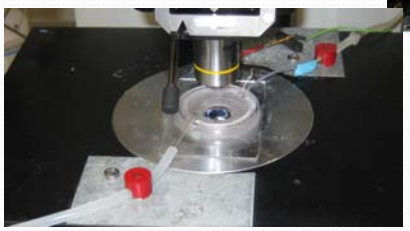
Theory:
Patch clamp technique

Patch clamp technique simplified

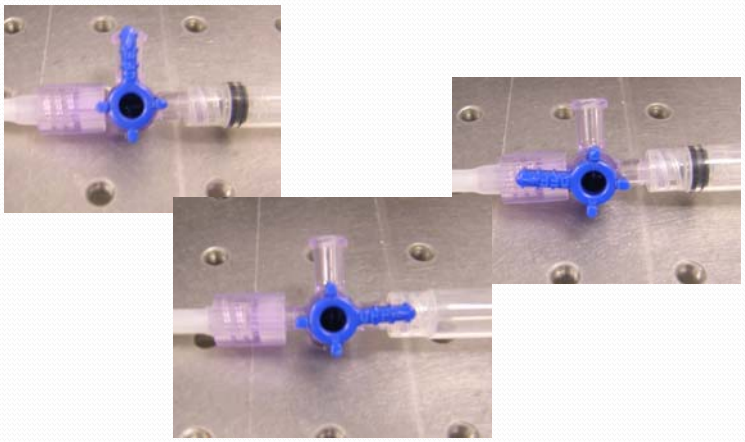
- Prepare brain slices
- Care for and maintain brain slice
- Find a cell
- Approach cell with micropipette tip
- Seal pipette tip to cell
- “Break in” – rupture membrane inside tip to gain electrical access to cell
- Run your experiment, make major discovery
- Become famous, win Nobel Prize

Preparation and maintenance of brain tissue

- Extraction and slicing
  - Ice cold ACSF with low Na+ and low Ca++
- Warm to 37 °C
- Maintain at room temperature in oxygenated ACSF (Saline)
- On the rig – maintained in a constant perfusion of oxygenated ACSF

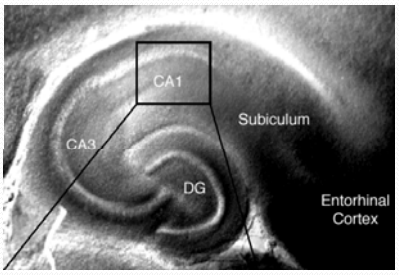


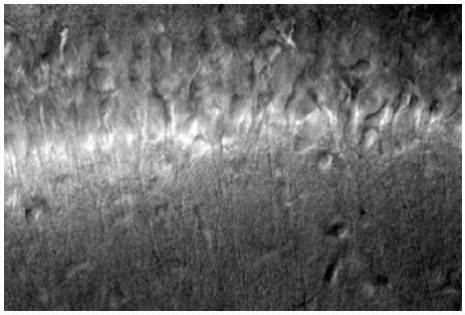
The most complicated piece of equipment in the room:



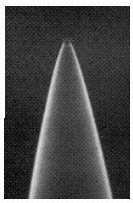
Find a Cell

- Start at 10x
- Center cell body layer of CA1 in field – use eyepieces (occulars)
- Switch to 40x – bring cells into focus using video monitor



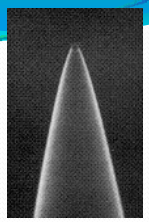


Glass patch pipettes

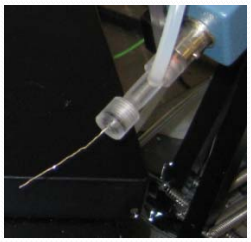


- Pipette fabrication
  - Precise application of heat to melt glass
  - Monitor velocity as glass separates
  - Stop heat at the correct velocity
  - Jet with cool air to solidify glass
  - Repeat these steps 2-4 times with different parameters
  - each time

Using glass micropipettes

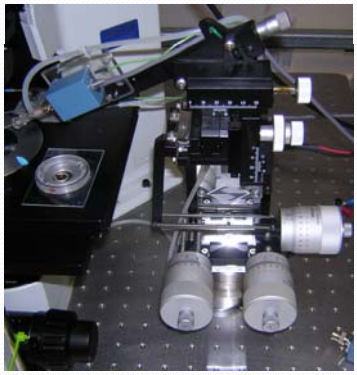


- Tips are delicate!
- Tip must be perfectly clean: they cannot be reused
- Backfill with Internal Solution
  - Internal solution must be perfectly clean
  - Tap to release all bubbles from tip
- Insert into pipette holder and tighten collet to seal
- Apply pressure before tip enters the recording bath
- You will break LOTS of tips at first
- They are cheap and easy to make – we will make more when you need them



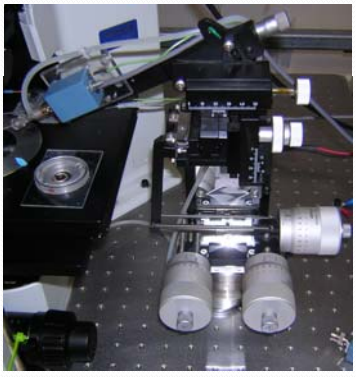
Find the pipette tip at 40x

- The hardest thing you will learn to do this semester
- Raise 40x objective
- Move tip into light path with coarse adjustments
- Find the “glow”
- Lower tip as close to tissue as possible under visual guidance
- Look for the “shadow” through the microscope occulars
- Bring the tip into focus



Approach the cell with coarse controls

- Focus downward
- Lower pipette into focus
- Repeat until tip is just above tissue surface



Form the gigaseal and break in

- Switch to fine controls
- Drop pipette tip on to cell surface
  - Focus up & down between pipette tip and cell surface to guide pipette down
- Watch for the “dimple”
- Apply slight negative pressure
- Watch test pulse to monitor seal formation
- Apply stronger negative pressure to “break in”