

# Foundations I

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## INTRODUCTION, OVERVIEW OF RECORDING TECHNIQUES AND BASIC CONCEPTS IN CIRCUIT ANALYSES

### I. Overview of Modern Recording Techniques

**A. Intracellular Recording** - Modern *in vivo* intracellular recording techniques and protocols were pioneered and exploited to great advantage by Sir John C. Eccles (a student of Sir Charles Sherrington) in the 1940s and 1950s to study synaptic transmission in cat spinal cord. The use of microelectrodes, very fine glass micropipettes with submicron tips filled with a conductive solution (usually KOAc, KMethylsulfate or KCl, 1-3 M), to obtain intracellular recordings from nerve and muscle cells, was first introduced by Ling and Gerard in 1949. The electrodes have impedances ranging from a low around 5 M $\Omega$  (for large tipped microelectrodes for recording from very large neurons like spinal motor neurons) to extremely high impedance electrodes over 100 M $\Omega$  for recording from small cells or large dendrites or axons. Intracellular microelectrodes can record full sized spikes 70-100 mV in amplitude, as well as subthreshold synaptic potentials less than a mV in size. The recordings are monopolar, referenced to an extracellular "ground", unfiltered and DC coupled so baseline voltages that represent the absolute membrane potential (the potential difference between the inside and outside of a cell) have meaning. Intracellular recording uses one of two general methods: 1) "*Current Clamp*" recording in which the membrane potential is measured as it varies either spontaneously or due to injected current or synaptic activity or 2) "*Voltage Clamp*" recording in which the membrane potential is "clamped" or held at a particular value by intracellular current injection and transmembrane currents resulting from action potentials or synaptic potentials are studied. More on voltage clamp at a later date.

#### 1. Advantages

Good for evoked responses

Can see subthreshold synaptic events and very slow events

Can do *in vivo*

Can directly measure membrane potential, membrane currents, input impedance etc.

Can directly stimulate (depolarize or hyperpolarize) the cell with intracellular current injection

Can fill microelectrodes with dyes (HRP, biocytin, neurobiotin, Lucifer Yellow) to identify cell after the recording by iontophoretically ejecting the dye out of the pipette

#### 2. Disadvantages

Not the method of choice for spontaneous activity measurements since impalement may damage or otherwise affect the neuron

Recordings difficult to obtain and maintain particularly in small CNS neurons *in vivo*

### 3. *In vivo* vs. *in vitro* recording

Under many circumstances, *in vitro* recordings can afford much greater stability than *in vivo* recordings (since there is no movement artifact introduced by the respiration and heartbeat of the animal), making it possible to maintain stable recordings for longer. The typical preparation uses slices of brain (these are actually more properly called *ex vivo* recordings), ranging from about 200  $\mu\text{m}$  ("thin slices") to around 400  $\mu\text{m}$  ("thick slices") in thickness, superfused in a buffered artificial oxygenated CSF containing a variety of ions and glucose. *In vitro* recordings are also made from acutely dissociated cells, especially for certain voltage clamp biophysics experiments where the truncation of the dendrites during dissociation helps maintain an adequate voltage clamp on the neuron.

The *ex vivo* and *in vitro* technique allows the experimenter to alter the extracellular ionic concentration and add drugs at will, making it an extremely powerful method for investigating responses to drugs and neurotransmitters. However, since most *long-distance* internuclear connectivity is lost in brain slices\*, the method is not generally available for studying synaptic responses elicited by stimulation of distant structures, although it works quite well to obtain paired recordings of synaptically connected neuron with a nucleus.

\*[Clever investigators, by doing careful tract tracing to delineate axonal trajectories have been able to circumvent this to a certain degree by cutting slices at oblique orientations, e.g., not quite true parasagittal or coronal, that were dictated by the results of their tract tracing studies. This has become quite common by now.]

*In vitro* recordings are also performed in cell culture, but in contrast to brain slices (or, more properly, *more* in contrast to brain slices which are, of course, different from neurons *in vivo*), cultured neurons may be abnormal in several respects, including which particular receptors are expressed and so care must be taken to ensure that the phenomena studied are relevant to neuronal systems *in situ*. (But don't say this too loudly around folks who record from cultures for a living – you might get hit.). Cultures come in several varieties. In some cases, fetal or neonatal cells are acutely dissociated and then cultured for a few weeks, during which time they mature and form synaptic contacts. Cultures can also be made from brain slices (slice cultures). Most recently, a number of investigators have put together slices of related brain areas, such as cortex and thalamus, or cortex, striatum and substantia nigra in an attempt to recreate complex systems *in vitro*. In many cases these so-called *organotypic slice cultures* have been very successful at retaining more of the *in situ* network properties than conventional acute brain slices.

### 4. Voltage clamp vs. Current Clamp

There are two principal ways of recording intracellular or transmembrane electrical events. In the first the microelectrode records the transmembrane voltage, often in response to an intracellular stimulus consisting of the injection of a transient current pulse, or an extracellular stimulus that evokes a synaptic response. This is called *current clamp* recording. Current clamp recordings are especially useful when one wants to observe action potentials as well as slower changes in membrane potential. In the second type of recording, a special differential feedback amplifier is used to inject current into the cell in order to prevent the voltage recorded at the tip of the intracellular electrode from varying from a command potential, thereby *clamping* it at a specified voltage. This is called *voltage clamp* recording, and what is actually measured is the current needed to prevent the cell's membrane potential from varying. This type of recording is especially useful when trying to

isolate the different conductances that underlie various types of neuronal activity and to obtain accurate measurement of the time and voltage dependence of the conductances.

**B. Patch Clamp Recording** - The most recent electrophysiological recording innovation was introduced by Neher and Sakmann in the late 1970s (in part for which they were awarded the Nobel Prize in Physiology and Medicine in 1991). The pipette is more similar to an extracellular glass micropipette than an intracellular pipette with a 1-3  $\mu\text{m}$  smooth (sometimes fire polished) tip, and a correspondingly low impedance, typically 3-6 M $\Omega$ . When the pipette is applied to a cell *in vitro* (dissociated from other neurons or in slice and under visual control under a compound microscope using differential interference contrast (DIC) optics and infra-red trans-illumination), a little suction is applied and a very high resistance seal ( $1-6 \times 10^9 \Omega$ ) forms around the tip. The "clamp" refers to the fact that these types of recordings are often performed in voltage clamp mode. The extremely high seal resistance allows measurement of currents flowing across the membrane under the patch. With smaller tips, single channel recordings are possible. Like the intracellular *in vitro* technique, patch clamping is ideally suited for many types of pharmacology due to good control over both the extra and intracellular environments. [Originally this could only be done *in vitro* with special preparations, for example cell culture or dissociated cells. The past few years have seen this technique routinely extended to slice recording, and to some *in vivo* preparations.]

**1. Patch Recording** - These recordings can be used to measure very small currents, sometimes resulting from the opening of a single ion channel, in the patch of membrane bounded by the gigaseal of the microelectrode. There are three main configurations of patch recording.

**a. On-Cell Patch (Whole Cell Patch)** - the membrane remains intact and connected to the neuron. This is a lot like extracellular or juxtacellular recording and is often used to monitor firing rate and pattern.

**b. Inside-Out Patch** - pulling the pipette slightly results in a small patch of membrane with the intracellular side facing outward becoming detached from the cell and remaining on the pipette tip with the intracellular membrane facing outward.

**c. Outside-Out Patch** - if after a little more suction is applied, the pipette is pulled back slightly, the membrane ruptures free of the cell and ends up attached to the pipette with the extracellular surface facing out.

**2. Whole Cell Recording** - if a little more suction is applied without moving the pipette, the membrane of the cell ruptures under the pipette and the intracellular compartment becomes continuous with the inside of the pipette. This makes it possible to inject various substances into the cell to control various internal biochemical processes. This type of recording is performed in current clamp or voltage clamp mode, and over the last few years has largely replaced conventional intracellular recording for many *in vitro* applications. The advantages of this method are increased stability of recording and the usual coupling of this method with IR-DIC optics which allows the experimenter to patch under visual control deep into the slice, thus rare cell types can be targeted if their morphology is distinctive enough under DIC illumination. Fluorescent labels can also be used to aid in targeting specific neurons if the cells can be induced to express a fluorescent protein, i.e., with genetic engineering.

One important drawback to whole cell recording is that since the interior of the electrode becomes continuous with the interior of the cell, the interior of the cell rapidly equilibrates with the filling solution in the pipette, essentially dialyzing out all the soluble components in the cell since the volume of the electrode filling solution is so much greater than that of the cell's interior. Thus, considerable care must be taken in selecting the composition of the filling solution, and to ensure that the cell's behavior does not change after time, as the dialyzation proceeds ("run-down"). In addition, whole cell recording makes it impossible to determine the true IPSP reversal potential for GABA<sub>A</sub> IPSPs, since the intracellular [Cl<sup>-</sup>] which dictates the reversal potential rapidly becomes whatever the experimenter put into the pipette.

**3. Perforated Patch Recording** - The drawbacks of whole cell recording can be compensated for by using the *perforated patch* technique in which an antibiotic like gramicidin or nystatin is dissolved in the internal solution. The experimenter then makes an on-cell patch and over the course of tens of minutes the antibiotic inserts itself into the membrane making small, pseudo-selective pores that are large enough to pass small ions like Na<sup>+</sup> and K<sup>+</sup> etc., but not intracellular biochemicals or Cl<sup>-</sup>. The main drawback to this is that the antibiotic makes it more difficult to obtain a gigaseal in the first place.

**C. Optical Recordings** - A very different type of "recording" takes advantage of certain voltage- or ion-sensitive dyes. Once introduced into a neuron (either by intracellular injection or diffusion, either *in vivo* or *in vitro*), the changes in membrane potential or intracellular ion concentration can be monitored by fluorescent video microscopy. Modern *endoscopes* are small enough to be inserted directly into the brain and to visualize dozens or hundreds of neurons simultaneously

1. Some voltage-sensitive dyes are fast enough for dozens of absorption measurements per ms, so the temporal resolution is excellent. In principle these dyes are difficult to work with because as will be discussed later, the majority of the charge separation during membrane voltage changes and thus the resulting fluorescent signal is only within a micron or so of the cell membrane.

2. **Fura-2** for Ca<sup>2+</sup> measurement. Since most calcium entry into neurons is by way of voltage-gated calcium channels, monitoring of intracellular calcium levels is an indirect way to monitor electrical activity (i.e., depolarization). Fura-2 changes its fluorescence when it binds calcium; thus the fluorescence intensity of a neuron viewed under high magnification is a measure of the relative intracellular calcium concentration. The absolute calcium concentration can be calculated by taking advantage of the fact that Fura-2 increases its fluorescence intensity when it binds calcium if illuminated at 340 nm but decreases its fluorescence intensity when calcium binds and it is illuminated at 380 nm. By taking a ratio of the fluorescence at the two wavelengths (*ratiometric imaging*) and making a few assumptions one can calculate the absolute calcium concentration.

3. **GCaMP** for Ca<sup>2+</sup> measurement. GCaMPs consist of a circularly permuted enhanced green fluorescent protein (EGFP), which is flanked on one side by the calcium-binding protein calmodulin and on the other side by the calmodulin-binding peptide M13. In the presence of calcium, calmodulin-M13 interactions elicit conformational changes in the fluorophore environment that lead to an increase in the emitted fluorescence. It is increasingly used for *in vivo* calcium imaging.

**a. Multiphoton imaging** Two-photon excitation of fluorescence is based on the principle that two photons of longer wavelength are simultaneously absorbed by a fluorochrome that would normally be excited only by a (single) photon with a shorter wavelength. The nonlinear optical absorption property of two-photon excitation limits the fluorochrome excitation to the point of focus. Limiting the excitation light to the point of focus rather than exposing the entire sample greatly reduces total photobleaching and photodamage. This is one major advantage to using two (or multi-) photon excitation. Multi-photon microscopy has several other advantages over confocal microscopy; optical sections can be obtained deeper within a specimen, a detection pinhole is no longer necessary, thus not limiting the number of photons being detected. In addition, the use of UV fluorophores is no longer limited to UV corrected objectives. Two-photon excitation utilizes longer visible or infrared wavelengths to excite UV fluorescent stains and indicators, therefore the objectives do not have to pass UV light (this makes everything much less expensive except of course for the lasers which currently run around \$150,000).

## **D. Extracellular recording**

**1. Single Units** - Extracellular single unit recordings measure the field potentials generated by action potentials of individual neurons. They are usually obtained with metal microwires, 20-40  $\mu\text{m}$  in diameter (used mostly in chronically implanted, freely moving animals), with metal in glass electrodes (etched tungsten wires are a popular choice) or micropipettes filled with a NaCl solution. The micropipette tips are much larger ( $\sim 0.5\text{-}3\ \mu\text{m}$ ) than those required for intracellular recordings, and consequently the impedances are much lower (1-10 M $\Omega$ ). These monopolar recordings are, like intracellular recordings, also referenced to a distant extracellular ground, although differential recording between two very closely spaced electrodes are sometimes used. The spikes recorded in this way are much smaller than with intracellular recordings, only 0.1 - 2 mV or so. Because of this, extracellular recordings typically require much higher gain, are band-pass filtered and AC coupled, producing poor low frequency response, so slow phenomena like synaptic potentials and membrane potentials cannot be detected.

[Extracellularly recorded action potential waveforms resemble the first derivative of intracellularly recorded action potential waveforms.]

### **a. Advantages**

Good for measurements of spontaneous and evoked spiking activity

Good for pharmacological studies

Easy to obtain and maintain recordings in vivo for long periods of time

Can easily obtain large sample sizes

Can use for chronic recording in freely moving animals

### **b. Disadvantages**

Can't measure subthreshold (synaptic) events

It is difficult to conclusively identify the single cell that was recorded (although the general recording *site* can be easily marked with extracellular dye injection (micropipettes) or the creation of a small lesion (metal electrodes). However, a relatively new technique called *juxtacellular labeling* is almost as reliable as intracellular filling for marking the cell that was recorded. In juxtacellular labeling an extracellular pipette is carefully advanced right up to the cell membrane of a neuron, usually at the soma. As the pipette is advanced, continuous rhythmic current pulses are applied and the investigator watches for a response from the neuron. When the electrode is very close (juxtacellular), passing current will cause the cell to fire in phase with the current injection.

Can't directly measure membrane potential or conductance

Can't directly manipulate level of polarization with intracellular current injection

## 2. Multiple Unit Recordings

**a. Small Groups of Neurons** - For multiple unit recordings, the electrodes are even larger and of lower resistance. Metallic electrodes (microwires) are most frequently used. The tips are several microns in diameter and the impedances range from several hundred k $\Omega$  to around 1 M $\Omega$ . Such recordings are particularly useful for understanding the behavior of population of neurons, and are most often used in chronic recordings in freely moving animals. There may be problems with recruitment and de-recruitment of neurons if one is studying the effects of drugs or stimulation, since individual units may not be discriminable. This latter issue is usually only a problem when recording from a relatively large number of neurons. For smaller numbers, a window discriminator can be used to isolate neurons based on the amplitudes and waveforms of their action potentials.

Today, most of these recording are done with tetrodes. Tetrodes are constructed by bundling together four very small electrodes; each wire is generally less than 30  $\mu\text{m}$  in diameter. Tetrodes are used to classify extracellular action potentials into sets generated by the individual neurons, as each channel of the tetrode is usually close enough to a cell such that action potentials emitted by that cell are detected on each of the four channels, but because of the spatial distribution of the individual channels, the amplitude of the signal varies across the four channels. Thus, individual neuron can be "triangulated" to improve identification and separation of individual single units.

Coupling tetrodes with a fiber optic carrying a laser pulse (optrodes) can allow the use of *optogenetics* to further identify the genetic identity of neurons by using viral transduction to infect specific neuron types in Cre mice with a light sensitive opsin so that a brief laser pulse will excite or inhibit the neuron, thereby identifying its genetic makeup.

Tetrodes can also be made with solid-state silicon probes that have 4 (tetrode) or more active (recording) sites at fixed locations along the probe. By comparing the waveforms recorded at multiple sites at different distances from each other, it is possible to "triangulate" on a neuron of interest and separate it from others also recorded from the same tetrode.

**b. Evoked Potentials (EP) and Event-Related Potentials (ERP)** - These recordings use even larger electrodes with even lower resistances. A primary difficulty may arise in attempting to identify the specific source (generators) of potentials. Because the signal to

noise ratio of the potentials of interest is often extremely low, signal averaging is usually used wherein an identical stimulus is presented hundred or thousands of times and the evoked responses averaged. This is the primary electrophysiological method used by cognitive neuroscientists to study brain activity in relation to cognitive tasks.

**c. EEG and evoked response** - These are essentially the same as evoked potentials, except instead of using depth recordings (insertion of electrodes into the brain), large metallic electrodes are placed on the surface of the skull or even on the scalp. The latter is the principal method used in human electrophysiology, since it is non-invasive. Under these conditions, what is measured usually arises from the summed synaptic responses [not action potentials] of hundreds of thousand or millions of individual neurons. [How could one know this?]

**d. Magnetoencephalography (MEG)** - A form of non-invasive recording of brain activity utilizes superconducting quantum interference device (SQUID) magnetometers to measure changes in the magnetic field surrounding the brain that result from electrical currents at activated synapses. It has superior spatial accuracy to EEG, but is most sensitive to activity in cortex that produces fields that are parallel to the skull.

## II. Basic Properties of Electrical Circuits

### A. Units

#### 1. Electrical Potential (E or V)

Electrical potential is a measure of the work to move electrostatic charges: Positive work moves like charges together; negative work moves like charges apart and vice versa.

Work is the integral of force\*distance so:

$$E = \int f(r) dr$$

where  $f$  = electrostatic force

and  $r$  = distance between the two charges

1 V = work to move 1 coulomb 1 meter against 1 newton

In an hydraulic analogy where current flow is thought of as water flowing through a pipe, voltage can be thought of as the water pressure.

**2. Current (I)** - Current is the rate of flow of charges

**1 ampere (A) = 1 coulomb/sec - where the charge on a proton or electron is**

**$1.6 \times 10^{-19}$  C.**

Current flows whenever there are two points of different electrical potential connected by a conductor. Note that Amps are defined by flow of positive charges, and "conventional current" is said to flow from a region of higher potential to region of lower potential (i.e. more positive to less positive) which is in the opposite direction to the flow of electrons in wires. In biological systems, current is carried by ions, not electrons. Current flows almost

instantaneously, near the speed of light, but neuronal current flow (action potentials and electrotonic potentials) is much slower.

**3. Resistance** - is the frictional force against flow of current, and is measured in Ohms. Resistance is the constant of proportionality between voltage and current, first described by Ohm in the relation known as Ohm's Law:

$$V=IR$$

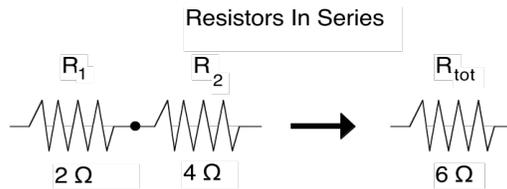
Ohm's law is arguably the most important equation in all of neurophysiology and the relation it describes is used in everything from the calculation of a cell's input resistance to the measurements taken by a voltage clamp amplifier.

The resistivity of a material is an intrinsic property of the material and is called  $\rho$  (rho)

$$R=\rho \cdot \text{length}/\text{area}$$

Resistances connected in series add linearly:

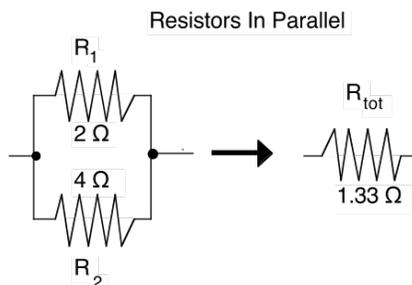
$$R_{\text{tot}}=R_1+R_2$$



Resistances connected in parallel add reciprocally:

$$1/R_{\text{tot}}=1/R_1+1/R_2$$

$$1/R = 1/2 + 1/4 = 3/4$$



Conductance is the reciprocal of resistance, formerly measured in mhos ( $\Omega^{-1}$ ), now siemens (S). Sometimes (for instance when talking about ion channels that open or close during synaptic activation) it is more convenient to talk about conductance ( $g$ ) rather than resistance. Note that since it is reciprocal of  $R$ , series and parallel additions are reversed. Thus, conductances in series add reciprocally:

$$1/g_{\text{tot}}=1/g_1 + 1/g_2$$

whereas conductances in parallel add linearly:

$$g_{tot} = g_1 + g_2$$

**4. Capacitance** - A capacitor is a device that separates and stores charges.

A capacitor consists of two conductive plates separated by an insulating material (dielectric). The capacitance (capacity) of a capacitor is given by:

$$C = \sum \sum_0 A/d$$

where

$\sum$  = dielectric constant (measure of insulatability)

$\sum_0$  = polarizability of free space ( $9 \times 10^{-14}$  f/cm<sup>2</sup>)

A = surface area of plates

d = distance between plates

This formula indicates that capacitance is directly proportional to the area of the conductive plates and inversely proportional to the distance between the plates.

The units of capacitance are Farads (f) = coulombs/volt

Thus a larger capacitor stores more charge at a given potential than smaller capacitor

Since we know the capacitance of biological membrane is around 1  $\mu$ F/cm<sup>2</sup> for all membranes, from the above equation the thickness of biological membrane (d) can be calculated:

$$d = \sum \sum_0 A/C$$

$$\sum = 5 \text{ for lipids}$$

$$= (9 \times 10^{-14} \text{ f/cm}^2)(5)(1 \text{ cm}^2)/(1 \mu\text{F/cm}^2)$$

$$= (9 \times 10^{-14})(5)/1 \times 10^{-6}$$

$$= 4.5 \times 10^{-7} \text{ cm} = 45 \text{ \AA}$$

or about twice the length of a membrane lipid molecule [The entire membrane is 75-100 $\text{\AA}$  because the entire membrane is not lipid [only about 33% is lipid].

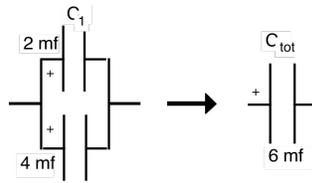
*Thus, the high capacity of biological membranes ( $\sim 1 \mu\text{F/cm}^2$ ) is due to their thinness.*

The addition rule for capacitors is the opposite of that for resistors:

Capacitance adds in parallel:

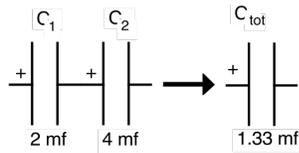
*(note that unlike resistors, the polarity matters)*

$$C_{tot} = C_1 + C_2$$



Capacitance decreases (adds reciprocally) in series:

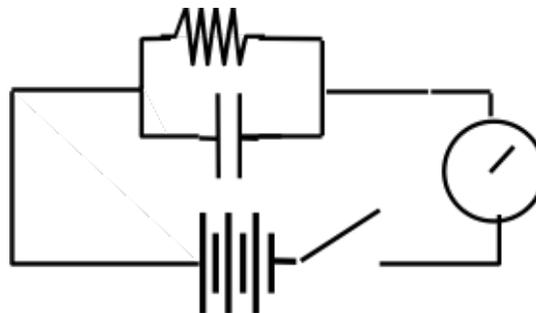
$$1/C_{\text{tot}} = 1/C_1 + 1/C_2$$



Current doesn't really flow through a capacitor - but as charges build up or dissipate on either plate, the rest of the circuit behaves as if current is flowing

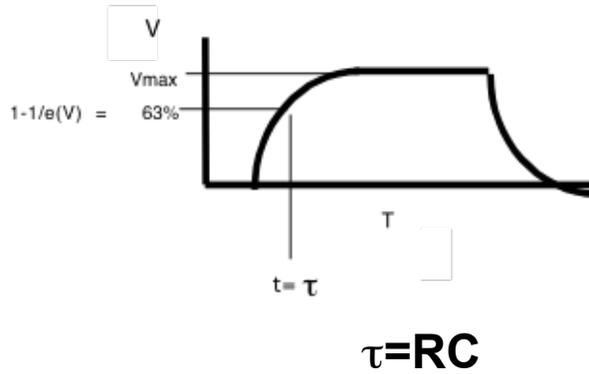
Thus, capacitors only 'pass' current when charging or discharging - thus capacitive circuits can act as "filters" that pass high frequency signals but not low frequency or DC signals. [What is the relevance of this to DC coupling in intracellular recording and AC coupling in extracellular recording circuits?]

Ideal capacitors will charge and discharge at speed of light, but no capacitive circuit has zero resistance.



Circuits containing resistor and capacitors in series or parallel take a characteristic time to charge up and down, called  $\tau$

For circuits with resistors and capacitors in parallel, the time for the potential to charge or discharge to  $1/e$  of its max/min value is the time constant,  $\tau$  and is given by:



Voltage at time  $t$  ( $V_t$ ) is given by:

$$V_t = IR(1 - e^{-t/\tau})$$

note that when  $t = \tau$ , this equation simplifies to

$$V_t = IR(1 - 1/e)$$

or

$$V_t = 0.632 IR$$

thus, the time it takes for the voltage to rise to 63% (or fall to  $1/63\% \sim 37\%$ ) of its maximum or minimum steady state value is  $\tau$ , the time constant.