Experimental methods in nerve muscle physiology

- Voltage clamp, patch clamp
- Recording from mixed nerves: Compound action potential, Strength duration curve
- Nerve conduction studies
- Electromyography

Methods in single neuron preparations

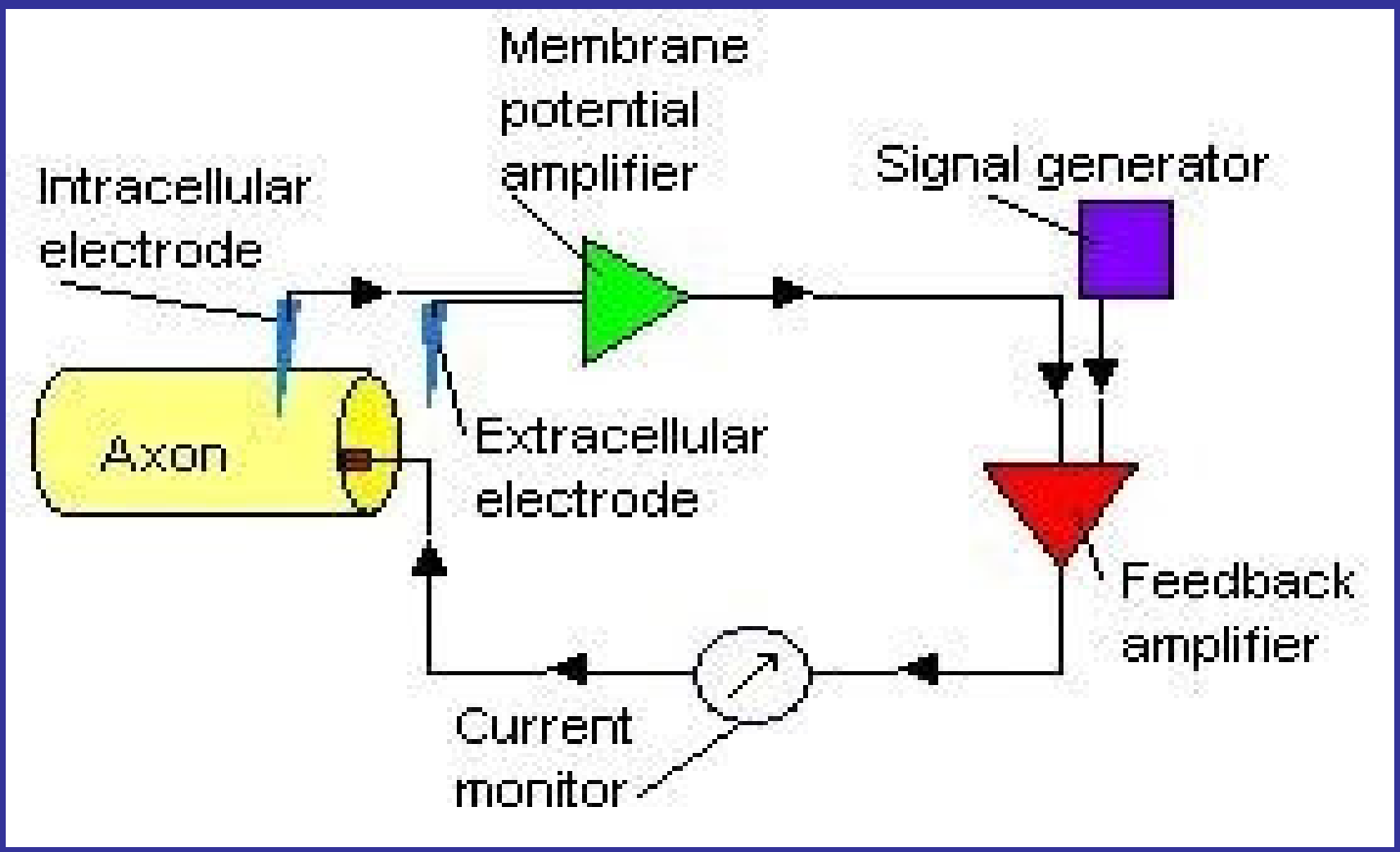
Voltage clamp

Julius Bernstein
(1839-1917)

K. S. Cole
1900-1984

- Most bioelectric events , voltage and current flowing through the membrane change simultaneously. Difficult to study experimentally
- The voltage clamp holds the voltage down(“clamps” the voltage) at one predetermined value and studies the current flowing through the membrane at this level.
- Uses electronic feedback to maintain the voltage balancing the current carried by flowing ions so the potential does not change.

Voltage clamp



Voltage clamp

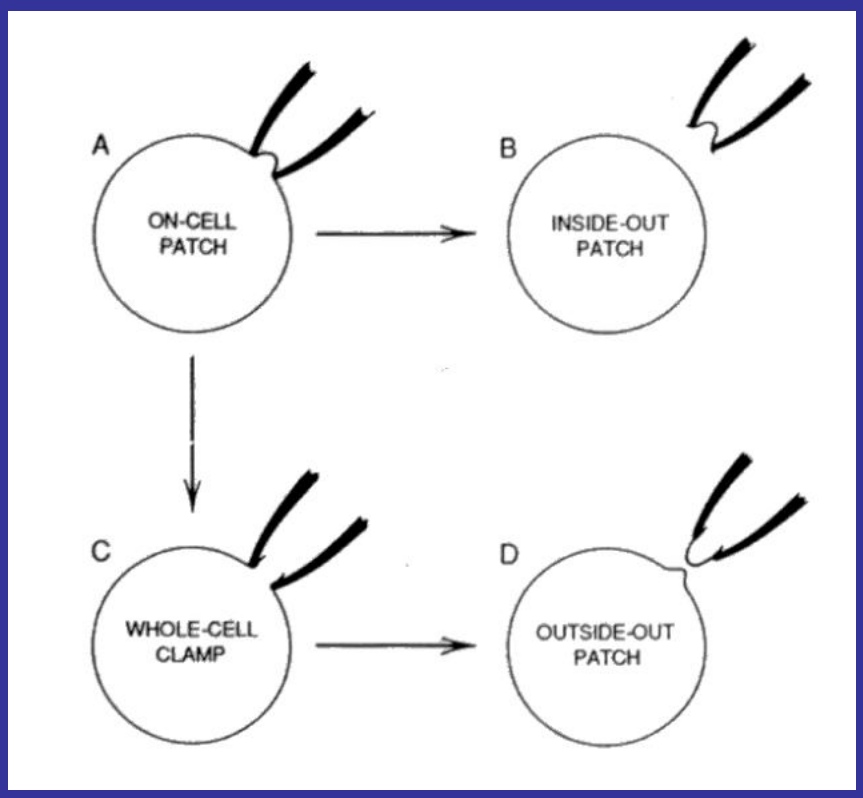
- The voltage clamp is a current generator with two electrodes, both of which are placed inside a cell.
- Transmembrane voltage is recorded through a "voltage electrode", relative to ground, and a "current electrode" passes current into the cell.
- The experimenter sets a "holding voltage", or "command potential", and the voltage clamp uses negative feedback to maintain the cell at this voltage.
- The electrodes are connected to an amplifier, which measures membrane potential and feeds the signal into a feedback amplifier. This amplifier also gets an input from the signal generator that determines the command potential, and it subtracts the membrane potential from the command potential (Vcommand - Vm), magnifies any difference, and sends an output to the current electrode.
- Whenever the cell deviates from the holding voltage, the operational amplifier generates an "error signal", that is the difference between the command potential and the actual voltage of the cell.
- The feedback circuit passes current into the cell to reduce the error signal to zero. Thus, the clamp circuit produces a current equal and opposite to the ionic current. This can be measured, giving an accurate reproduction of the currents flowing across the membrane.

The squid giant axon – up to 1mm in diameter
Rediscovered in 1930s by JZ Young

DEATH BY FUGU

TTX is 10,000 deadlier than cyanide

Patch clamp (Neher and Sakmann 1976)

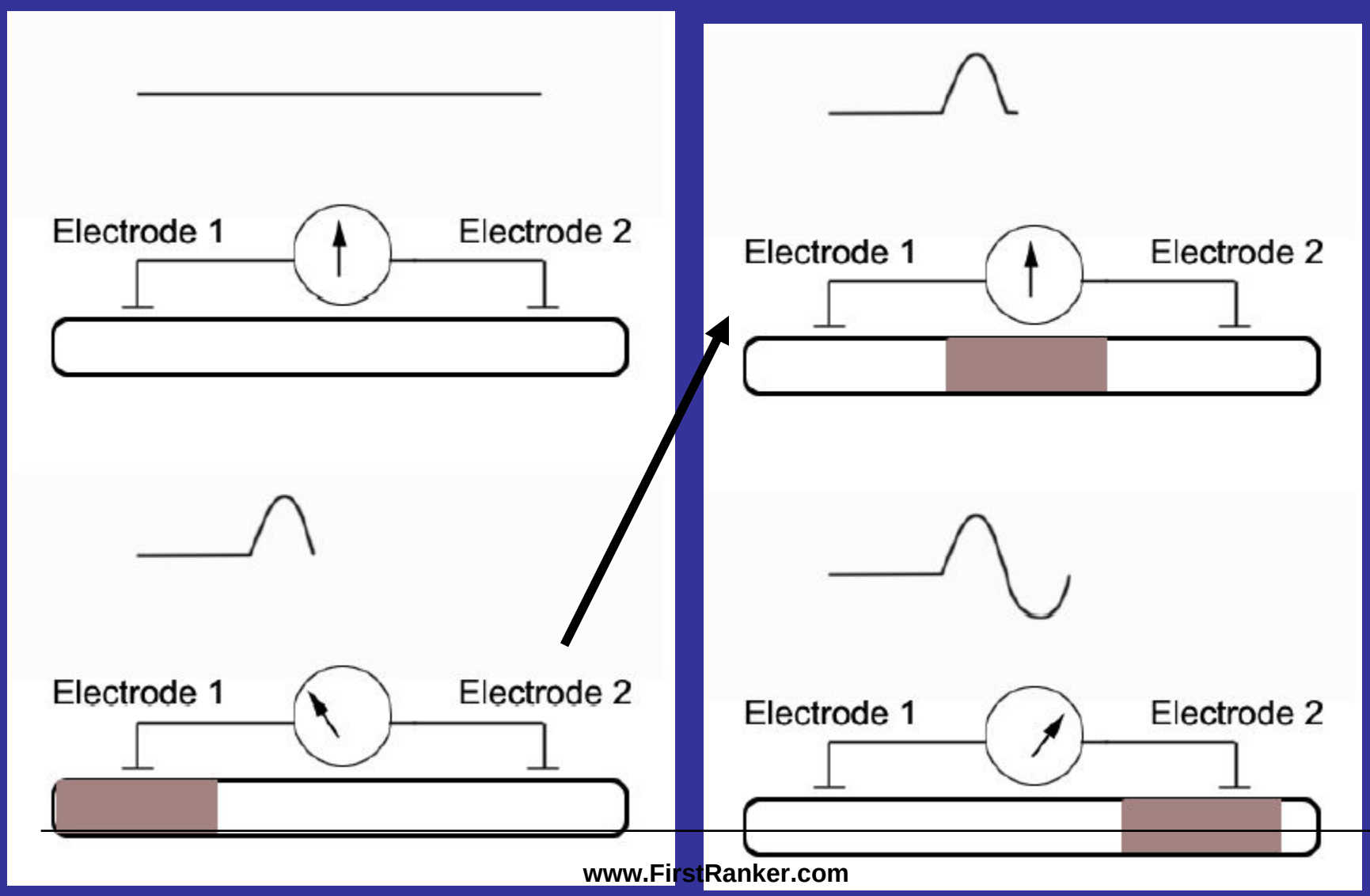


- For studying current flows through single channels
- uses a single electrode both to control membrane potential and to measure current
- amplifier is highly sensitive and is able to resolve the tiny currents (pA) flowing thru single channels

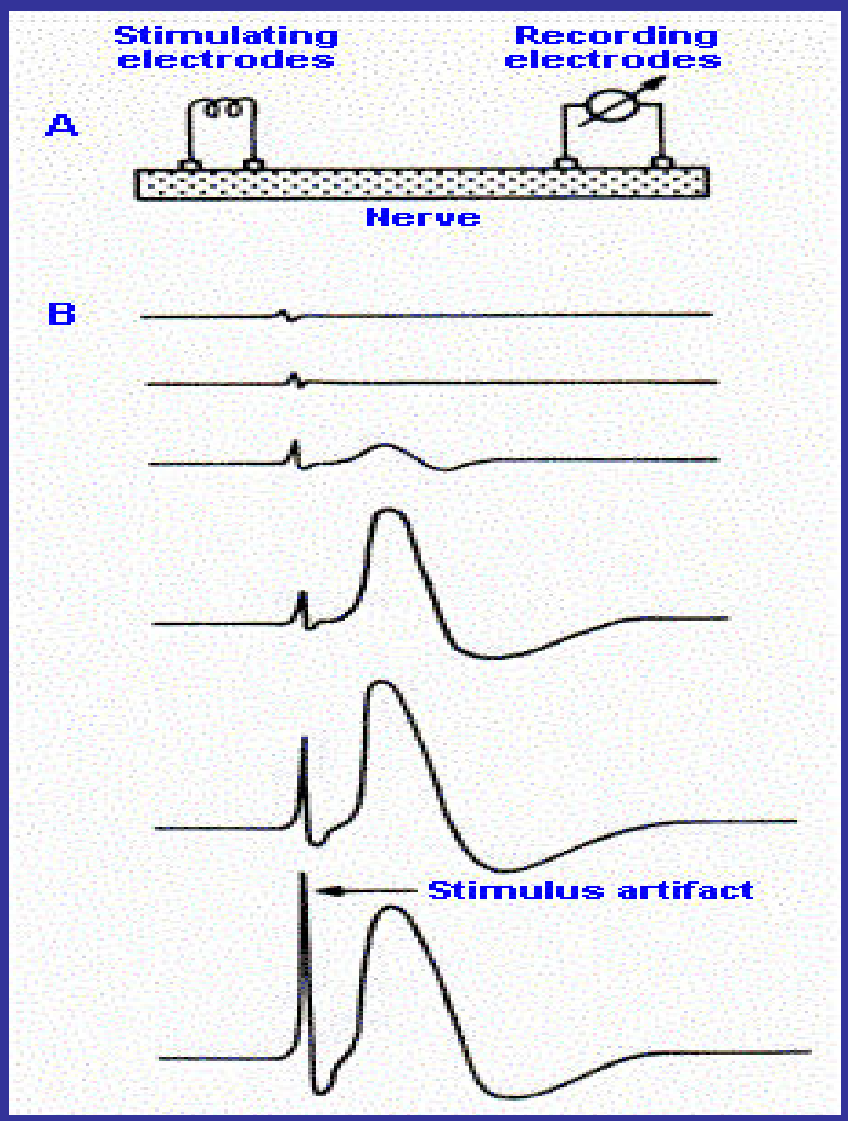
Studies on mixed nerves

Or…
Why Frogs Hate Scientists

Biphasic action potential



Compound action potential



Compound action potential

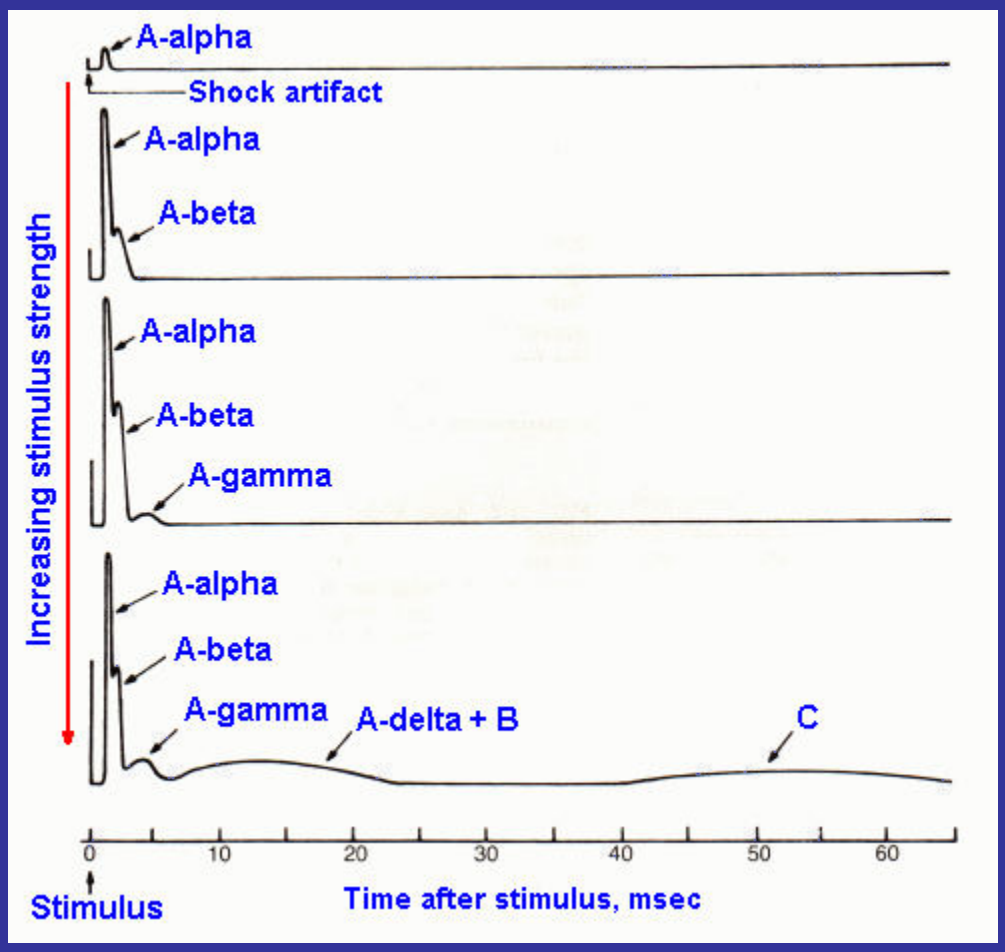


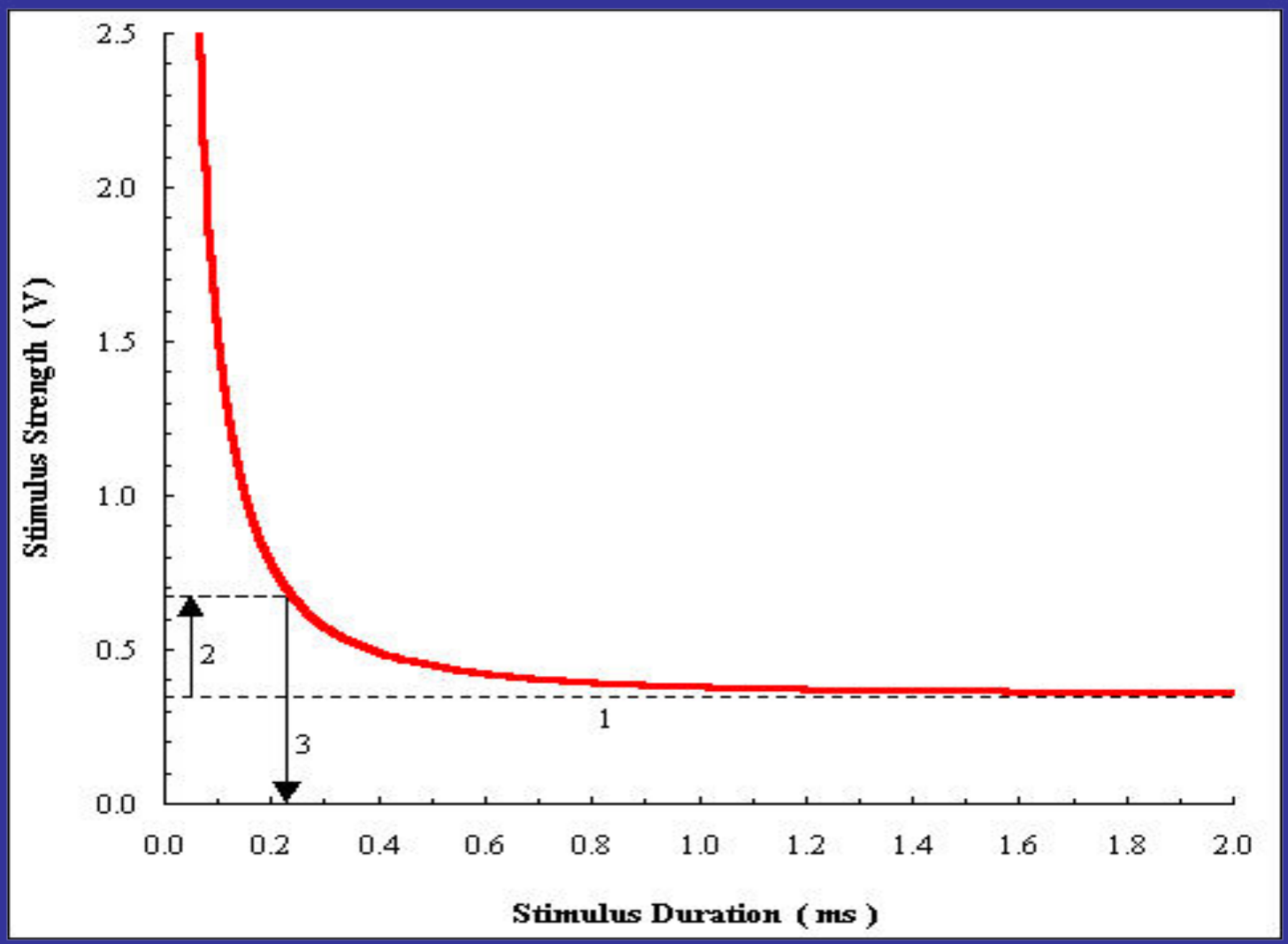
Table 1 - Mammalian Axon Properties*

Fiber Types	Fiber Diameter (μm)	Conduction Velocity (m/sec)	Action Potential Duration (msec)	Absolute Refractory Period (msec)	Functions
Aα motoneurones	12-22	70-100	0.4-0.5	0.2-1.0	Efferent alpha Afferent muscle spindles, tendon organs
Aβ	5-13	30-70	0.4-0.5	0.2-1.0	Afferent, cutaneous, Touch, pressure
Aγ	3-8	15-40	0.4-0.7	0.2-1.0	Gamma motoneurons
Aδ	1-5	12-30	0.2-1.0	0.2-1.0	Afferent, fast Pain, temperature
B	1-3	3-15	1.2	1.2	Efferent, autonomic **Only Preganglionic**
C (unmyelinated)	0.2-1.2	0.2-2.0	2	2	Afferent, "slow" Pain, Efferent Autonomic postganglionic

Susceptibility of Different Types of Fibers to Conduction Block by Various Agents

Effect	Most susceptible	Intermediate	Least susceptible
Block by hypoxia	B	A	C
Block by pressure	A	B	C
Block by local anesthetics	C	B	A

Nerve excitability: Strength duration curve



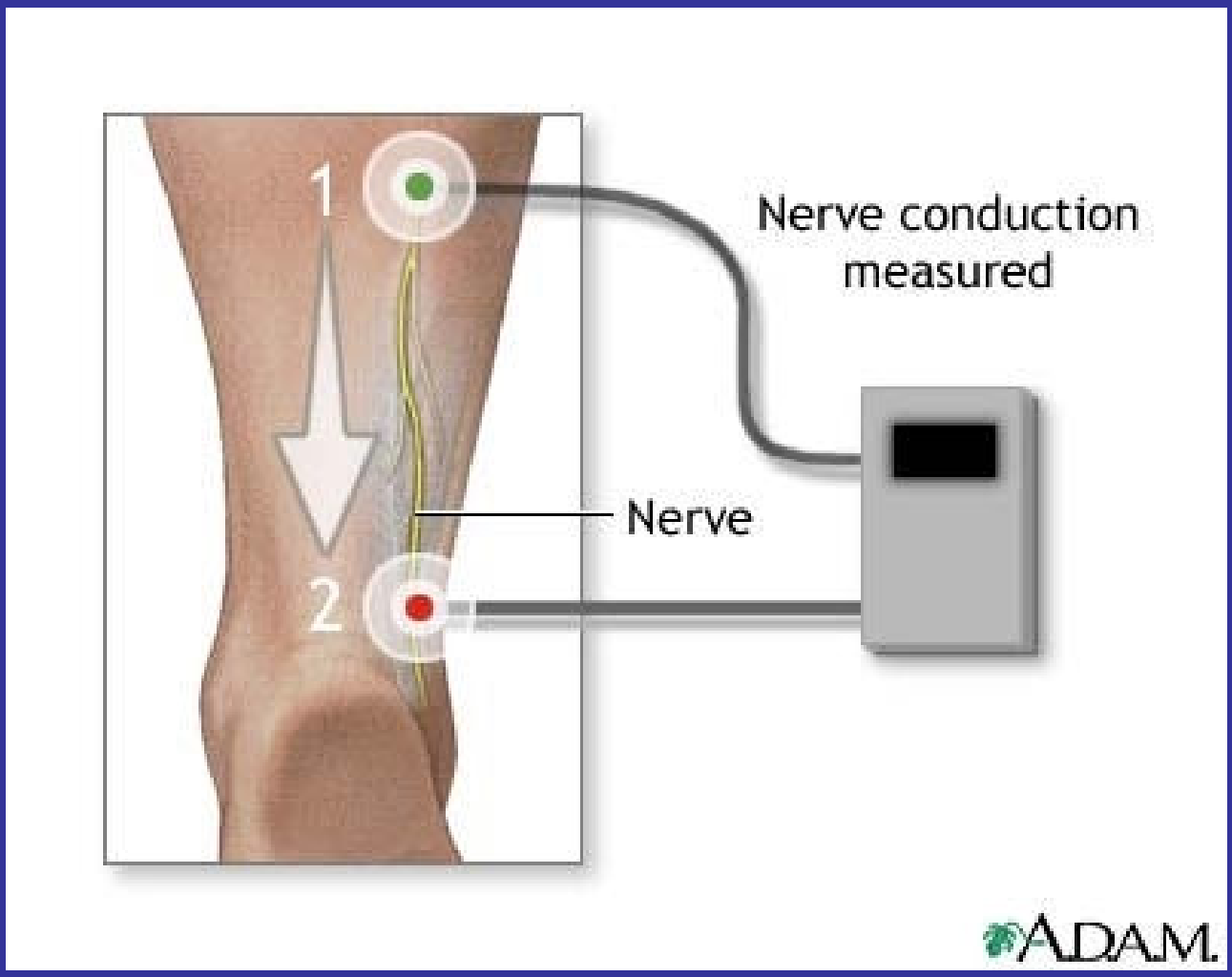
- Rheobase: the minimum stimulus strength which when allowed to pass through the excitable tissue for an infinite period of time , excites the tissue
- Chronaxie: minimum time required to stimulate excitable tissue when the current used is twice the rheobase

Nerve conduction velocity

Nerve conduction velocity

- **Nerve conduction velocity (NCV) is a test of the speed of conduction of impulses through a nerve.**
- **The nerve is stimulated surface electrodes, placed on the skin over the nerve at various locations. One electrode stimulates the nerve with a very mild electrical impulse.**
- **The resulting electrical activity is recorded by the other electrodes. The distance between electrodes and the time it takes for electrical impulses to travel between electrodes are used to calculate the nerve conduction velocity.**

Nerve conduction velocity



Utility of nerve conduction studies

Most often, abnormal results are caused by some sort of neuropathy (nerve damage or destruction) including:

- **Demyelination (destruction of the myelin sheath)**
- **Conduction block (the impulse is blocked somewhere along the nerve pathway)**
- **Axonopathy (damage to the nerve axon)**

Some of the associated diseases or conditions include:

- **Alcoholic neuropathy**
- **Diabetic neuropathy**
- **Nerve effects of uremia (from kidney failure)**
- **Traumatic injury to a nerve**

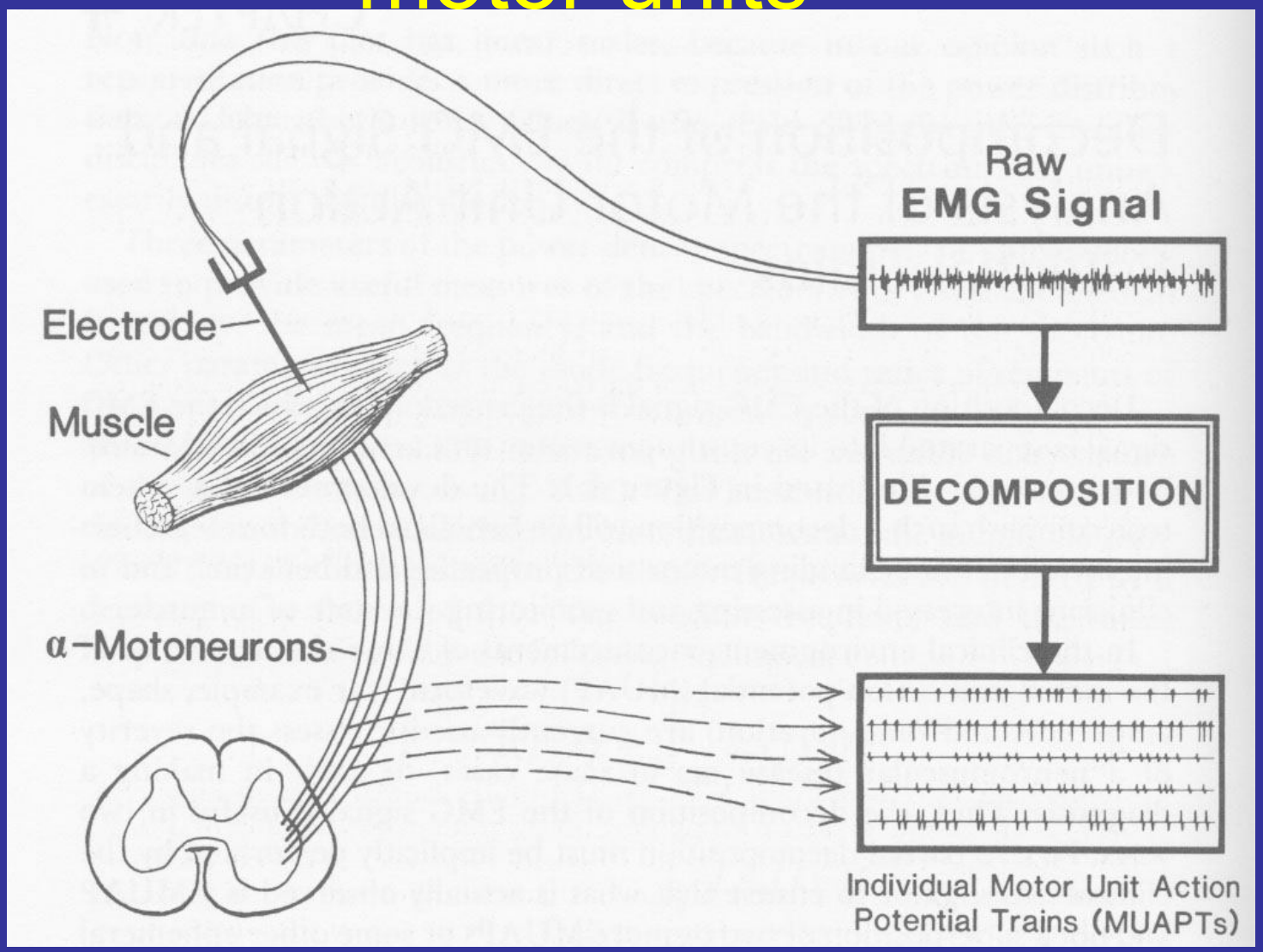
Electromyography

- Study of muscle function through the examination of the muscle's electric signals
  - 'Electro' – electric
  - 'Myo' – muscle
  - 'Graphy' – to graph
- Why EMG?
  - Determine *in vivo* muscle forces for various activities
  - Quantify muscle pathology

EMG

- Skeletal muscle performs mechanical work. It is stimulated to contract when the brain or spinal cord activates motor units.
- An action potential in the motoneuron causes activation of muscle fibers.
- The activation of motor units by action potentials generates a voltage signal in the muscle.
- EMG is a high gain amplifier to which electrodes are connected which records these voltages

EMG
Mixture of signals from different motor units



EMG types

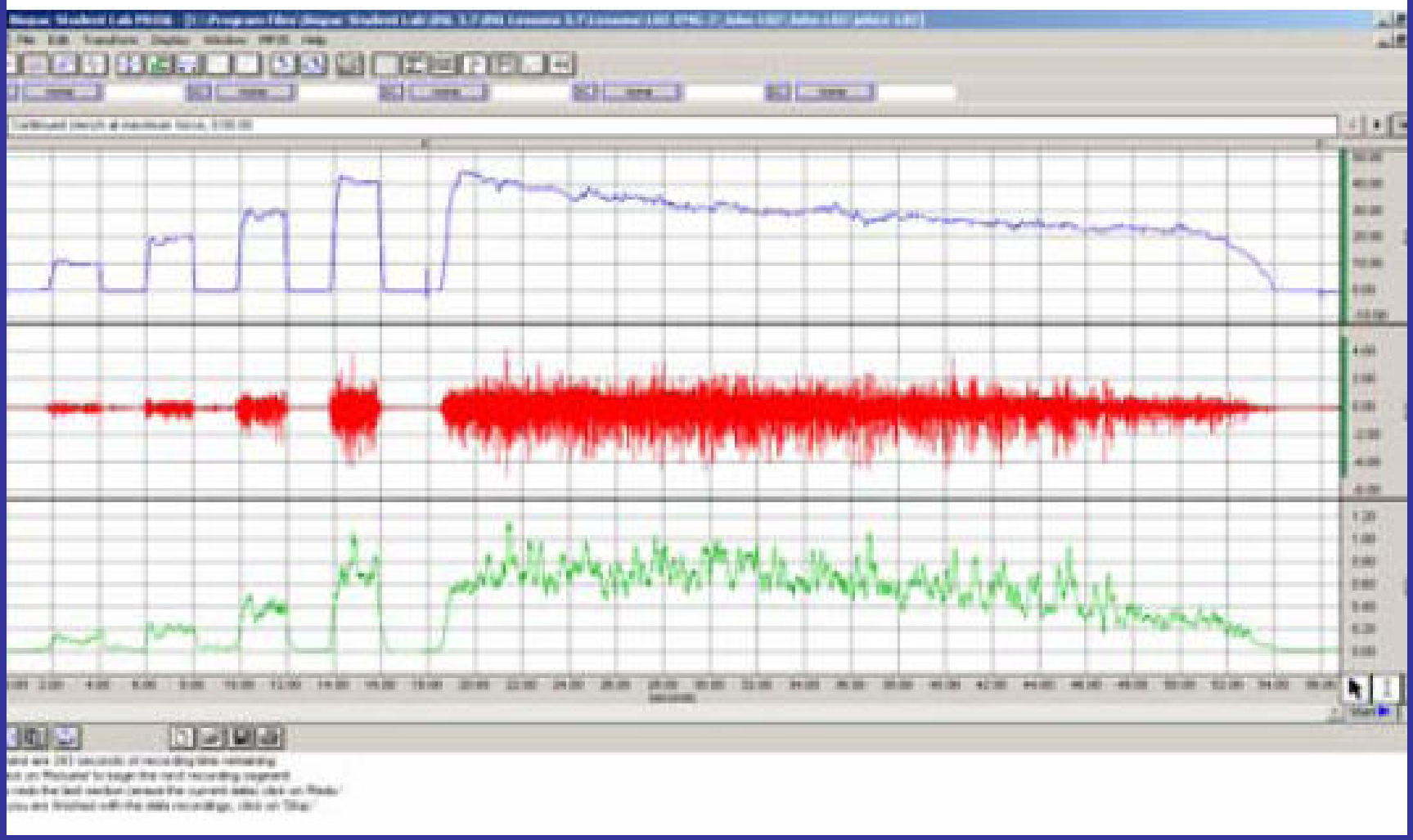
- Surface EMG (SEMG) – Electrodes are applied to the surface of the skin.
  - Used to measure muscle signals in large muscles that lie close to the surface of the skin
- Indwelling EMG – Electrodes are inserted into the muscle (usually via a needle)
  - Used to measure muscle signals in small or deep muscles, which can not be adequately monitored using SEMG.

Noise

- EMG signals are very small
- External noise
  - Electronics noise
    - Recording/measuring equipment
  - Ambient noise
    - TV, radio, overhead lights
  - Motion artifact
    - Movement of electrodes or wires

EMG with surface electrodes

EMG – sample recording



EMG

- Electromyograms are obtained at
  - Rest : no muscle activity
  - During slight muscle contration to asses the size and duration of ativiy of motor units
  - During maximal contraction to study recruitment
  - Abnormal patterns at rest

    -fibrillation

    -fasciculation