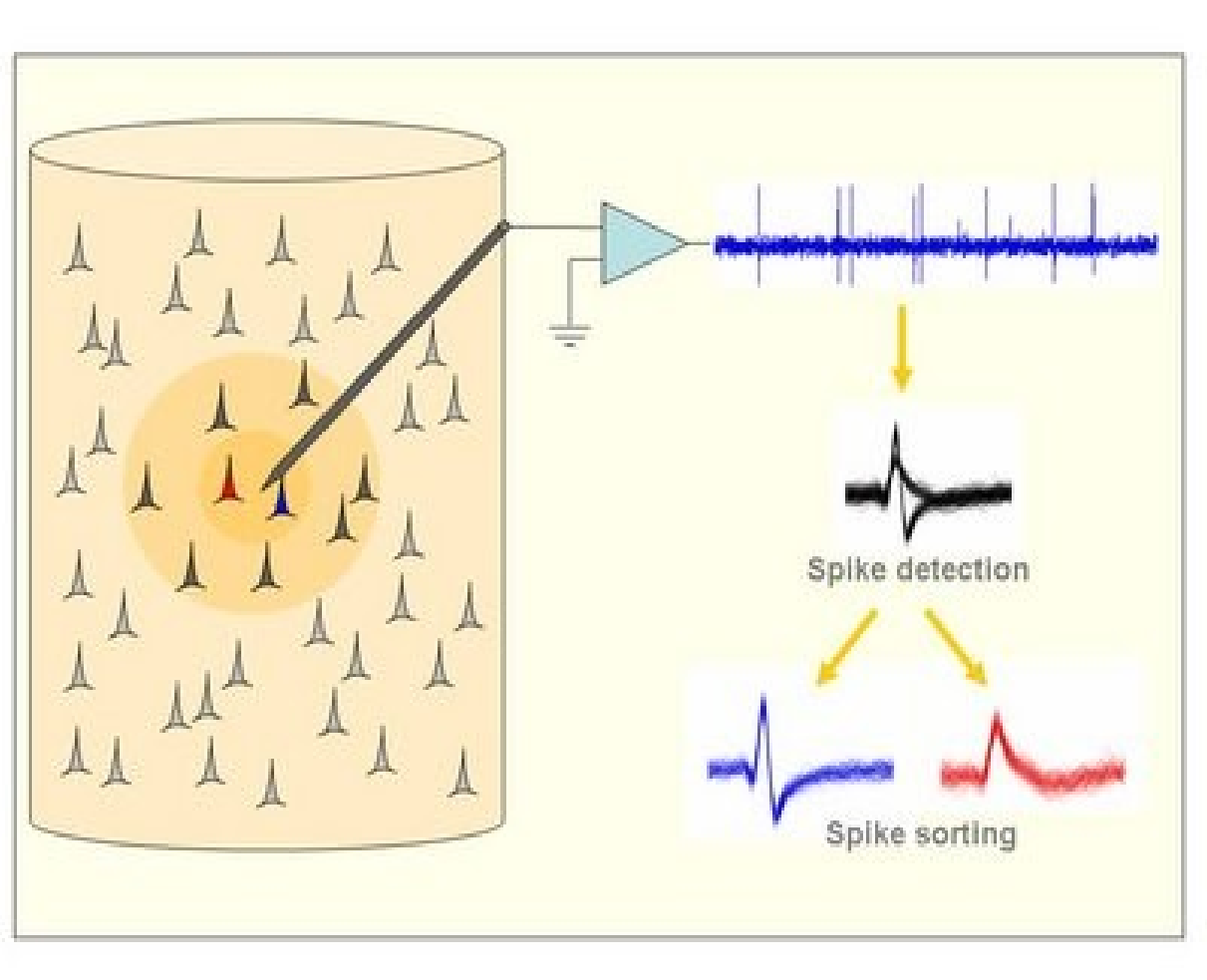
Biology Experiment 1

Extracellular Recordings and Measuring Field potentials using Microelectrode array (MEA) chips

Introduction to Extracellular Recording (adapted from CNS Tutorial 2012)



- Electrical signals from individual cells are acquired by inserting a conductive microelectrode near the cell membrane, which then provides a source or sink for the trans-membrane currents associated with an action potential.
- Each spike appears as a stereotyped waveform on the voltage trace recorded from the microelectrode.
- Neural tissue in the brain is dense enough that a probe inserted at random into the gray matter will lie close to many cell membranes, and will couple to currents across all of them.

Figure 1: Extracellular Recording (from CNS 2012 Tutorial)

Different stages of getting the desired signal

- The signal from the microwire (Fig 1) is amplified and band-pass filtered and the firing of the nearby neurons appears as spikes on top of background activity.
- Spikes are detected using an amplitude threshold and then sorted according to their shapes.
- For neurons close to the electrode tip - about 50 to 100 microns the signal-to-noise ratio is good enough to distinguish the activity of each *single unit* .
- For more distant neurons up to about 150 microns - spikes can be detected but the difference in their shapes is masked by the noise (*multi-unit* activity).
- Spikes from neurons further apart cannot be detected and they contribute to the background *noise* activity.

Illustration of Extracellular Recording at single cell level

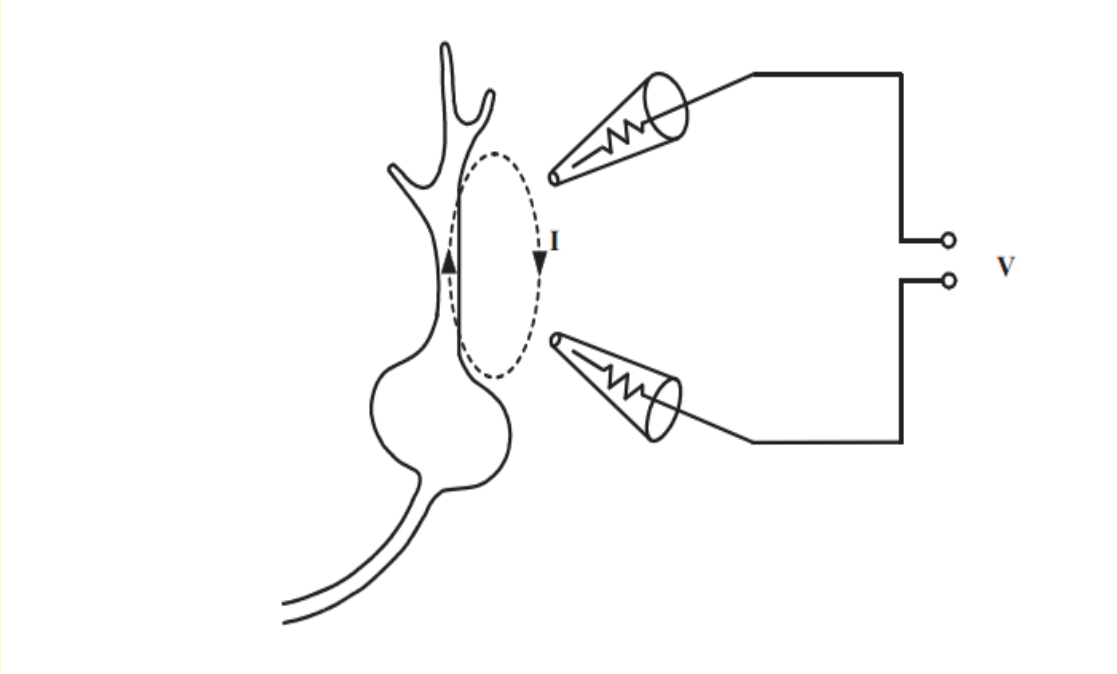


Figure 2: IR Drop (From Axon Guide)

- In an extracellular recording experiment: the current I that flows between parts of a cell through the external resistance R produces a potential difference ΔV, which is usually less than 1 mV .
- As the impulse propagates, I changes and, therefore, ΔV changes as well.

Volume conductor theory

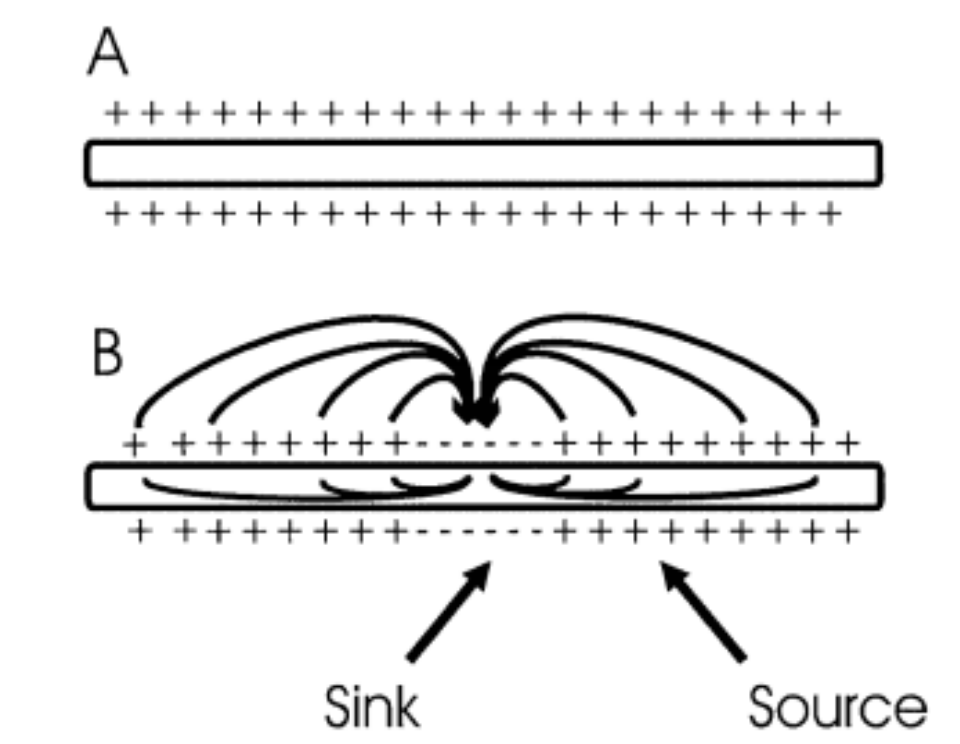
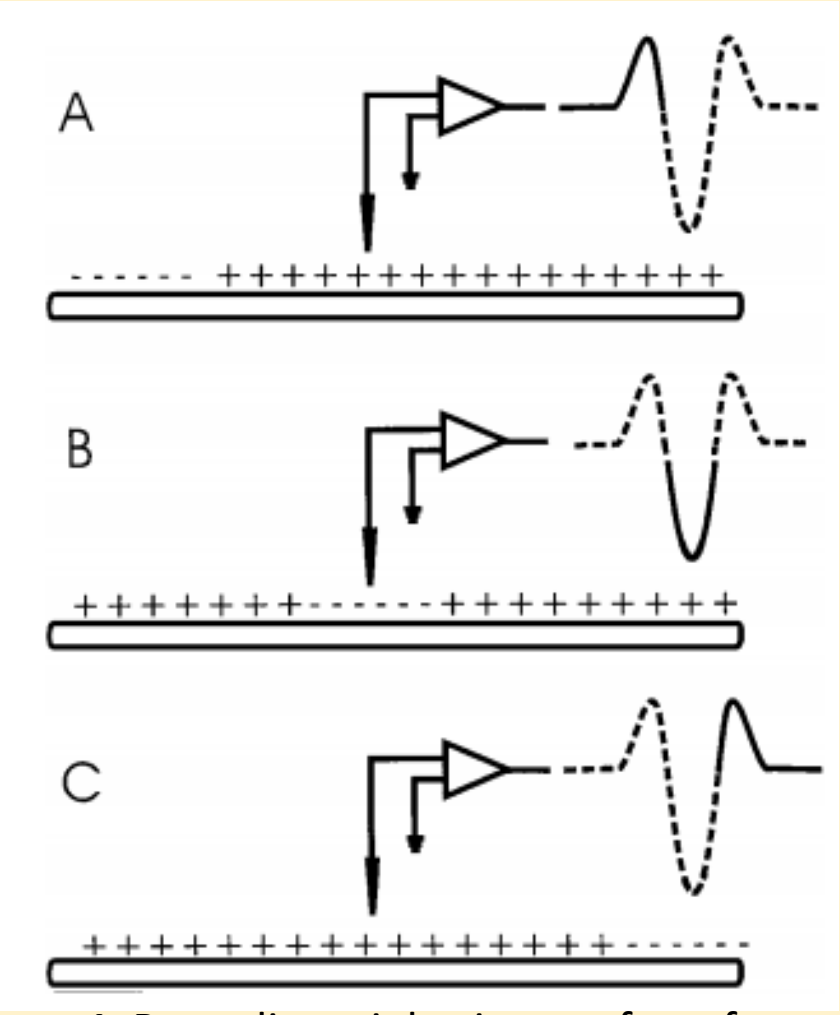


Figure 3: Current flow around an axon (Wikipedia)

- A : when the axon is at rest, the membrane potential is uniform, and no current flows
- B: Current will flow when a segment of the membrane is depolarized. The flow is inward at the depolarized region (“sink”) and outward at adjacent regions, which acts as a (“source”) of the current sink.

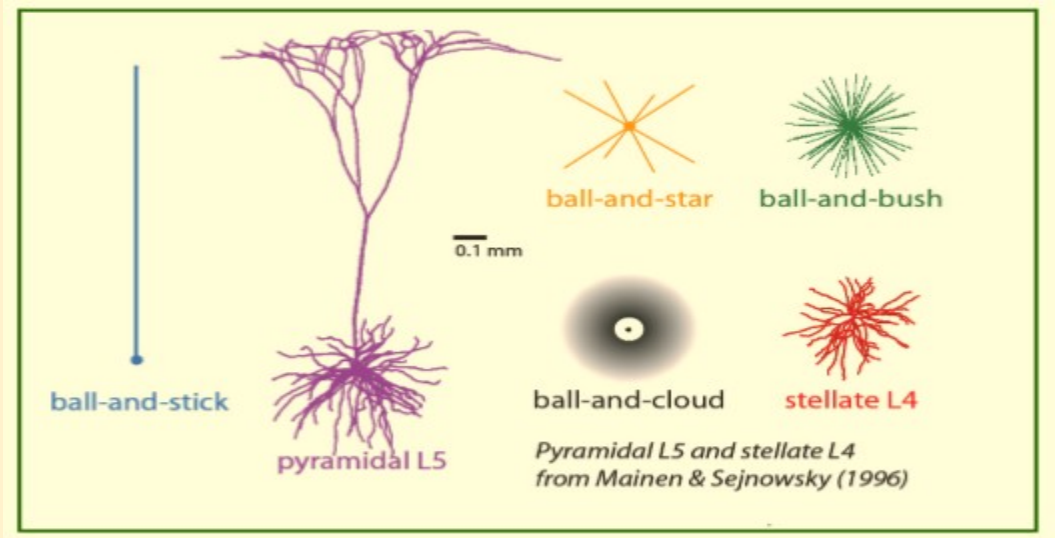
Model of sources and sinks predicts that a triphasic wave will be recorded from an isolated axon



- A . As the action potential approaches the region underneath the electrode, that membrane serves as a source, and the electrode sees a positive potential relative to a distant indifferent electrode.
- B. When the action potential reached the membrane underlying the membrane, the electrode records a negative potential.
- C. As the action potential continues down the axon, the membrane under the electrode once again acts as a source, and as a consequence, the electrode records a positive potential.

Figure 4: Recording triphasic waveform from an axon (Wikipedia)

How does the extracellular signature of action potentials depend on neuronal morphology



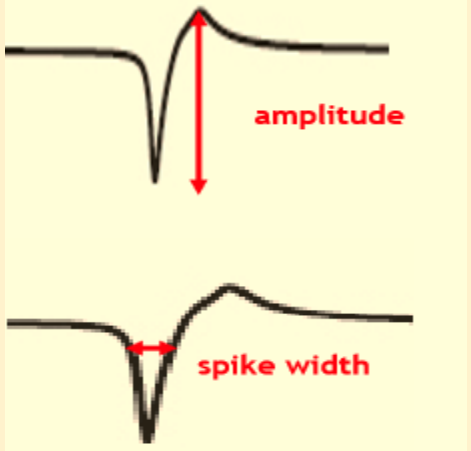


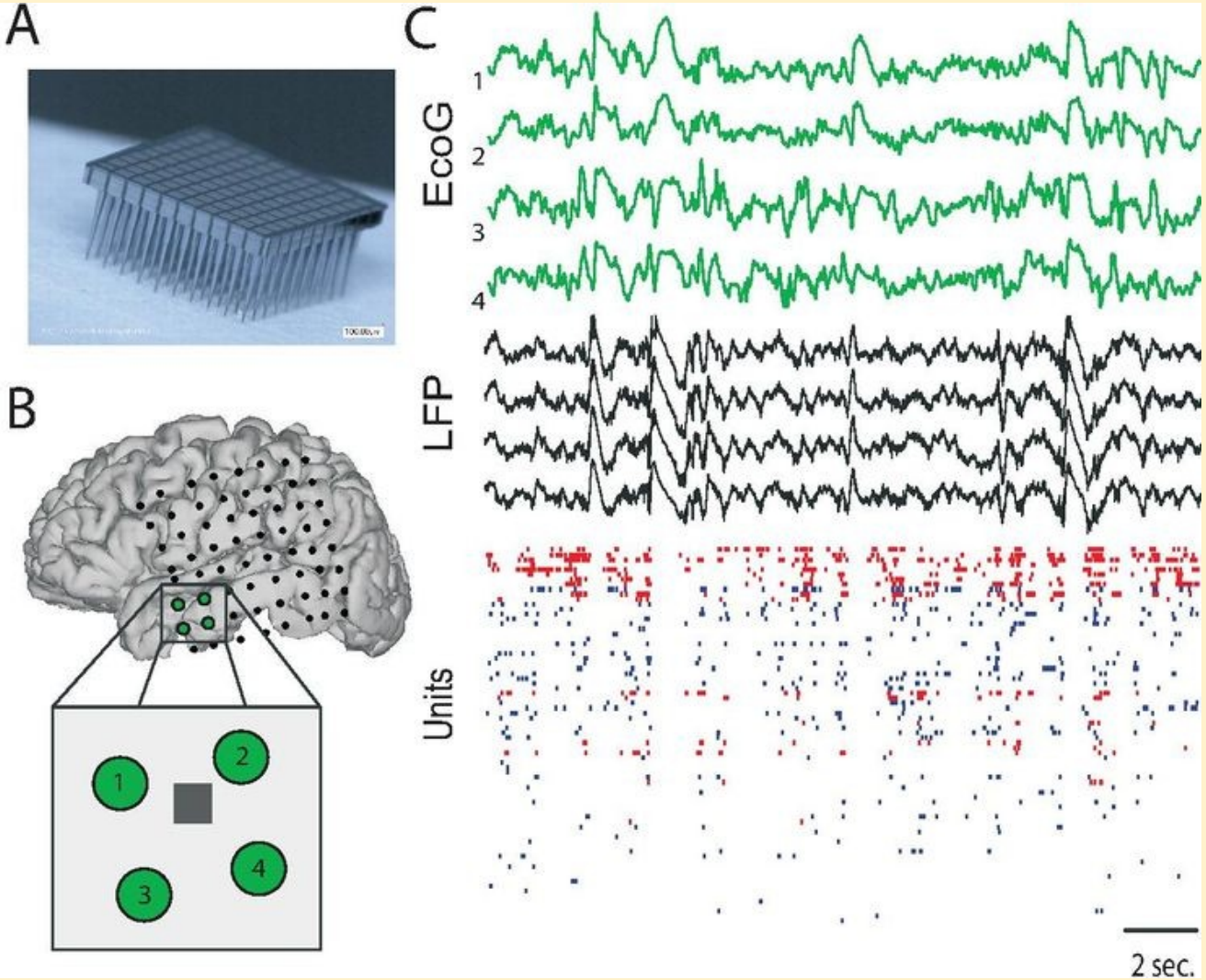
Figure 5 - Above : Different cells with different morphologies,
Left : Characteristics of Extracellular Signals
(Adapted from CNS Tutorial 2012)

- Amplitude is (i) roughly proportional to sum of cross-sectional areas of dendrites connected to soma, (ii) independent of membrane resistance Rm, … (check)
- Spike width increases with distance from soma, i.e., high-frequency dampening also with simple ohmic extracellular medium.

Advantages and Limitations of Extracellular Recordings

- Extracellular recordings are attractive. Why?
- They produce a (whole) lot of data with “moderate” tissue damage.
- They potentially provide sub-millisecond resolution of neuronal spiking activity.
- They are relatively cheap to implement.
- But ...
- They require a lot of processing.
- They cannot provide some very important pieces of information like the neuronal cell type, the sub-threshold activity, the morphology of the recorded cells.

Local Field Potential (Adapted from Scholarpedia)



- The **local field potential** (LFP) refers to the electric potential in the extracellular space around neurons.
- A. Array of 100 silicon electrodes with 400µm inter-electrode distance.
- B. Plan of Implementation (black dots)
- As distance from microwire increases the recordings will pick up slower activity. We can record action potentials from unit recordings as shown.
- C. Typical recordings obtained.
- ECoG: Electrocorticogram

Figure 3: Local field potentials recorded in humans using an array of extracellular electrodes.

Typical Data Analysis after measurement using microelectrode array (MEA) chip

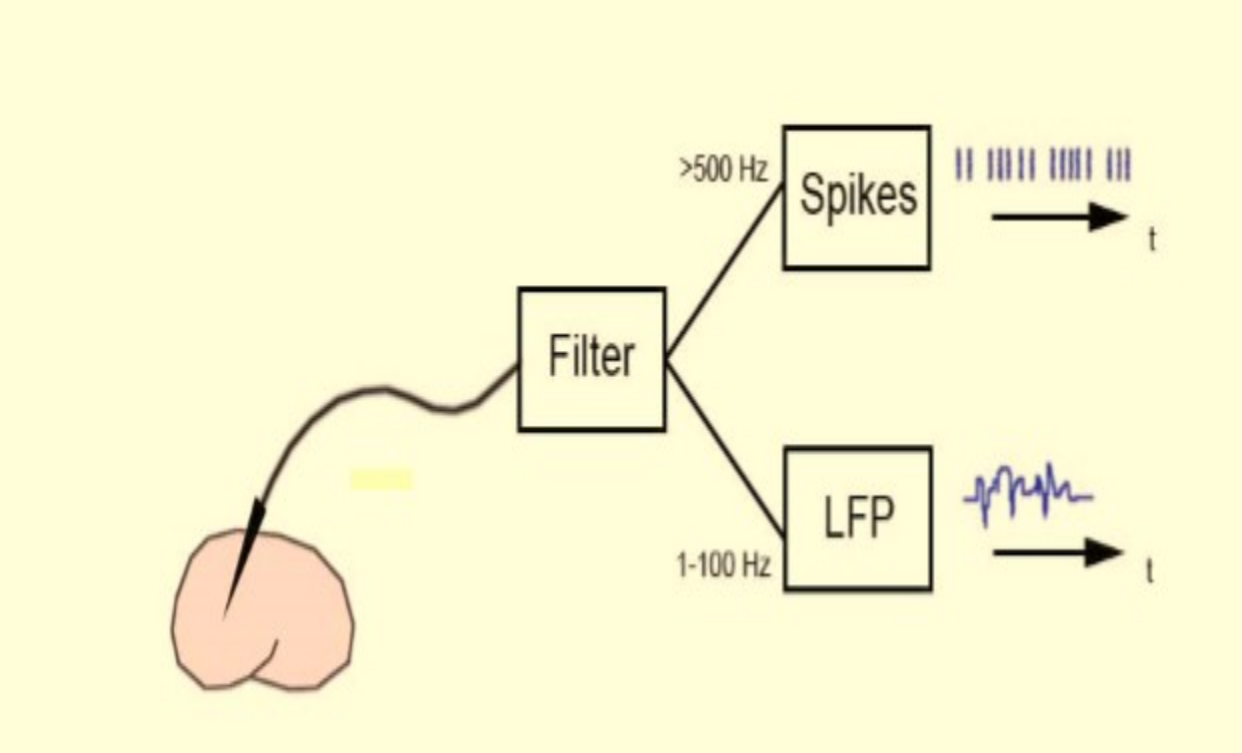


Figure 4: Recorded Signal (From CNS Tutorial 2012)

- Neuronal recordings split into two frequency bands:
- High-frequency band (>~ 500 Hz): Multi-unit activity(MUA), measures spikes in neurons surrounding electron tip
- Low-frequency band (<~300 Hz): Local field potential(LFP), measures subthreshold activity
- LFP represents the summed excitatory and inhibitory signals and other types of slow activity such as voltage dependent oscillations or spike after potentials
- “slower” processes, synapses, leak currents, capacitive currents etc.

The simplest model of LFP (Adapted from Scholarpedia)

- Assumptions: LFP is generated by transmembrane currents, Neurons are embedded in perfectly resistive medium, Electrical conductivity (σ) and permittivity (ε) of the extracellular medium are constant and independent of frequency.
- Electrical Potential generated is V(r)

$$V(r) = \frac{1}{4\pi\sigma} \frac{I_0}{|r - r_0|},$$

Where V(r) is the extracellular potential at a position r in extracellular space, I_o is the current source, and $|r-r_o|$ is the absolute distance between r and the position of the current source r_o

The potential resulting from a set of current sources is given by :

$$V(r) = \frac{1}{4\pi\sigma} \sum_j \frac{I_j}{|r - r_j|}.$$

This expression can be used to calculate the LFP resulting from a network of neurons or complex morphologies.

Frequency filtering properties of LFPs

- The fact that action potentials contribute little to the LFP may be explained by frequency-filtering properties of the extracellular medium.
- If the medium acts as a low-pass filter, it may attenuate more severely the high frequencies (greater than ≈ 100 Hz), such as that produced by action potentials, while the attenuation will be less severe for lower frequencies such as synaptic events.
- As a consequence, an extracellular electrode at a given position will "see" only the action potentials for cells immediately adjacent to the electrode.
- On the other hand, the low-frequencies of the LFP will be a compound signal of slower events (such as synaptic events) from a population of cells around the electrode.

BIO LAB 1 ASSIGNMENT (5 questions)

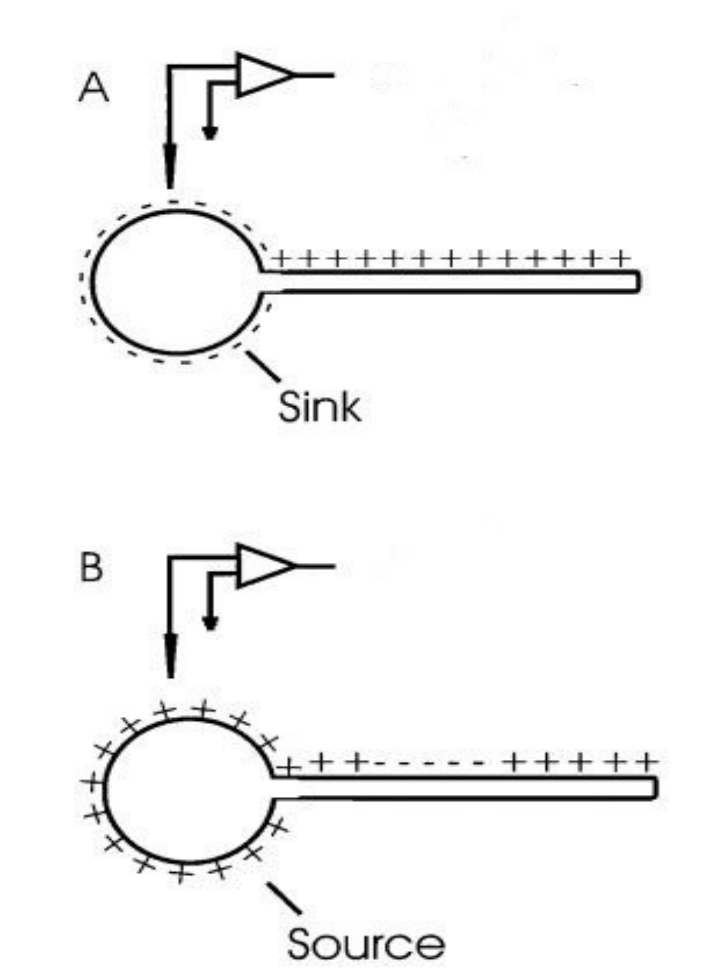
Q1 : **LFP using MEA**

a. Go to the following website http://vlab.amrita.edu/index.php?sub=3&brch=43&sim=135&cnt=2108

b. Click on the “Animation”. Participate in a full animated walk-through to complete the feel for a real LFP recording. You will have to click/move using the computer mouse to pass through the various stages of the experiment.

c. Finish “Self-evaluation” and get a screenshot for your answers, and provide it as solution to exercise 1.

Q2: Action potential propagation



- Sketch the membrane voltage (extracellular voltage recorded by the electrode in the figure) as measured in A (AP generated in the soma) and B (AP propagates to the axon).
- What is amplitude of a typical extracellularly measured signal?
- Is the resultant waveform biphasic or triphasic? Why?

Q 3) The amplitude of the measured voltage is different for intracellular and extracellular recordings. Explain clearly why.

Q 4) The extracellular recording can change polarity when the electrodes are placed in a different location. Why does this happen? Why does this not happen with intracellular recordings?

Q 5) Why does LFP only capture slower currents, i.e., only subthreshold activity. Why does it not capture spikes or high frequency activity?

References

- Axon guide
- Modeling and interpretation of extracellular potentials
- Neurophysiology Virtual Lab: http://vlab.amrita.edu/?sub=3&brch=43
- Principles of Extracellular Single- Unit Recording. CNS2012 Tutorial
- Scholarpedia : http://www.scholarpedia.org/article/Local_field_potential
- Wikipedia : http://en.wikipedia.org/wiki/Multielectrode_array

Video References:

http://www.youtube.com/watch?v=E8l3_kAUe0w

http://www.youtube.com/watch?v=RTgoRmDJvmE