

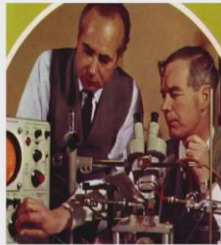
Introductory Neuroscience & Neuro-Instrumentation
Professor Mahesh Jayachandra
Center for Bio-Systems Science and Engineering
Indian Institute of Science, Bangalore
Lecture No. 12
The Action Potential (2)

So, Introductory Neuroscience & Neuro-Instrumentation, the action potential lecture 2.

(Refer Slide Time: 0:27)

Hodgkin & Huxley found how Action Potentials are generated in the Squid giant axon

- 1) Hodgkin and Huxley used the voltage-clamp technique to find mechanisms of AP generation in the squid giant axon.
- 2) Axons and neurons have a threshold for the initialization of an action potential of about -45 to -55 mV.



Mahesh Jayachandra MBBS, MD, PhD Action Potential-2 03 Sept, 2020

So, Hodgkin and Huxley, I keep talking about them. They were high (0:32) of Neuro Physiology, Electro Physiology as (0:35). And you see, on the right, Hodgkin is on the extreme right and Andrew Huxley is on the left and the apparatus they use, you have a cathode ray oscilloscope, you have an operating microdissection microscope and then things to infuse an electrode to record so on and so forth.

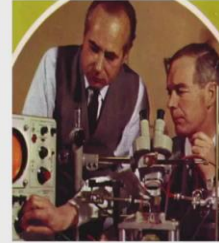
So, Helan Hodgkin or Sir Helan Hodgkin and Sir Andrew Huxley, they found out how the action potentials are generated in the squid giant axon and it was remarkable to reforce when they did it because though a lot of evidence had been accumulating, they were the first to prove the actual mechanisms, physiologist. So, they used the voltage-clamp technique which was developed by Cannel Curl at Woods Hole in Massachusetts to find mechanisms of action potentials generation, the squid giant axon.

So, this is one of the nice things about science that people collaborate and are very generous and share their techniques and advances occur. Axons and neurons have a threshold for the initialization of action potential. So, the resting membrane potential is about minus 60 millivolts and if it goes up depolarises goes to a 0 to about minus 45, you have an action potential.

(Refer Slide Time: 2:06)

Hodgkin & Huxley found how Action Potentials are generated in the Squid giant axon

3) Increasing the voltage from -60 to 0 mV produces a large, transient, flow of positive charge into the cell (inward current). This is followed by a sustained flow of positive charge out of the cell (outward current).



4) Voltage clamping experiments by Hodgkin and Huxley demonstrated that the inward current is carried by Na^+ ions flowing into the cell, and the outward current is carried a flux of K^+ ions moving out of the cell.

Maresh Jayachandra MBBS, MD, PhD

Action Potential -2

03 Sept, 2020

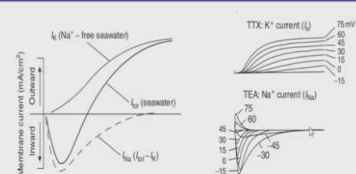
So, increasing the voltage from minus 60 to 0, produces a large transient flow of positive charges into the current is called inward current. This is followed by a sustained flow of positive charges out of the current, out of the cell, and is called outward current. So, voltage clamping experiments by Hodgkin and Huxley demonstrated that the inward current is due to sodium ions flowing into the cell and the outward current is caused by potassium ions moving out of the cell.

(Refer Slide Time: 2:44)

Dissecting channels by blocking them selectively

1) Na^+ and K^+ currents (I_{Na} and I_{K} , respectively) can be blocked, allowing each current to be examined in isolation.

2) Tetrodotoxin (TTX), a powerful poison found in the puffer fish *Spheroïdes rubripes*, selectively blocks voltage-dependent Na^+ currents. *The puffer fish is a Japanese delicacy.*



Above left: Inward current in sea-water and Na^+ -free seawater (no inward current).

Right: TTX blocks all Na^+ currents and TEA blocks all K^+ currents at all Command voltages (from Hodgkin & Huxley).

Maresh Jayachandra MBBS, MD, PhD

Action Potential -2

03 Sept, 2020

So, these currents, the sodium current or the potassium current, typically they are denoted by I subscript Na for sodium a current, and I subscript K for potassium current. Now, they can be selectively blocked and this block does not affect the other ionic flows. The block occurs

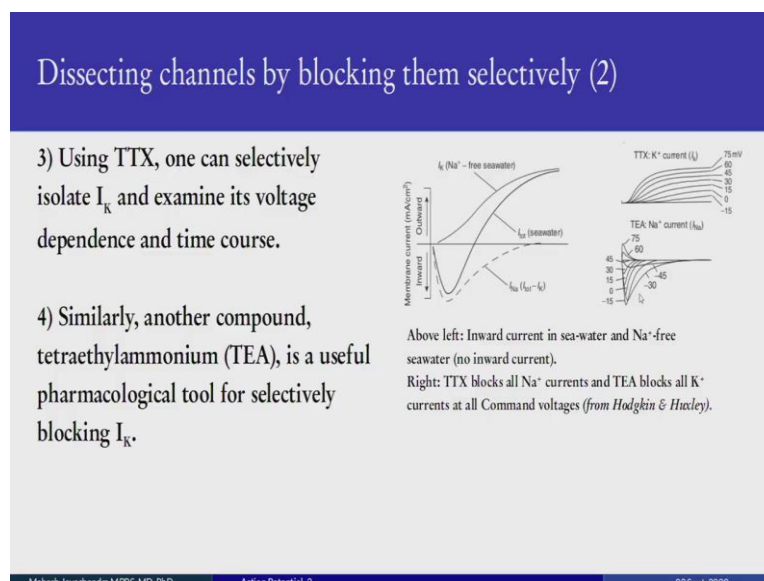
at the sodium channel, there is a sensitive area, it is kind of a lock and key mechanism and there is this chemical compound called Tetrodotoxin, a short name to TTX.

Now, this is a very powerful poison that is found in the Japanese pufferfish. It is a delicacy, they eat it but they carefully remove the TTX before eating, but even so every year many people die in Japan of TTX poisoning because it blocks the sodium channels at microscopic concentrations 10 to the minus 5 molar, only sodium channels are blocked and it is reversible.

So, similarly, with potassium, you have specific channel blocks for potassium. It only affects potassium channels. It is called TEA, Tetra Ethyl Ammonium. So, if you consider the plots on the right, you have an inward current. Look at the central thick line, you have an inward current and followed by an outward current. This is during the action potential. Now, suppose, you replace the sodium outside and make it sodium free seawater, you do not have any inward current, but you still have the outward current.

And in this panel on the extreme right, the plot above is where you have a TTX blocked happening and you have only the potassium currents and these are the different command voltages. It is a voltage clamp minus 15, 0, 15, 30, 45, 60. So, you see it occurs later. When you block the potassium channels with TEA, you only have the sodium current, the inward current and that is the panel below. And these are the command voltages of the voltage clamp minus 15, 0, 15, 30, 45. And it is very fast (5:09) and comes back to normal quickly.

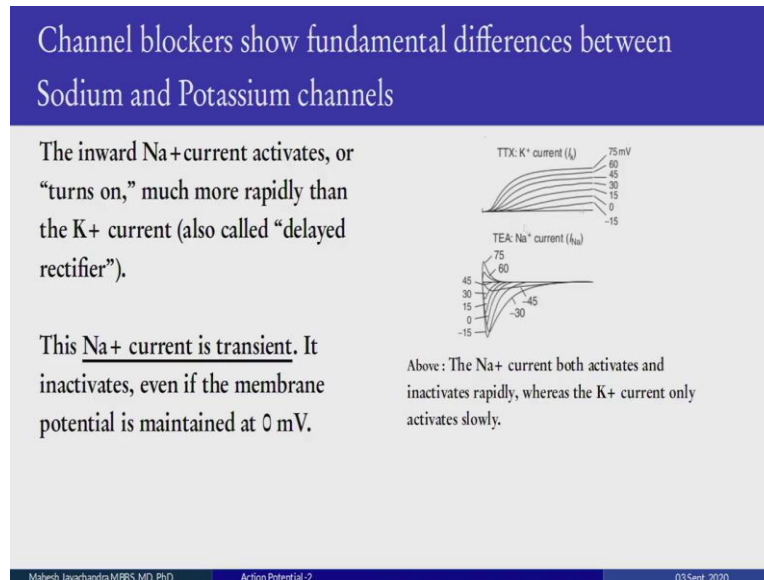
(Refer Slide Time: 5:13)



So, using TTX we can selectively isolate the potassium current and examine its voltage dependence here. And similarly, in TEA tetraethylammonium we do the same thing and look

at only the sodium channels because as sodium currents because you have blocked the potassium channels.

(Refer Slide Time: 5:35)



So, these channel blockers show some fundamental differences between sodium and potassium channels. The inward, let us consider the potassium I mean the sodium currents which are below. So, you have an inward current and it occurs fast but also rapidly inactivates and is transient. It inactivates even when the membrane potential is 0. The potassium currents, on the other hand, take some time to occur, and then they are long-lasting.

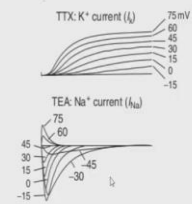
And that is why they are called delayed and it is also called a rectifier, delayed rectifier. When you hear the word, term delayed rectifier, you think of potassium currents. So, the sodium current both activates and inactivates rapidly. While the potassium current only activates slowly like so over here.

(Refer Slide Time: 6:32)

Channel blockers show fundamental differences between Sodium and Potassium channels (2)

In contrast, the outward K^+ current, once activated, remains “on” as long as the membrane potential is clamped to positive levels; that is, the K^+ current does not inactivate but is sustained.

These fundamental properties of the underlying Na^+ and K^+ channels allow the generation of action potentials.



The figure contains two graphs. The top graph is labeled 'TTX: K^+ current (I_K)' and shows several curves of current (pA) versus voltage (mV) ranging from -15 to 75. The curves show a slow activation and then a sustained outward current at positive voltages. The bottom graph is labeled 'TEA: Na^+ current (I_{Na})' and shows several curves of current (pA) versus voltage (mV) ranging from -15 to 75. These curves show a very rapid activation followed by a rapid inactivation, returning to zero current at positive voltages.

Above: The Na^+ current both activates and inactivates rapidly, whereas the K^+ current only activates slowly.

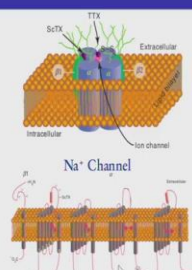
Maresh Jayachandran MBBS, MD, PhD Action Potential -2 03 Sept, 2020

And the potassium current, there is an interesting property, as long as the membrane potential is clamped at this particular voltage, it is activated. It keeps happening. And it does not inactivate, it is sustained. But the sodium channel is very different, it activates and deactivates fast and the interplay between these two processes causes the action potential.

(Refer Slide Time: 7:02)

Channel Activation and Inactivation

- 1) Hodgkin and Huxley proposed that K^+ channels possess a voltage-sensitive “gate” that opens by depolarization and closes by the subsequent repolarization of the membrane potential. This process of “turning on” and “turning off” the K^+ current is known as activation and deactivation.
- 2) Na^+ current also exhibits voltage-dependent activation and deactivation, but the Na^+ channels also become inactive despite maintained depolarization.



The diagram shows a cross-section of a cell membrane with an Na^+ channel. The top part shows the channel in a closed state with a 'gate' that can open or close. The bottom part shows the channel in an open state, allowing Na^+ ions to flow through. Labels include 'Extracellular', 'Intracellular', 'Ion channel', and 'Na+ Channel'.

Maresh Jayachandran MBBS, MD, PhD Action Potential -2 03 Sept, 2020

So, at the time when they found this out, this physiological phenomenon, we were not sure, they were not sure, nobody was sure of what is the cellular mechanisms? What happens and the beauty of electrophysiology is you do not need to know to figure out what is happening, but if you want to go to the molecular level, you have to have some ideas. So, they speculate some kind of enzyme related activity, and that fit in.

Subsequently, with molecular biology and the tools of molecular biology and structural chemistry, protein structure, we have now found that the sodium channel, there is a channel and it is sensitive to voltage and it inactivates and activates. And it is on the membrane. So, these channels have a voltage-sensitive gate that opens with depolarization and closes with subsequent repolarization of the membrane potential. So, this process of turning on and turning off the potassium current is known as activation and deactivation.

Simple enough. But the sodium channel is slightly different. It also exhibits voltage-dependent activation and deactivation, but the sodium channels become inactive also despite maintained depolarization, unlike the potassium channels. And on the right, you see the molecular structure, the postulated molecular structure of the sodium channels. It has 4 units and there is a place where TTX acts, Tetrodotoxin, and blocks it.

And there is another blocker called saxitoxin similar to Tetrodotoxin blocks and below is the actual conformational, the biochemical. This would be the secondary, tertiary, and quaternary structures of the sodium channel.

(Refer Slide Time: 8:57)

Channel Activation and Inactivation

Thus, the Na^+ current not only activates and deactivates, but it also exhibits a separate process known as inactivation, whereby the channels become blocked even though they are activated.

Removal of this inactivation is achieved by removal of depolarization and is a process known as deinactivation. Thus, Na^+ channels possess two voltage-sensitive processes:

- Activation-deactivation, and
- Inactivation-deinactivation.

Na⁺ Channel

Maresh Jayachandran MBBS, MD, PhD Action Potential-2 03 Sept, 2020

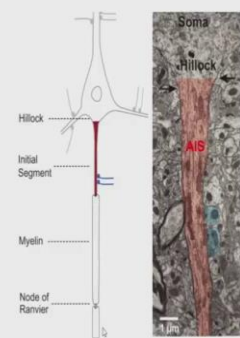
So, channel activation and activation so the sodium channel not only activates and deactivates as mentioned earlier but also exhibits a separate process called inactivation, whereby the channels become blocked even though they are activated. So, removal of this inactivation is achieved by the removal of depolarization and is a process known as deinactivation. So, just to make it a little more complex, the sodium channels possess two voltage-sensitive processes; one is activation-deactivation and the other one is inactivation-deinactivation.

(Refer Slide Time: 9:31)

Where is the Action Potential initiated?

In most cells, each action potential is initiated in the initial portion of the axon, known as the axon initial segment (right).

The initial segment of the axon has the lowest threshold for action potential generation because it typically contains a moderately high density of Na^+ channels and it is a small compartment that is easily depolarized by the in-rush of Na^+ ions.



The diagram on the left illustrates the structure of a neuron, labeling the Soma, Hillock, Initial Segment, Myelin, and Node of Ranvier. The electron micrograph on the right shows a cross-section of the axon with labels for the Soma, Hillock, and AIS (Axon Initial Segment). A scale bar of 1 μm is present in the micrograph.

Maresh Jayachandra MBBS, MD, PhD Action Potential -2 03 Sept, 2020

So, let us step back. Where is the action potential initiated generally? So, if you remember the microscopic lecture on the microscopic anatomy of the central nervous system, you have a cell. This is a pyramidal cell and you have all the dendrites which are not shown over here and then you have the axon going down covering myelin and stuff like that.

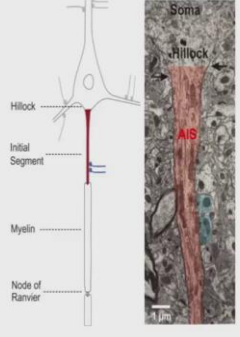
Between the cell body, the soma, and the axon, there is a segment called IS, the initial segment. And that has a very high density of sodium channels compared to the rest of the soma and it is a very small compartment and it depolarises easily. So generally, action potentials are initiated in the initial segment and this is an electron microscopic image of the initial segment which is a kind of light red and hillock and the soma. The action hillock is where the initial segment begins and the initial segment ends where the axon with the myelination starts.

(Refer Slide Time: 10:39)

Action Potential initiation (2)

Once a spike is initiated (e.g., about 30–50 μm down the axon from the cell body in cortical pyramidal cells), this action potential then propagates:

- Forwards (orthodromically) down the axon to the synaptic terminals, where it causes release of transmitter, and
- Backwards (antidromically) back through the cell body and into the cell dendrites, where it can modulate intracellular processes.



Mahesh Jayachandran MBBS, MD, PhD Action Potential -2 03 Sept, 2020

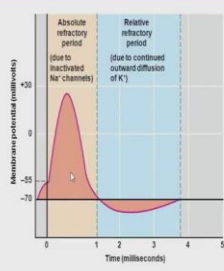
So, once a spike is initiated about 30 to 50 μm down the axon from the cell body in cortical pyramidal cells, this then propagates, so it moves. Now, it can move forward down the axon to the synaptic terminals where it causes the release of neurotransmitters or it can move backward antidromic. So, the first one is orthodromic where it moves forward. The second process where it moves backward is antidromic and it goes and into the cell body, into the cell dendrites and there it can modulate intercellular processes.

(Refer Slide Time: 11:15)

Refractory Periods

Absolute Refractory Period:

Immediately after the generation of an action potential, another action potential cannot be generated regardless of the amount of current injected into the axon - Na^+ inactivation.

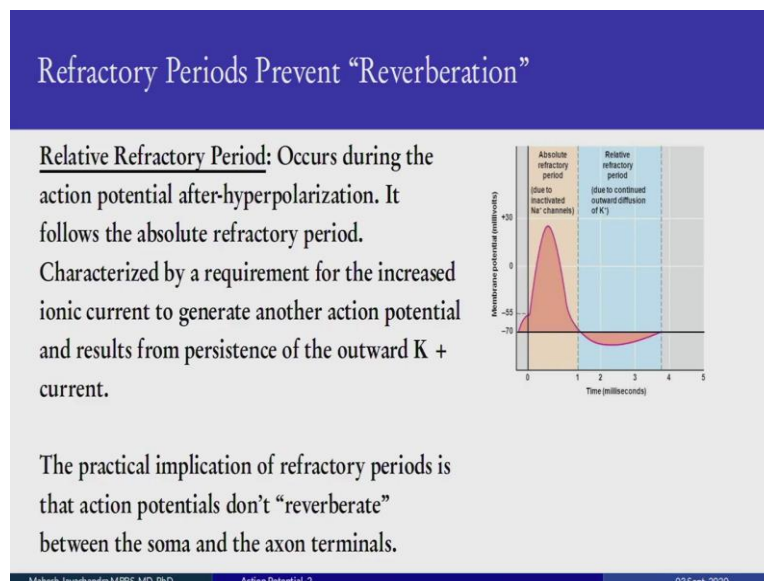


Mahesh Jayachandran MBBS, MD, PhD Action Potential -2 03 Sept, 2020

So, the refractory period. So, immediately after an action potential, for 1 millisecond, during that period, the cell is refractory. It will not be able to fire another action potential. This is because of the inactivated sodium channels. They have to recover. And regardless of the

amount of current you gave, it will not be able to fire. So, this is the absolute refractory period of an axon.

(Refer Slide Time: 11:45)



The relative refractory period follows the absolute refractory period and here it is due to the there is after-hyperpolarization, the potassium channels act as a delayed rectifier. So, there is a continued outward diffusion of potassium. So, here is relatively refractory in the sense that if you use the same stimulus threshold to get the AP, that will not fire, but if you increase the stimulus current and you can force it to fire.

So, the relative absolute refractory period is about 1 millisecond, the relative refractory period is about 2, 3, 4 milliseconds. It depends on the cell, but one is that this prevents the electrical activity from reverberating in an ensemble, in a network of neurons. Because otherwise, you could have reverberation occurring and if you have reverberation, then you have epilepsy.

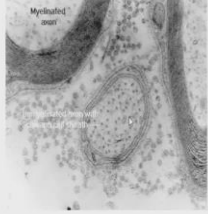
So, normally these processes prevent uncontrolled positive feedback reverberation from happening in the network. The other interesting thing is if you look at the absolute refractory period where it cannot be fired, that gives us an upper estimate of what is the maximum firing rate of a neuron. It has to be limited by the absolute refractory period. So, approximately over here. If it is 1 millisecond, and it would be the firing rate, the absolute firing rate of a neuron anywhere from 500 to 1000 hertz and that is it. It cannot be higher than that.

(Refer Slide Time: 13:24)

The Speed of Action Potential Propagation Is Affected by Myelination

Axons may be either myelinated or unmyelinated. Invertebrate axons or small vertebrate axons are typically unmyelinated, whereas larger vertebrate axons are often myelinated.

Sensory and motor axons of the peripheral nervous system are myelinated by specialized cells (Schwann cells) that form a spiral wrapping of multiple layers of myelin around the axon.



A black and white micrograph showing a cross-section of a myelinated axon. The central part is a dark, circular axon, surrounded by a lighter, multi-layered ring of myelin. Labels point to the 'Myelinated axon' and the 'Myelin sheath'.

Maheesh Jayachandran MBBS, MD, PhD Action Potential-2 03 Sept, 2020

This speed of action potential propagation is affected by myelination. So, axons can either be myelinated where they have a covering of myelin which is insulation, we will study this in detail. Or it can be unmyelinated where it just has a cell membrane but there is no myelin. So, sensory and motor neurons of the peripheral nervous system, they are myelinated by a special glial cell called a Schwann cell.


So, this forms a spiral wrapping of multiple layers of its cell wall around the axon. And typically, small vertebrate axons, like for example, Sea fibers that absorb pain, and invertebrate axons are not myelinated. Whereas, large vertebrate axons are often myelinated. We will get into the details of the advantages of myelination in just a little bit.

(Refer Slide Time: 14:21)

The Speed of Action Potential Propagation Is Affected by Myelination (2)

Several Schwann cells wrap around an axon along its length; between the ends of successive Schwann cells are small gaps (nodes of Ranvier).

In the central nervous system, a single oligodendrocyte, a type of glial cell, typically ensheaths several axonal processes.



A diagram illustrating the process of myelination. It shows three stages: 1. A Schwann cell with a nucleus approaching an axon. 2. The Schwann cell beginning to wrap around the axon. 3. The Schwann cell fully wrapped around the axon, forming a myelin sheath. Labels include 'Nucleus', 'Axon', 'Myelin', and 'Node of Ranvier'.

Maheesh Jayachandran MBBS, MD, PhD Action Potential-2 03 Sept, 2020

So, in the peripheral nervous system, which is the part of the nervous system outside the brain and the spinal cord, several Schwann cells wrap around the axon along its length and leave small gaps in between the curl nodes, nodes of Ranvier. So, here on the right, you have a Schwann cell and it is wrapping its membrane around the axon, keeps wrapping it so you have multiple layers.

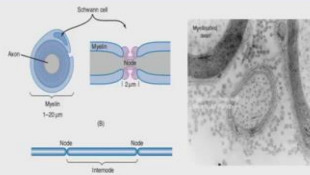
And in between two different such wrappings, you have a node where the axon is exposed and it is called the node of Ranvier. So, these are all names of anatomist Ranvier Schwann so on and so forth. In the central nervous system instead of the Schwann cell, you have oligodendrocyte, which is a kind of glial cell and each oligodendrocyte typically ensheaths multiple axons, Schwann cell on the one axon and the periphery oligodendrocyte multiple axons. But otherwise, it is the same purpose and function insulates the axon.

(Refer Slide Time: 15:21)

Myelination (contd.)

Myelination of the axon reduces its membrane capacitance by moving the electrical charge differences between the inside and outside of the axon further apart and thus reducing their influence on each other.

This significantly increases the passive length constant (λ) of the axon.



Above, left: Myelination schematic. Right: EM images of myelinated and unmyelinated nerves.

Maresh Jayachandran MBBS, MD, PhD Action Potential-2 03 Sept, 2020

So, we will study this a little more detail in neuronal biophysics, but for now, this myelination of axon reduces its membrane capacitance by moving the electrical charge differences between inside and outside further apart, therefore, reducing their influence on each other. And this significantly increases the passive length constant of the axon. We will, the length constant briefly is the distance where the electrical potential decreases to one to the eth of its initial value that is, 37 percent of its initial value. We will get into details more but this increases the length constant.

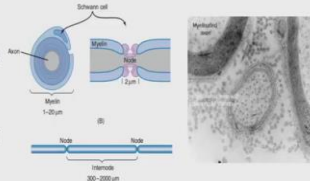
And here you have the details. A cross-section of an axon with its myelin cell, the Schwann cell is with its myelination layers. The Schwann cell which produces myelination is outside and in between myelin layers, you have this node. It is about 2 μ , 2 microns. It is a node of

Ranvier. And typically, the distance between I mean differs in different axons but the internodal distance is anywhere from 300 to 2000 micron which is 0.3 to 2 millimeters.

(Refer Slide Time: 16:42)

Myelination (contd.)

Na^+ channels are concentrated at the nodes of Ranvier. The generation of an action potential at each node results in depolarization of several adjacent nodes and subsequently generation of an action potential with an internode delay of only about $20 \mu\text{s}$, referred to as saltatory conduction.



Above, left: Myelination schematic. Right: EM images of myelinated and unmyelinated nerves.

Demyelination of axons causes conduction failure of action potentials, e.g., Multiple Sclerosis and Guillain-Barre syndrome.

Maresh Jayachandran MBBS, MD, PhD Action Potential-2 03 Sept, 2020

So, the sodium channels which allow the upstroke of the action potential, they are concentrated at the nodes. So, the generation of an action potential at each node causes depolarization of several adjacent nodes and subsequent generation of action potential with an internode delay of only 20 microseconds. This is referred to as saltatory conduction.

Demyelination of axons causes conduction failure of action potentials. For example, multiple Sclerosis where your latency is increased and finally it stops conducting or it could be that it could be that genetic and it could be post-viral infection cause the Landry Guillian-Barre syndrome where you have demyelination occurring and loss of nerve function, conduction failure.

(Refer Slide Time: 17:38)

Some characteristics of CNS neurons

1. Neurons of the CNS Exhibit a Wide Variety of Electrophysiological Properties
2. Neurons Have Multiple Active Conductances
3. Na^+ Currents are Both Transient and Persistent
4. K^+ Currents Vary in Their Voltage Sensitivity and Kinetics

Mahesh Jayachandra MBBS, MD, PhD Action Potential -2 03 Sept, 2020

So, some characteristics of central nervous system neurons. So, we talk mainly about the squid but this what happens in the squid as far as the action potential resting membrane, all these are pretty much the same if not very similar in all the neurons vertebrate, invertebrate, mammalian human study so far. But the details vary. So, in the human central nervous system neurons exhibit a wide variety of electrophysiological properties.

They have multiple conductances, it is not just sodium and potassium, they may also have calcium. And you have different types of sodium, different types of potassium conductance, different types of calciums, and conductances. Now, in the squid we saw sodium currents are transient but they can be transient, they can also be persistent, and likewise, potassium currents they vary a lot in the voltage sensitivity and kinetics in different cells in this human central nervous system.

(Refer Slide Time: 18:41)

Some characteristics of CNS neurons

5. Neurons Possess Multiple Subtypes of High-Threshold Ca^{2+} Currents.
6. Low-Threshold Ca^{2+} Currents Generate Bursts of Action Potentials
7. Hyperpolarization-Activated Ionic Currents Are Involved in Rhythmic Activity

Maresh Jayachandra MBBS, MD, PhD Action Potential-2 03 Sept, 2020

So, neurons talking of potassium, they have multiple subtypes of high threshold potassium currents. And there are low threshold potassium currents and they generate bursts of action potentials and after this when you have hyperpolarization, that also the process of high polarization activates ionic currents which are involved in rhythmic activity, central pattern generators in the brain stem which control heart rate, control respiration, rhythmic activity.

(Refer Slide Time: 19:15)

Thank you!

In the next session we shall consider aspects of Axonology and Neuronal Biophysics.

Maresh Jayachandra MBBS, MD, PhD Action Potential-2 03 Sept, 2020

So, thank you very much. In the next session, we shall consider aspects of Axonology and Neuronal Biophysics.