Goal of Electrophysiological Recording

Neuronal Signals in Real Time (μs-hours)
Sub-Threshold and Action Potentials

- Current clamp
  - measure membrane potential
  - PSPs, action potentials, ‘resting membrane potential’

- Voltage clamp
  - measure membrane current
  - PSCs, Voltage- ligand-activated conductances
Electrodes

- In circuits, we use wires
- In biology, nature uses liquids
- Electrodes are used to transform current flow from electrons to ions
Electrodes & Pipettes

- Reversible electrode
  - silver wire coated with Ag and AgCl
  - forward flow: electrons from wire convert AgCl to Ag atoms and Cl⁻ ions, the Cl⁻ become hydrated and enter solution
  - reverse flow: Ag atoms give up electron and combine with Cl⁻ from solution
  - solution must contain Cl⁻
  - okay for some silver to be exposed
  - if AgCl exhausted, Ag will leak into solution and poison cells

- Glass micropipettes
Conventions—Voltage

• Positive Potential
  – a positive voltage at the headstage input with respect to system ground

• Transmembrane potential, $V_m$
  – $V_{\text{inside}}$ relative to $V_{\text{outside}}$

• Depolarizing Potential
  – is a positive shift in $V_m$

Conventions—Current

• Positive Current
  – flows out of the amplifier into the electrode
  – flows out of the tip of the electrode into the cell

• Inward current
  – flows from the outside surface of membrane to the inside surface

• Positive and Negative currents and voltages are always based on the headstage’s perspective
**Single Electrode Voltage Clamp**

\[ i_{1(0)} = \frac{V_0}{R_s} \]

\[ i_s = \frac{V_0}{(R_s + R_M)} \]

\[ R_M \gg R_S \implies R_M = \frac{V_o}{i_{SS}}, \quad R_S = \frac{V_o}{i_1(0)}, \quad C_M = \frac{\tau}{R_S} \]

**Two Electrode Voltage Clamp**

Use two electrodes to share tasks
Negative feedback system
Digitizing Analog Signal

8 bit = $2^8$ = 256 values
12 bit = $2^{12}$ = 4,096 values
16 bit = $2^{16}$ = 65,536 values

8 bit = 78.4 mV
12 bit = 4.88 mV
16 bit = 0.305 mV

Low-Pass Analog Filter

Nyquist Theorem:
Setting the Sampling Frequency to 2X the Pass Band
Optical Setup

- IR-sensitive digital camera
- Magnification: 0.63x
- Camera controller
- Video monitor
- Analyser
- DIC prism
- 40x W objective
- WD: 3 mm NA: 0.8
- Patch pipette
- Condenser
- DIC prism
- Polariser
- IR filter

Mechanical Setup
position the pipette above the slice
approach and cell contact
seal formation
break-in

The basic circuit

Bath ground
Ag wire
Pipette holder
pipette

bath

Pre-amplifier
Amplifier
AD converter
Computer

Pipette Pressure

position the pipette above the slice
approach and cell contact
seal formation
break-in

test-pulse

pressure

weak pressure
zero pressure line
release and slight suction
strong pressure
pulses of strong suction
Getting a recording

1. Find a “healthy” cell.
2. Fill a pipette, place in holder and apply positive pressure.
3. Put pipette in the bath.
4. Get the test pulse going (check things look OK).
5. Zero the offset.
6. Position the pipette above the cell.
7. Verify positive pressure and advance into slice.
8. Push pipette tip into cell and release pressure.
9. Apply slight suction and a negative holding potential (-60 mV).
10. If everything is right you will get a Gohm seal.

Pipettes

1. Fabrication
   - pullers (2-stage or multi-stage pullers)
   - type of glass (quartz lowest noise but difficult to pull)
   - filament?
   - measurement of tip size (resistance, “bubble” number)
   - what size do I want? (Rs, wash-out)
   - fire polishing?
   - sylgard? (bath level, dental wax an alternative)

2. Internal solutions
   - Osmolarity, pH, blockers, dyes
Intracellular solutions

“Standard” intracellular (patch) solution:

135 K-Gluconate or K-MeSO₄, 10 HEPES, 7 NaCl, 2 Na₂-ATP, 2 MgCl₂ (pH 7.2 with KOH)

Osmolarity - ~10% lower than extracellular (higher is better for lower Rᵢ)

Could also add/replace:
- GTP (0.3 mM)
- Phosphocreatine (10 mM)
- Fluorescence dyes or biocytin
- Cs instead of Gluconate/MeSO₄ to block K⁺ currents
- High Cl to increase inhibitory responses at resting membrane potentials (eg. replace K-Gluconate with KCl)
Visually guided or blind?

<table>
<thead>
<tr>
<th>Visually guided</th>
<th>Blind</th>
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</thead>
<tbody>
<tr>
<td>Expensive</td>
<td>Cheaper</td>
</tr>
<tr>
<td>Expensive microscope &amp; camera required, better manipulators (?)</td>
<td>Only need dissecting microscope &amp; no camera</td>
</tr>
<tr>
<td>Best for cells near the surface</td>
<td>Can patch deep cells (also in vivo)</td>
</tr>
<tr>
<td>Typically lower series resistance</td>
<td>Typically higher series resistance</td>
</tr>
<tr>
<td>Can record from multiple cells</td>
<td>Difficult to record from multiple cells</td>
</tr>
<tr>
<td>Can record from multiple locations on the same cell</td>
<td>Impossible (?) to record from multiple locations on the same cell</td>
</tr>
<tr>
<td>Can identify cells before recording (morphology, fluorescence)</td>
<td>Identification only possible after experiment (but can use electrical cues)</td>
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Technical issues

1. Loosing the seal on break-in (“leaky” cell; depolarised membrane potential, (How do I know: negative (downward) shift, “jump” in holding current)
2. Series resistance (VC/CC, filtering, voltage-drop/error, compensation)
3. Noise (60 Hz line frequency, grounding, high-frequency noise – single channel)
4. Wash-in/wash-out (perforated patch recording: Amphotericin or Gramicidin?)
5. Offsets (junction potentials, Ag/AgCl electrodes)
6. Recording issues (amplifier gain, AD boards, saturation, sample rates)
7. Space clamp
Patching dendrites with IR-DIC

Dendritic recording pipette

Somatic recording pipette

Stuart & Sakmann 1994