**Lab Book Layout**

A close-up of a notebook

AI-generated content may be incorrect.

**“Cell Health” Table** **(top right)**

Rt: resistance of the recording pipette in the bath (generally ranges from 4.0-6.0 MΩ)

speed: of forming seal when positive pressure is release but before the cell is broken into

Ra: access to the cell, generally ranges from 1.0-2.0 MΩ

Vm: resting membrane potential (mV)

hold: current (pA) needed to hold the cell at -70 mV

coordinates: (x, y) from the top of the third ventricle (µm)

**Recording Parameters (bottom right)**

Cm: capacitance (pF) tells us about the size of the cell

Rm: membrane resistance (### MΩ to # GΩ) tells us about

Ra: access to the cell, generally ranges from 1.0-2.0 MΩ

Tau: membrane time constant (µs)

Hold: holding current (pA)

**Orientation of Slice in Bath**

X

**Getting Electrodes In**

Find the ventricle under 5x

↓

Find potential DMH cell under 40x (**no** **more** moving the stage when this is done)

↓

Put in the stimulating (left) electrode, find under 5x by moving the electrode, focus the tip by moving the electrode

↓

Bring stimulating electrode down to bath

↓

Find stimulating electrode under the 40x by moving the electrode, focus the tip by moving the electrode

↓

Move stimulating electrode and objective down to the slice

↓

Positive pressure when recording (right) electrode goes in bath

↓

Membrane test in bath mode in voltage clamp, offset the pipette (record Rt)

↓

Find recording electrode under 5x

↓

Find recording electrode under 40x

↓

Move recording electrode and objective down to the slice

**Software Settings**

In voltage clamp (VC): primary output should be membrane current (0.5 V/nA) and secondary be membrane potential (10 mV/mV)

In current clamp (IC): primary output should be membrane potential (10 mV/mV) and secondary be membrane current (0.5 V/nA)

**How to Get a Cell**

Positive pressure when recording electrode is in bath

↓

Voltage clamp

↓

Offset pipette

↓

Membrane test set to bath mode at 10 kHz

-looks like: square waveform

↓

Dimple in membrane

↓

Release positive pressure

↓

Dimple should flatten out and reach GΩ seal (can suck in a little to help if needed)

↓

Then hold at -70 mV (still in voltage clamp) and switch to cell mode of membrane test (10 kHz)

↓

Lip suck to break into cell and stop membrane test

-looks like: #

**When You Have a Cell**

**Do not** stop holding at -70 mV in voltage clamp

↓

Switch to current clamp mode, don’t hold anything in current clamp

↓

Note **resting** Vm (mV)

↓

Gap free (C1) recording for ~30 seconds

↓

Find what current (pA) is needed to **hold** at -70 mV and hold that

↓

Current clamp steps (C2) recording

↓

Stop holding in current clamp

↓

Switch to voltage clamp (should still be holding at -70 mV)

↓

Look for currents using CA1

↓

5-minute intervals: membrane test at 10 kHz: record Ra and hold, switch back to recording (C3) at 1 kHz

↓

When done with the voltage clamp recordings, never stop holding at -70 mV when going back to current clamp mode

↓

Switch to current clamp mode, don’t hold anything in current clamp

↓

Note resting Vm (mV)

↓

Repeat gap free (C1) recording

↓

Find what current (pA) is needed to hold and -70 mV and hold that

↓

Repeat current clamp steps (C2) recording

↓

Stop holding in current clamp

↓

When totally done all recording… stop holding in voltage clamp

↓

Measure coordinates of cell from the top of the third ventricle

**Recording Types**

Gap Free (C1): us not doing anything, in current clamp mode

Current Clamp Steps (C2): 1 second sweep times 10 (250 msec of nothing then 500 msec of hyperpolarized, then 250 msec of nothing) each of the 10 sweeps the middle 500 msec gets more depolarized.

PPR 1.0 (C3): 5 second sweeps of paired stimulations of 0.2 Hz, 50 msec apart. In voltage clamp.

High Frequency Stimulation (C4): 100 Hz for 4 seconds, repeated twice, 20 seconds apart. In voltage clamp.