EXCITABLE TISSUE ELECTROPHYSIOLOGY

ANAESTHESIA TUTORIAL OF THE WEEK 173

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QUESTIONS:

Before continuing try to answer the following questions. The answers can be found at the end of the article.

1. Properties of the Action Potential

a) The action potential is carried by free electrons
b) The action potential diminishes over distance
c) The frequency of action potentials encodes important information
d) The action potential has a variable size, but fixed duration
e) The speed of action potential conduction varies according to nerve type.

2. The Resting Membrane Potential

a) The cytoplasmic surface of the neuronal membrane is negative with respect to the outside surface.
b) For every two potassium ions taken up into the cell by the sodium potassium ATP pump, three are extruded
c) The Resting membrane potential for a typical mammalian neurone is around 70mV
d) At rest the cell membrane of the typical mammalian neurone is more permeable to potassium than sodium
e) The Goldman constant field equation can be used to calculate a predicted equilibrium potential for single ions.

3. The Voltage gated sodium channel

a) Allows passage of ions other than sodium
b) Is open in the resting state
c) Is open for 0.7ms during an Action Potential
d) Is blocked by Ryanodine
e) Is composed of four separate protein units.

4. The Movement of Ions

a) Anions are positively charged
b) Ions typically pass directly through a phospholipid bilayer
c) Anions move towards the Positively charged cathode
d) Electrical resistance may be described as 1/conductance
e) Voltage is the resistance to a charged particle

5. The ionic basis of the Resting membrane potential

a) At rest the cell membrane is more permeable to sodium ions than potassium.
b) Each individual ion has an individual equilibrium potential that can be predicted using the Goldman Constant Field equation
c) The absolute temperature is a component of the Nernst equation
d) The equilibrium potential for Potassium is -80mV
e) A large change in absolute ion concentrations occurs across the membrane during an action potential.

EXCITABLE TISSUES

The primary function of excitable tissue is to move information over distance. In a copper electrical wire this is brought about by the movement of electrons along the wire. Air acts as the insulator.

In excitable tissue, such as nerves, information is carried not by free electrons, but by ions as the **Action Potential** (Nerve impulse).

The Action Potential has several important properties:

- In contrast to passively conducted signals, the action potential does not diminish over distance.
- It is of fixed size and duration
- The speed of conduction is *slower* than that of electricity along a wire
- The information encoded by an action potential is coded by the frequency of impulses (λ) and by the distribution of the neurons transmitting the potentials.

An analogy can be drawn between the transmission of an action potential along a nerve axon and that of a flame being applied to the end of a trail of gunpowder where particles ignite along the trail immediately in front of the flame.

Nerves and muscle (including, importantly, cardiac muscle) can both generate and propagate action potentials. They are therefore excitable cells. Excitation of these tissues may be electrical, chemical or mechanical.

The ability of excitable tissue to generate and propagate action potentials depends upon the electrical properties of the cell membrane at rest.

The Excitable Cell Membrane at Rest

When a cell with an excitable membrane is not transmitting impulses it is said to be at rest. In the resting neuron, the cytosol along the inside surface of the membrane has a negative charge compared to the outside. The difference in electrical charge across the membrane is called the **Resting Membrane Potential.**

The action potential is simply a brief reversal (around 1/1000 of a second) of this situation so that the inside of the cell becomes positive with respect to the outside.

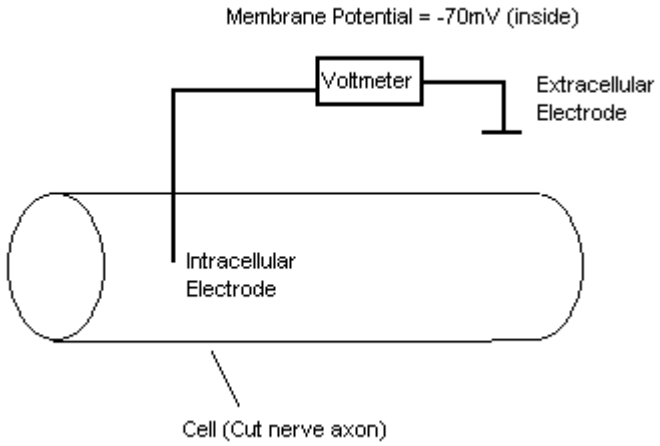

Fig.1a: The Resting membrane potential

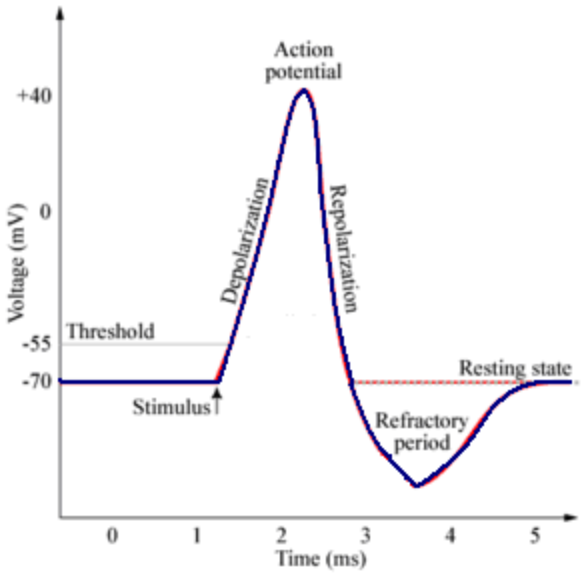

Fig.1b: The Action potential.

The Chemical composition of the cytosol and the extracellular fluid, as well as that of the cell membrane itself, are crucial to its electrical properties.

Water

Water carries an uneven distribution of charge and is therefore a *polar* molecule. The two hydrogen ions and the oxygen atom are bound together covalently and share electrons. The Oxygen atom, however, has the greater affinity for electrons and therefore carries a net negative charge. The Hydrogen atoms as a result carry a net positive charge.

The polar nature of water makes it an effective solvent of polar or charged molecules.

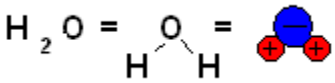

Fig 2: Water is a polar molecule

Ions

Ions are atoms or molecules that have a net electrical charge. NaCl (Table salt) in crystal form is held together by the electrical attraction of oppositely charged atoms (ionic bond). Salt dissolves readily in water because the charged portions of the water molecules have a stronger attraction for ions than they do for each other.

$NaCl \leftrightarrow Na^{+} + Cl^{-}$

The clouds of water molecules that surround each ion are called spheres of hydration and effectively insulate ions from each other.

Cell Membrane

Cell membranes are phospholipid bilayers, two molecules thick, with the hydrophilic ("water loving") polar heads on the outside and the hydrophobic ("water fearing") lipid tails on the inside. The membrane isolates the cytoplasm from the extracellular environment. A simple membrane with no protein channels is impermeable to ions.

Integral proteins transverse the membrane, whilst others sit on the intra and extra cellular surfaces. The resting membrane potential is determined by the action of protein channels and ion "pumps" that determine the ionic permeability of the membrane and therefore the electrical potential across it.

Ion Pumps

These are protein structures that use energy generated by the breakdown of ATP to transport certain ions across the membrane.

Ion pumps are central to the generation of the resting membrane potential, the most important example by far being the *Sodium-Potassium ATP pump*.

At the normal resting membrane potential of -70 to -80 mV both the electrical and chemical gradients of sodium tend to push it into the cell, and despite the relative impermeability of the membrane to this ion, some sodium does get in.

The Na^+/K^+ ATP pump actively extrudes sodium in exchange for potassium and consumes ATP in the process.

The pump exchanges sodium and potassium in unequal ratios, with two potassium ions being taken up for every three sodium ions extruded. The pump therefore generates an electrical charge (it is ***electrogenic***) as it **extrudes more positive charge than it admits**. One molecule of ATP is broken down for every three molecules of sodium extruded.

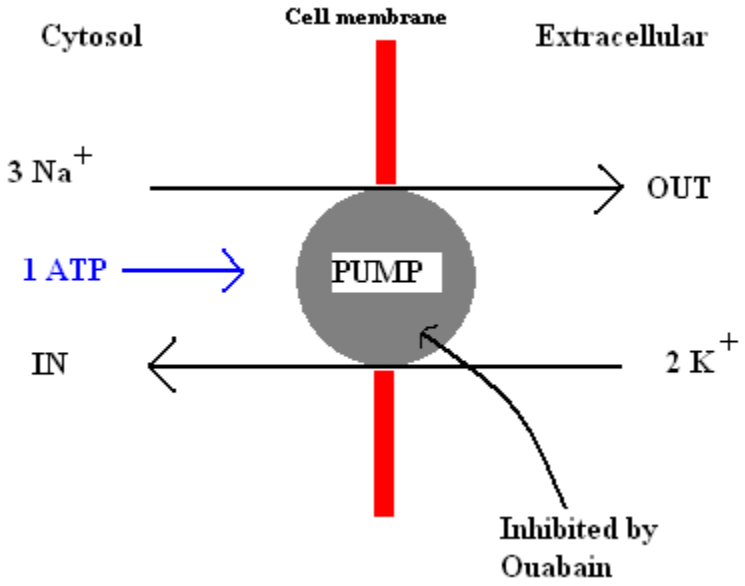

Fig 3: The Sodium-Potassium ATP pump

The Calcium pump is also of importance. This pump actively transports Ca^{2+} out of the cytoplasm across the cell membrane. Intracellular calcium binding organelles and proteins further decrease intracellular free calcium to a very low level (0.0002 mM)

These pumps ensure that ionic concentration gradients are established and maintained.

Ion Channels

An ion channel is a protein "pore", composed of four to six membrane spanning protein molecules, which allows the passage of an ion or ions across the cell membrane. The composition of the subunits varies from channel to channel.

The diameter of the pore and the nature of the *R* groups that lines it determines its ion selectivity. Ion channels are often gated (opened or closed) by changes in the local membrane microenvironment.

The basis of the action potential is that depolarisation opens sodium, potassium or calcium channels that are gated by the membrane voltage. The "voltage clamp" and the "patch clamp" techniques have been used to investigate the function of ion channels.

Voltage clamping involves the passage of a current through the membrane; the current required to maintain a constant membrane potential reflects the ionic flow through channels. In patch clamping a small area of membrane is voltage clamped so that individual channels can be observed.

Voltage gated Ion channels are so integral to the generation of the Action Potential that it is worth spending a few minutes looking at one of the most important examples of them.

The Voltage Gated Sodium Channel

This channel is selective and filters ions passing through it. During the action potential it is open for 0.7ms. It is an internal membrane protein with *four* transmembrane domains each containing *six* α helices (S1 to S6). S4 has a positive charge, S1 to S3 have negative charges. The protein forms a pore lined by the negatively charged helices.

In the resting state the channel is closed. When the membrane depolarises the channel undergoes conformational change and the central pore opens allowing passage of mainly sodium ions. Subthreshold depolarisations cannot open the pore and therefore no action potential can be generated. After activation the pore is temporarily inactivated by the inactivating particle.

The channel is opened by membrane depolarisation, blocked by **tetrodotoxin** (a poison produced by puffer fish, whose action on the sodium channel can be likened to that of a cork in a bottle) and inactivated by a particle that can be removed by the internal application of the enzyme pronase.

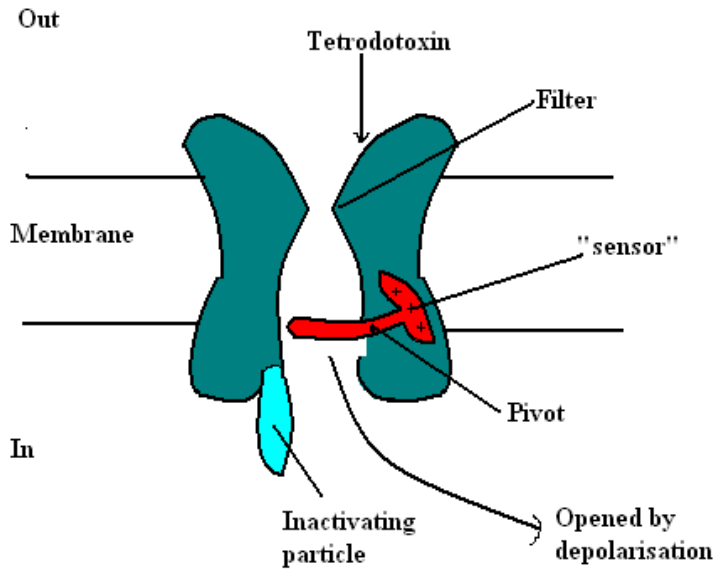

Fig. 4 The Voltage gated sodium channel

The Movement of Ions

Ion channels *allow* movement of ions across the cell membrane, but do not *cause* it. That is to say that an open ion channel will not always result in the movement of ions across a membrane.

An external force is required to drive movement.

The two main forces applicable to ion movement through an ion channel are chemical gradients (and therefore diffusion) and electrical gradients across the membrane (e.g. the movement of a positive ion to an area with a negative potential).

The Chemical Movement of Ions (diffusion)

Diffusion is the movement of a substance from an area of high concentration to an area of low concentration along its *concentration gradient* (The difference in concentration between an area of low and high concentrations of a substance).

Although ions typically will not pass through a phospholipid bilayer directly, diffusion will cause ions to be pushed through channels in the membrane. For an ion to diffuse across a membrane two things are required:

- A concentration gradient across the membrane
- Channels permeable to the ion

The Electrical Movement of Ions

Ions are electrically charged particles and will therefore move when an electrical field is applied. This can be illustrated using a battery in a solution of NaCl where sodium ions (Cations) will move to the negatively charged cathode and chloride ions (Anions) to the positively charged anode.

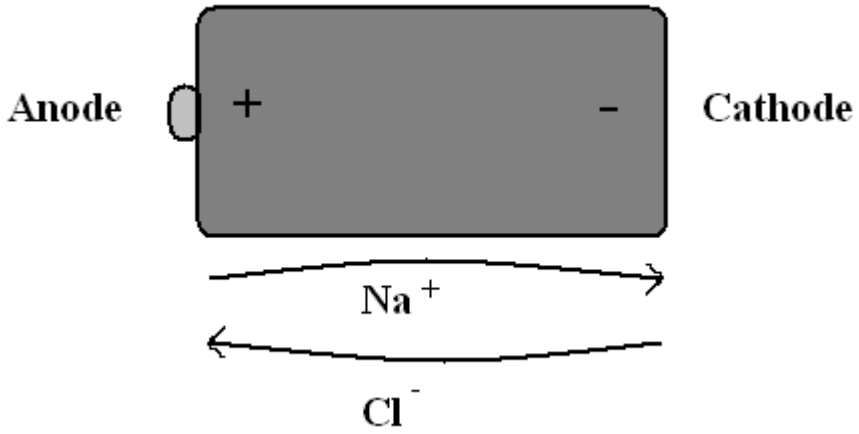

Fig 5. The Electrical movement of ions

The rate of movement of the electrical charge is the current (I) and is measured in amperes. By convention current flows in the direction of positive charge and so therefore in this example the flow is in the direction of Na^+ movement to the cathode.

The *Electrical Potential (Voltage)* and the *Electrical Conductance* determine how much current will flow.

The Voltage is the force on a charged particle and reflects the difference in charge between the anode and the cathode. As the voltage and therefore the difference in charge increases the current flow will increase.

Electrical conductance is the ease at which an electrical charge can migrate from one point to another. It is determined by the number of particles available to carry charge and by the ease with which these particles are able to travel through space. These properties are commonly expressed as *Electrical Resistance(R)* which is 1/conductance and measured in ohms.

The relationship between Voltage Current and Resistance is represented in *Ohm's law*

$$\mathbf{V = I \times R}$$

The Ionic Basis of the Resting membrane potential

Where are we now?

We know that there are electrically charged ions on either side of a phospholipid bilayer (membrane), and that these ions mostly can only pass across the membrane by means of protein channels.

We know that these channels can be highly specific for certain ions and gated by local changes in the microenvironment.

We know that movement of a given ion through a given channel will depend on both the electrical and concentration gradients across the membrane.

Lastly we know that at rest there is a negative resting membrane potential (V_m) across the cell membrane of around -70mV which is briefly reversed during the Action Potential. The negative resting membrane potential is an absolute requirement for a functioning nervous system and is generated by the distribution of certain important ions across the cell membrane.

Equilibrium Potentials

The concept of equilibrium potentials is central to the understanding of how the resting membrane potential is generated. At rest the cell membrane is more permeable to some ions than others (i.e *semipermeable to ions*). In practice this means the membrane is more permeable to Potassium ions than Sodium ions.

Let us take a hypothetical cell. Inside the cell a strong KCl solution is found. Outside the same KCl solution but twenty times weaker. The phospholipid bilayer has **no** channels or transport proteins. This would mean that despite the large concentration gradient from inside the cell to outside there would be **no** recordable membrane potential as there would be no net movement of ions. i.e. $V_m = 0mV$

Let us now make the cell membrane semipermeable to Potassium by inserting potassium channels. In this situation Potassium would be able to pass freely along its concentration gradient out of the cell. Negatively charged chloride ions however would not, hence a net negative charge would develop across the membrane.

The size of this negative charge would slowly increase until it starts to pull positively charged K^+ ions back into the cell along the electrical gradient. At *equilibrium* the electrical force pulling potassium ions back into the cell would be exactly counterbalanced by the concentration gradient pulling potassium ions out of the cell. In this example the V_m would = -80mV

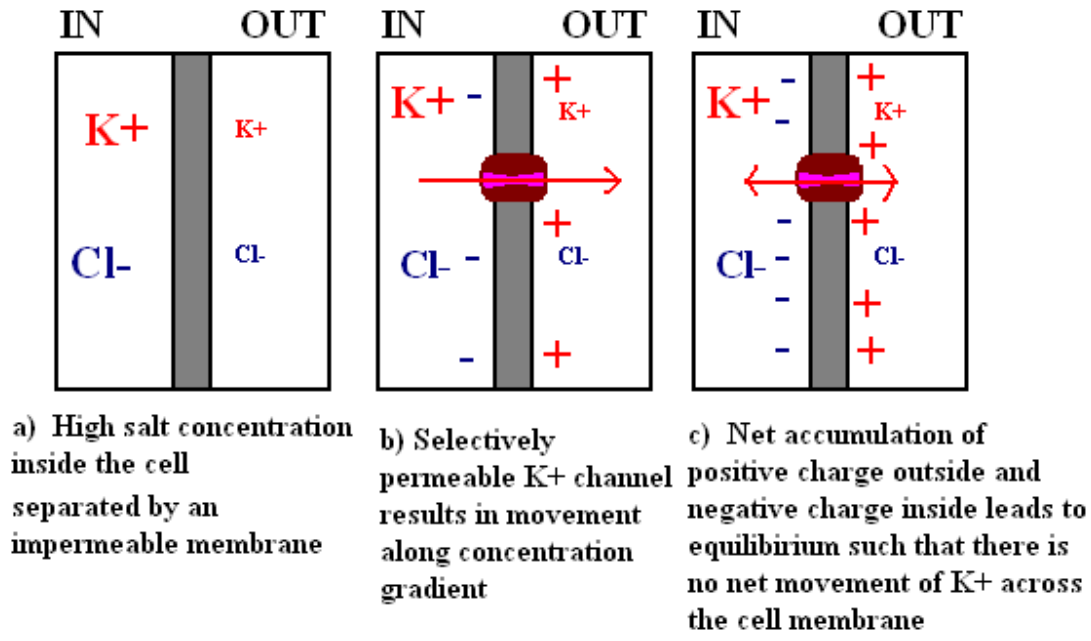

Fig. 6: Ion distribution across membranes

This model helps us to understand the following points about the development of an equilibrium potential:

- The absolute change in ionic concentration from in to outside of the cell changes very little in contrast to large changes in membrane potential. (a cell 50 micrometers in diameter containing 100 mM K^+ would require a concentration change of 0.00001 mM to generate a membrane potential of – 80 mV)
- The difference in electrical charge occurs at the inner and outer membrane surfaces . In this way the cell membrane acts as a capacitor as the positive and negative charges are attracted electrostatically to each other across the membrane.
- The rate at which ions are driven across the membrane is proportional to the difference between the membrane potential and the equilibrium potential (the ionic driving force)
- If the concentration difference for a given ion across a membrane is known then the equilibrium potential can be calculated for that ion. This is done using the **Nernst equation.**

The Nernst equation

Ionic concentration gradients exist across cell membranes, these are mainly established by the ion pumps discussed earlier. With knowledge of the relative concentrations of different ions across a cell membrane, the Nernst equation can be used to calculate the equilibrium potentials for different ions.

$$E_{ion} = 2.303 \frac{RT}{zF} \log \frac{[ion]_o}{[ion]_i}$$

where

F = Faraday's constant (96500 coulombs mol^{-1})
$\mathbf{E_{ion}}$ = ionic equilibrium potential
R = universal gas constant (8.314 J $deg^{-1}mol^{-1}$)
T = absolute temperature (degrees Kelvin)
Z = charge of the ion
$\mathbf{[ion]_i}$ = ionic concentration inside the cell
$\mathbf{[ion]_o}$= ionic concentration outside the cell

The equation can easily be understood by referring back to some of the principles already discussed.

The equilibrium depends both on the concentration gradient $\left(\frac{[ion]_o}{[ion]_i}\right)$
And the electrical gradient, where an increase in the electrical charge of an ion (z) will decrease the potential difference required to balance diffusion.

The rate at which a given ion moves along its concentration gradient will be governed by diffusion, the rate of this being increased by an increase in temperature (T).

As at rest and body temperature the cell membrane is mostly permeable to potassium, it is not surprising that the calculated equilibrium potential of -80mV for this ion is not much different from the resting membrane potential of -75mV that we see in many excitable cells.

The actual resting membrane potential can be predicted using the **Goldman constant field equation (Goldman-Hodgkin-Katz equation).** This formula takes into account the relative permeability of the membrane to other ions. (e.g. the relative permeability of the resting cell membrane to K^+ is forty times greater than that to Na^+).

$$\mathbf{Vm = 58 \log_{10} \frac{P_k[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_k[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o}}$$

Where P = permeability to each ion.

The importance of external Potassium concentration

As clinicians we are familiar with the importance of maintaining potassium concentrations within normal limits in order to avoid cardiac complications.

At rest the excitable cell membrane is mostly permeable to potassium. Because of this, changes in extracellular potassium concentration have a marked effect on the resting membrane potential. For example, increasing the $[K^+]_o$ from 5 to 50 mMol brings the RMP from -65mV to -17mV. That is, it *depolarises* the cell. In cardiac tissue for example this will markedly increase the likelihood of action potential generation and therefore increases the risk of arrhythmias.

In neuronal tissue Potassium homeostasis is very well maintained by neuroglia, particularly Astrocytes.

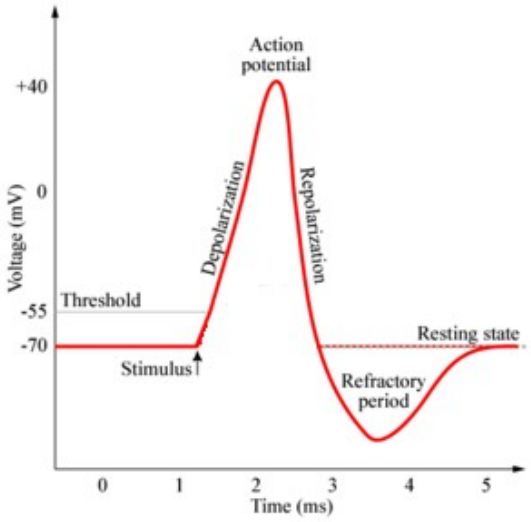

Fig 7: The Action Potential

The sequence of events resulting in the generation of an action potential can be summarised as follows:

1. Initial stimulus e.g. pain
2. Membrane depolarisation in nociceptive cell secondary to sodium influx (**the generator potential**)
3. Generator potential reaches **threshold** and action potential produced.

In other nerves the stimulus for generator potential production may be, for example, secondary to ion channel activation by neurotransmitters released at the synapse.

We can see from this that two types of physiochemical disturbance can be described.

- Local non propagated potentials (synaptic, generator or electrotonic)
- Propagated potentials (Action Potentials)

The Action Potential will then be transmitted along the axon to its termination.

The Components of an Action Potential.

When threshold has been reached the trace rises rapidly, overshooting the isopotential (zero potential) line to around +35 mV. The trace then rapidly reverses and falls back towards the resting level. This is called the **spike potential**.

At around 70% repolarisation the trace approaches the resting level more slowly. This is **after depolarisation.**

After reaching the previous resting level, the trace overshoots. This is known as **after hyperpolarisation.** This period coincides with the refractory period.

The All or Nothing Law

If a small current is applied to a neurone the minimal intensity of stimulating current (threshold current) that, acting for a given time, will produce an action potential can be determined.

A short intense current , or weak, but long, stimulus will both result in an action potential, providing threshold is reached. If the threshold current is not reached then an action potential will not be generated.

If the stimulating current rises too slowly then an action potential will not be generated as the neuron will adapt. This is known as **accommodation**.

Once threshold has been reached then further increases in stimulating current will not cause any increment in the action potential., or any change in its amplitude or form.

In short, unless threshold is reached, no action potential will be generated. Furthermore, any further increases in stimulation intensity will not result in any change in the action potential.

Generation of Multiple Action Potentials

Multiple Action Potentials will be produced if a stimulating current is applied to a neuron. The frequency of firing will increase in proportion to the magnitude of the stimulus current until a maximum firing frequency is reached (usually around 100 Hz).

The ionic basis of the Action Potential

At rest there is a large inward driving force on Na^+ as the membrane potential is so negative with respect to the equilibrium potential for sodium ions.

Once voltage gated sodium channels are opened, at threshold, sodium ions move into the neuron in a direction that takes V_m towards the equilibrium potential for sodium (65 mV). The membrane is depolarised.

Threshold can therefore be explained as the membrane potential at which enough voltage gated sodium channels are opened that the relative ionic permeability of the membrane favours sodium over potassium.

The Falling phase now needs explaining. This can be described in terms of the action of two types of channel. First, the voltage gated sodium channels start to close. Secondly, voltage gated potassium channels open (triggered by membrane depolarization. There is now a large driving force on potassium ions inward, hence potassium ions flow back into the cell bringing the membrane potential back towards the equilibrium potential for potassium.

Undershoot now occurs. This is due to relative membrane impermeability to sodium in comparison to the resting state, due to inactivation of voltage gated sodium channels. The membrane potential therefore approaches the E_K of -80mV.

This coincides with the *absolute* and *relative refractory* periods. Voltage gated sodium channels are inactivated by a strongly depolarised membrane. They cannot therefore be activated until the membrane potential has become sufficiently negative. This means that no action potential can be generated during this period.

The relative refractory period is associated with the period that the membrane is hyperpolarised due to the continued activation of voltage gated potassium channels, but voltage gated sodium channels are reactivated. During this period a greater than normal stimulus is required to reach threshold.

Against this background the Na^+/K^+ ATP pump continues to work to maintain the normal ionic concentration gradients.

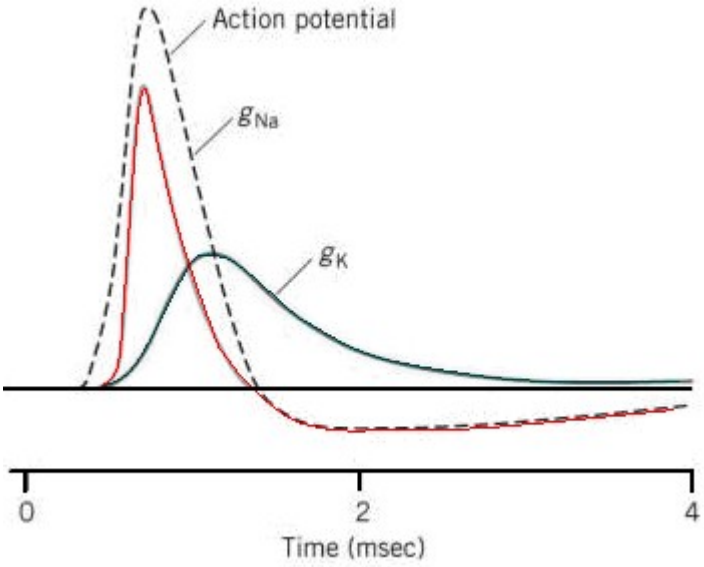

Fig 8. Relative membrane conductances for sodium and potassium during the Action Potential.

Transmission of the Action Potential along the neurone.

During the action potential the polarity of the neuronal membrane reverses briefly. Positive charges from the membrane ahead of and behind the action potential flow into the area of negative charge represented by the action potential. This is called **current sink**.

Current sink causes the membrane in front of the action potential to become slightly depolarised. This results in movement of the membrane potential towards threshold and subsequent depolarisation of that segment of the axonal

membrane.

This sequence of events moves regularly along an *unmyelinated* axon to the end. It is therefore, as discussed previously, a self propagating event and does not diminish with time.

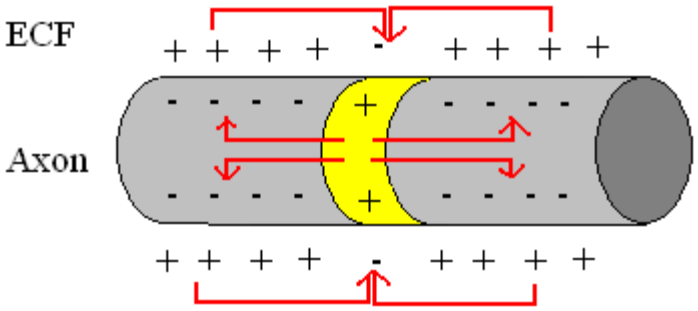

Fig 9. Local current flow around an axonal impulse

The action potential cannot move backwards along the membrane as the area immediately behind it will still be in its absolute refractory period.

An axon can however theoretically conduct in either direction. If an action potential is set up in the middle of an axon, two impulses travelling in opposite directions are generated.

In humans and other mammals conduction is normally in one direction only; from a receptor or synapse to their termination. This is known as **orthodromic conduction**. Conduction in the other direction (antidromic conduction) will be prevented by the first synapse encountered.

The speed at which an action potential propagates along the unmyelinated axon depends an how far ahead depolarisation spreads, which is in turn dependent on certain physical characteristics of the axon itself.

The first of these is axon diameter. The larger the axon diameter, the faster the conduction of the impulse. The second factor is whether the axon is Myelinated or not.

Saltatory Conduction

Myelinated axons transmit action potentials in a similar way to unmyelinated axons. However, myelin is an extremely effective insulator. This prevents the action potential from being transmitted regularly along the neuronal cell membrane to its end. Instead the action potential 'jumps' from one demyelinated area to the next (**saltatory conduction)**. These demyelinated areas represent the junctions between Schwann cells in peripheral axons and processes from oligodendrogliocytes in the central nervous system.

In this situation the current sink at one node of Ranvier acts to depolarise the cell membrane at the next node. In this way the area of depolarisation jumps from one node to the next (saltatory derives from the Latin saltare, meaning to dance).

Saltatory conduction velocities may be up to fifty times faster than unmyelinated nerve conduction velocities.

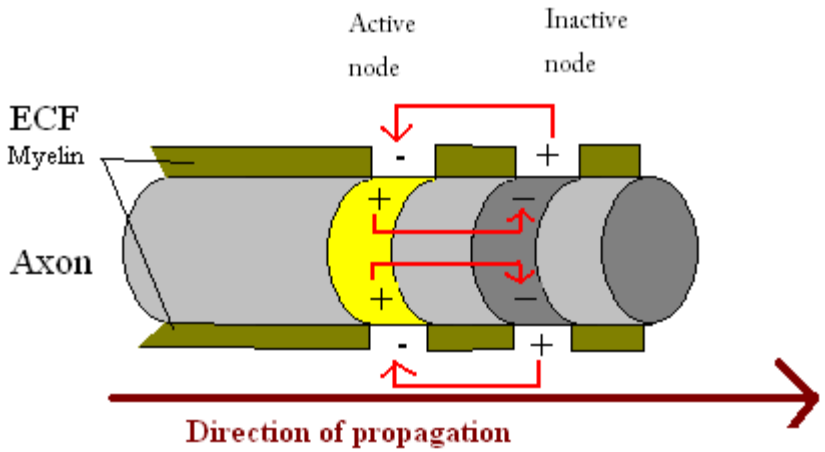

Fig.10 Local current flow; saltatory conduction

ANSWERS.

1. FFTTT
2. TFTTF
3. FFTFF
4. FFFTF
5. FFTTF

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