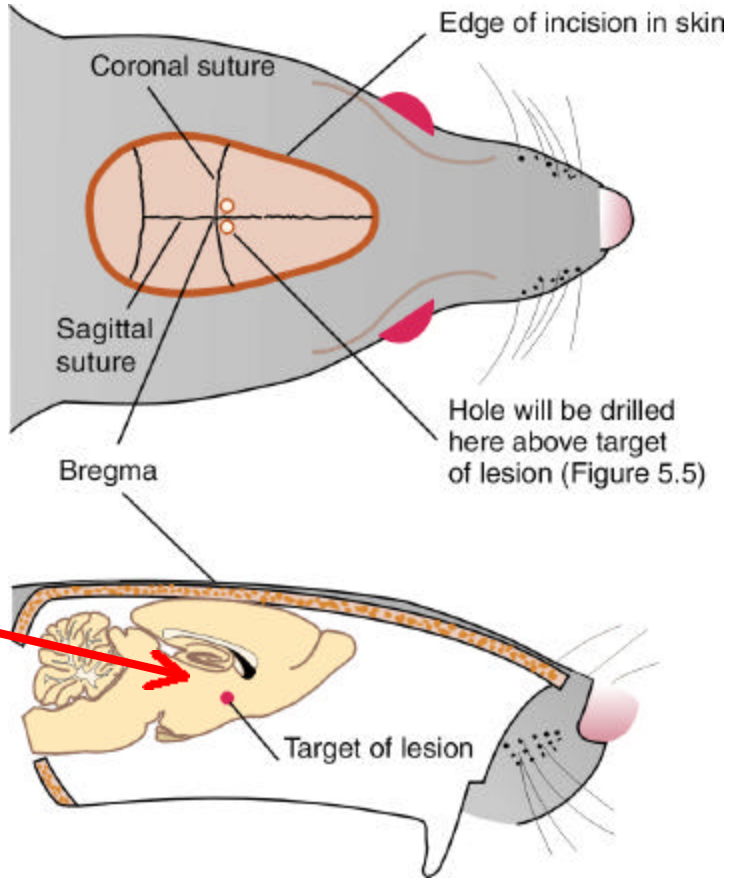
Stereotaxic Surgery

- A stereotaxic instrument holds the head in a fixed position
  - The instrument has an arm that can move in 3 dimensions
  - The surgeon can thus position an electrode or other device within a particular sub-cortical structure
- A stereotaxic atlas provides a series of drawings of brain structures
  - Each page is a section of brain relative to a landmark on the skull (such as bregma)



Using a Stereotaxic Atlas to Target a Brain Lesion





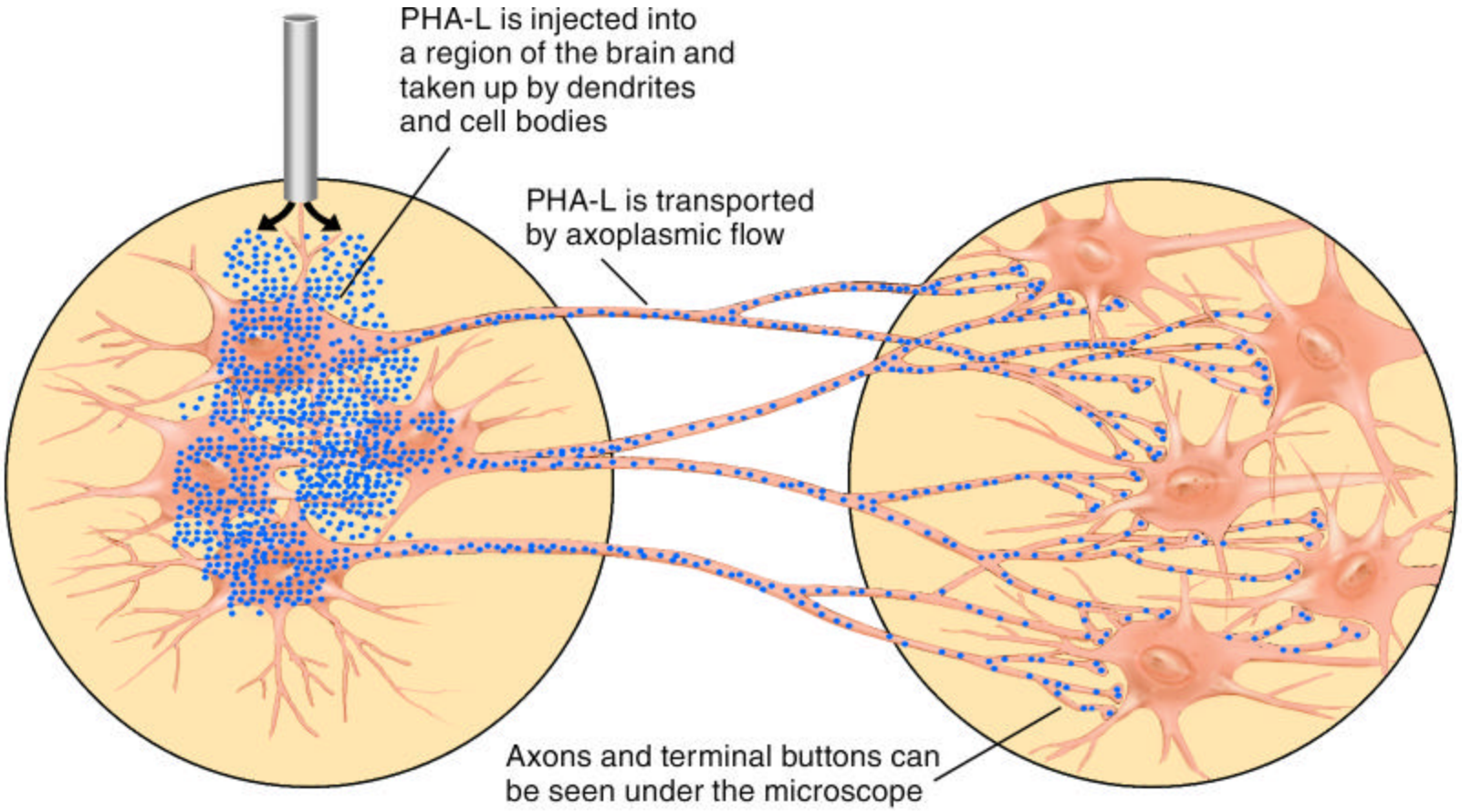
Histological Techniques

- Histological techniques are used to verify the placement of a lesion within brain
  - Perfuse (to remove blood from brain)
    - Remove brain
  - Fix brain in formalin to solidify tissue and to prevent autolysis
    - Slice brain into thin sections (10-80 microns thick)
  - Use stains to highlight selective neural elements
    - Myelin (Weil stain)
    - Cell body (cresyl violet: Nissl substance in cytoplasm)
    - Membrane (Golgi stain)

Defining Neural Connections

- Neurons in a given region send axonal outputs (efferents) to other brain regions and receive axonal inputs (afferents)
  - Tracing efferent connections is done using anterograde labels that are taken up by the cell bodies and transported to axons
    - "Forward: toward axons from cell bodies"
    - Inject the lectin PHA-L into a nucleus, wait several days, process brain tissue.
    - Immunocytochemistry uses a radioactive antibody to PHA-L in order to identify cells containing PHA-L
  - Tracing afferent connections is done using retrograde labeling
    - "Backwards: from axons to cell bodies"
    - e.g. fluorogold is a retrograde tracer

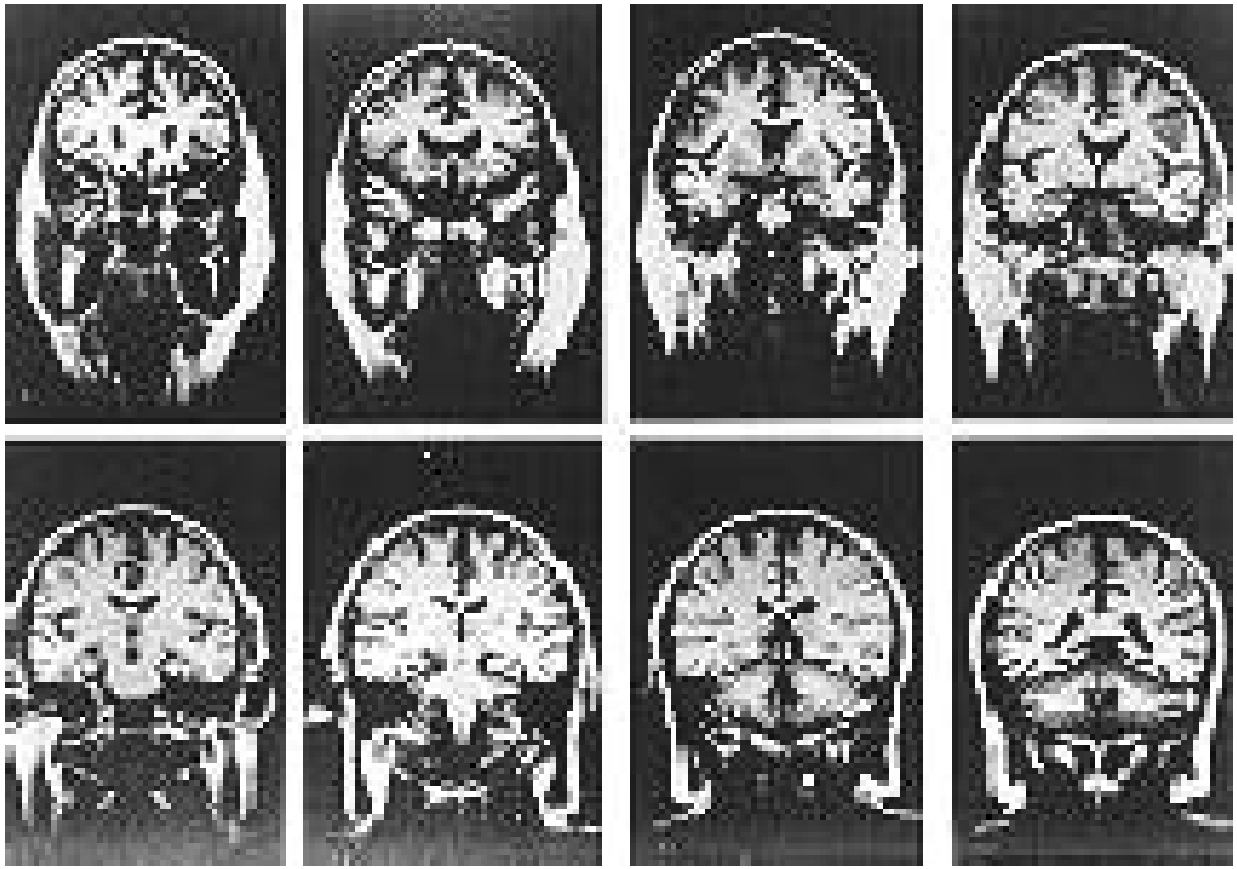
Anterograde Tracing



Visualizing a Living Human Brain

- Computerized tomography (CT) uses an x-ray beam to scan the brain from all angles, these scans are then summarized in an image of the skull and brain (in a horizontal plane)
- Magnetic Resonance Imaging (MRI) uses a magnetic field and radio waves to excite hydrogen molecules, the resulting information is combined to form an image of tissue

Human MRI (Normal)



Images courtesy of Dr. Nancy Andreason

Recording Neural Activity

- Axons conduct action potentials and neurotransmitters elicit postsynaptic potentials
- The electrical events of a discrete region can be recorded using glass microelectrodes (acute recording) or tungsten wire (chronic recording)
- Macroelectrodes record the summated electrical activity of large regions of brain
  - Surface electrodes placed on human scalp are used to record brain activity (electroencephalogram: EEG)

Recording Synaptic Activity

- Increases in neural activity are associated with increases in metabolic activity in a brain region
  - The 2-deoxy-glucose (2-DG) method measures relative glucose utilization
    - 2-DG cannot be metabolized, is trapped in cells and accumulates
    - Radioactive 2-DG is then quantitated using autoradiography
  - The c-***FOS*** method measures a nuclear protein (**Fos**) that is expressed when a neuron is activated
    - Neuronal activation is associated with activation of genes in the neuron nucleus- can localize *Fos* within the nucleus, indicates relative degree of activation

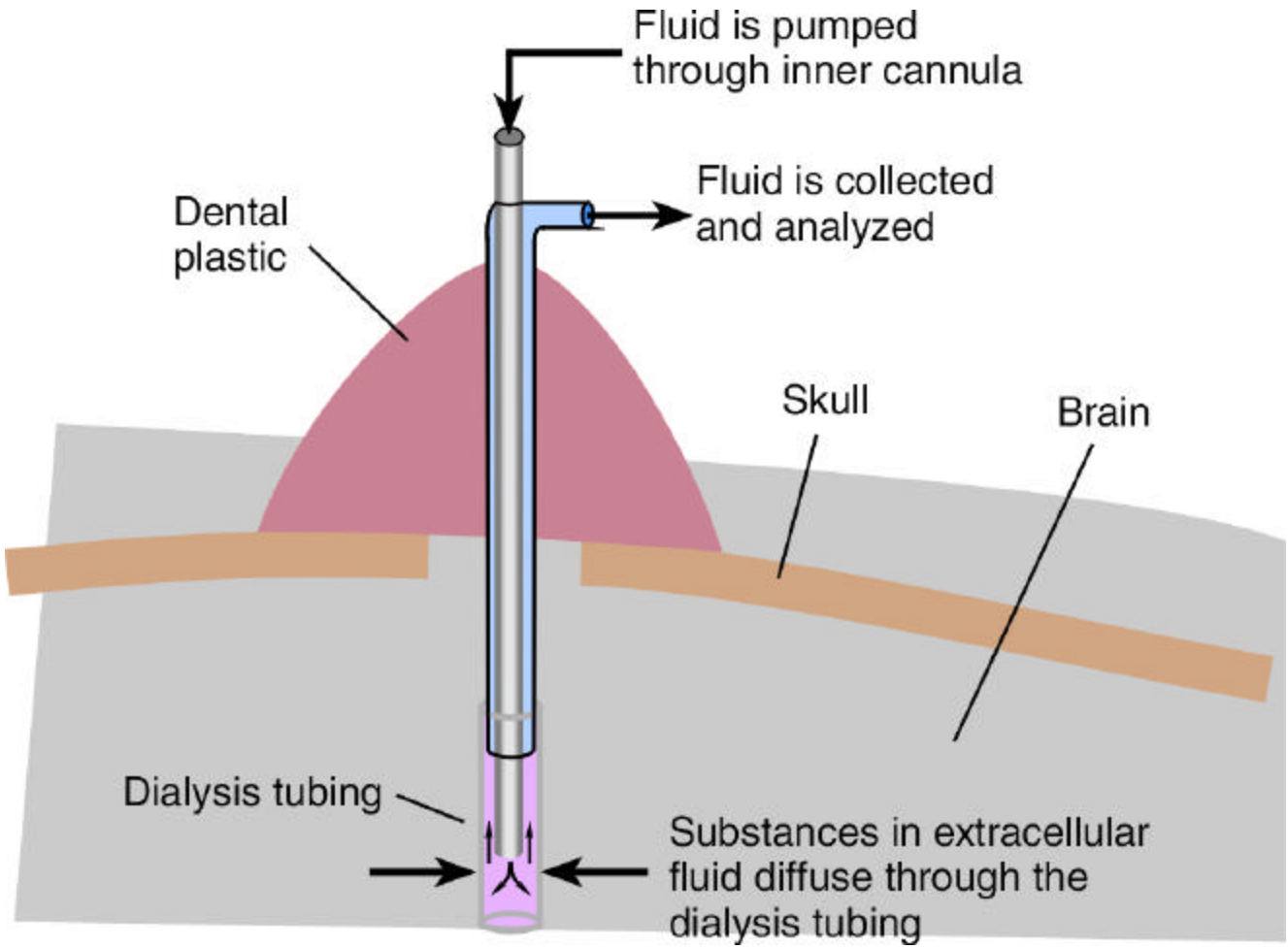
Human Brain Imaging

- The PET scan is a variation of the animal 2-DG technique
  - Human subject is injected with radioactive 2-DG, which is taken up by brain cells
  - As the radioactive molecules decay they emit positrons that can be detected by a scanner
  - A PET scan indicates the relative activity of different brain regions during mental states
- Functional MRI (fMRI) scans detect the level of oxygen in brain blood vessels
  - Current fMRI scanners have a higher resolution than do PET scanners

Microdialysis

- The secretion of neurotransmitter (NT) within a discrete brain region can be measured using the microdialysis technique
  - The tip of a microdialysis probe is positioned in a brain region, CSF is flowed inside the membrane, and NT can pass through the semipermeable membrane into the probe
  - An analytical technique is then used to quantitate the amount of NT in the dialysate

Microdialysis Probe Details



Artificial Stimulation of Brain

- Neurons in a region can be artificially activated to assess the role of that region in behavior
  - Electrical stimulation involves passing electrical current through a wire inserted into brain
  - Chemical stimulation can involve infusion of an excitatory amino acid such as glutamate into a region
    - A cannula implanted into a region can be used to deliver drug solutions into that region
    - Chemical stimulation can be more specific than electrical stimulation (glutamate activates cell bodies, not axons)

Localization of Neurotransmitters

- Three approaches to the localization of a neurotransmitter
  - Peptides are proteins, and proteins can be localized using immunocytochemistry
  - The enzyme that produces a nonpeptide NT can be assayed using immunocytochemistry
    - ChAT is the synthesis enzyme for ACh
    - Neurons that use ACh should contain ChAT
  - mRNA controls the production of an NT or enzyme
    - Brain tissue can be exposed to a radioactive solution containing the complement of the mRNA sequence, and autoradiography can be used to localize cells that produce the NT or synthesis enzyme

Receptor Localization Techniques

- Receptors can be localized in brain tissue using
  - Autoradiography:
    - Sections of brain are exposed to solutions containing a radioactive ligand (chemical that binds), washed, and placed on film
    - The resulting film image shows spots at which radioactivity exposed the film
  - Immunocytochemistry:
    - Antibodies are developed for the receptor protein, are tagged with a fluorescent dye
    - The tissue is exposed to the antibody/dye
    - The section is then examined under a microscope for the presence of dye in specific regions

Genetic Methods

- Genetic research methods seek to demonstrate the linkage between genes and behavior
- Twin studies examine the impact of varying degrees of genetic similarity on behavioral similarity
  - Identical twins (MZ) share 100% of their genes while fraternal twins (DZ) share about 50% of their genes
  - Concordance rate examines the likelihood of whether a twin shares a behavioral trait with the other twin
  - A higher concordance rate for MZ twins relative to DZ twins suggests a genetic influence for that characteristic

Genetic Methods

- Adoption studies examine the similarity with regard to a trait for an adopted person compared to their adopted parents and their biological parents
- Targeted mutations involve the insertion of defective (knockout) genes into the chromosomes of mice
  - The target of the mutation is often an enzyme that controls a chemical reaction or a protein that serves as a receptor for a specific neurotransmitter