Review of Electrophysiology:

Fundamental principles in electrical signaling and synaptic transmission

Slide 1

1) My name is Josh Lawrence, I am an Associate Professor in the Department of Pharmacology and Neuroscience. My expertise is in cellular and synaptic physiology. It is my pleasure to give you this review.
2) Why now? First, there were reports of missed questions on USMLE Step 1 about this material. Second, it's really part of Integrated Neuroscience. MS1 is not enough of a background for you. Third, this material provides an important conceptual foundation that will enable you to gain insight into disease processes and therapeutic mechanisms.
3) This review is based on chapters 2,3,5, and 8 of the Purves Neuroscience textbook (6th edition), which is required for Integrated Neuroscience. Most of the slides I am going to use come directly from the textbook. I don't think I can do justice to the chapter material but this lecture will perhaps motivate you to want to dedicate time to reading these chapters.
4) I'll be using Turning Point to probe your understanding of the material, so get your clickers out!

Slide 2

1) This slide will give you a sense for how this review's content aligns with the textbook material.
2) For each of the 4 chapters I'll touch on, I have structured the content into two learning objectives.

Slide 3

1) First, we'll cover the Chapter 2 objectives. The first objective is to understand how ion movements produce electrical signals.
2) For this objective, an important prerequisite is to know the Na+/K+ ATPase, which is critical for understanding how electrochemical gradients are established.
3) The second objective is to understand the ionic basis of action potentials.

Slide 4

1) This slide gives you an overview of the passive and electrical properties of neurons.
2) The configuration is in A here – you can inject current, in amps, with one electrode and record membrane potential, in volts.
3) For this, you need to know Ohm's lab. V = IR, where V= voltage, I = current, and R = resistance.
4) Here is an experiment to help you understand how passive and active properties are examined in a living neuron.

5) The first thing you notice is that when you insert a recording electrode, the membrane potential moves from 0 to -65 mV. This is the resting potential of a neuron.
6) When the voltage moves more positive to the resting potential, this is called depolarization; when the voltage moves more negative, this is called hyperpolarization.
7) Upon injection of a negative current, the neuron hyperpolarizes. A larger negative current gives a larger hyperpolarization. A small injection of positive current will give a subthreshold depolarization. These are considered passive responses, and are governed by Ohm's law, in which injection of any current, whether it's negative or positive, gives a predictable voltage that is determined by the specific resistance of the neuron.
8) At higher currents, the voltage reaches threshold. Here, everything changes. Rather than see a passive response, the current injection elicits an active response in the form of an action potential.
9) Larger currents cause more action potentials. This is often referred to as a "rate code".
10) This is the overview. Now we'll get into the ionic basis of the resting membrane potential.

Slide 5

1) To understand the basis of the resting membrane potential, we start with just one ion, the potassium ion, and a membrane that is permeable to potassium, as shown here in A. The voltmeter reads zero.
2) However, when you start with potassium being 10-fold higher on the inside than outside, the voltage changes. K ions flow down their concentration gradient, leaving behind impermeant anions. There is an accumulation of negative charge on the inside of the cell, which generates an electrical force that opposes the force that causes K to flow down its chemical gradient.
3) There is a voltage in which the electrical voltage exactly counterbalances the chemical gradient – this is called the electrochemical equilibrium or Nernst potential. It is approximately 58 volts for every 10-fold difference in concentration between outside and inside.
4) The more generalized Nernst equation is shown here, which takes into account the valence of the ion and the temperature.

Slide 6

1) From the Nernst equation, you can now understand the ionic basis of the action potential.
2) This table lists the intracellular and extracellular concentrations of important ions. For K, Ek = -84. For Na, ENa = +67. Remember the Na+/K+ pump is responsible for establishing K and Na gradients!
3) The action potential can be described as swinging form EK to ENa and back again.

4) At the resting membrane potential, permeability to K dominates, as so Vm is near EK.
5) During an action potential, there is a transient increase in Na permeability, which causes Vm to move towards ENa.
6) During the repolarization of the action potential, K permeability again dominates, and Vm is near EK again.
7) The relationship between K and Na ions is described by the Goldman equation. This equation also includes the relative permeability of each ion, termed PK and PNa.
8) You can see how domination of either P_K or P_{Na} can reduce the Goldman equation down to the Nernst equation for E_K or E_{Na}.

Slide 7

(Turning Point Question)

Slide 8

(Turning Point Question)

Slide 9

1) Now we're on to Chapter 3. The first objective is to understand how action potentials are generated through voltage-dependent membrane permeability.
2) The second objective is to understand how propagation of action potentials works.

Slide 10

1) To understand voltage-dependent membrane permeability, we come back to Ohm's Law.
2) I previously introduced Ohm's Law in the context of passive properties of neurons. You introduce a fixed current, whether it's positive or negative, and you get a fixed voltage response because of a constant resistance.
3) However, we now introduce the idea that the active properties of a neuron are non-Ohmic. The resistance is partly dependent on the membrane potential.
4) Also, we know that membrane permeability for sodium and potassium are occur depend on the voltage.
5) To incorporate this idea, we introduce the concept of conductance. Ohm's law can be re-written as I = V/R. Conductance, or g, is the inverse of resistance – you can view g as how leaky, or permeable, the cell is for a particular ion. So we end up with $I_{ion} = g_{ion}V_{ion}$.
6) Iion is the ionic current. Gion is the membrane conductance. Then you have the membrane potential Vm and the equilibrium potential, or Nernst potential, for a particular ion.

7) Vm-Eion is referred to as the electrochemical driving force. This term has to be not negative to generate a current, if the conductance is not zero.
8) $I_{ion} = g_{ion}(V_m-E_{ion})$ is a generic equation for any ion, but it can be customized for potassium and sodium conductances, because they have different permeabilities and different equilibrium potentials. So you have $I_K = g_K(V_m – E_K)$ and $I_{Na} = g_{Na}(V_m-E_{Na})$.
9) To give you a better sense for how the driving force is important, let's do some calculations. At the resting potential Vm, the membrane is mostly permeable to potassium and is therefore near EK. However, if you calculate the driving force on sodium, it's large: -151 mV. So if gNa suddenly changes, then it will generate a large inward (negative) current.
10) At the peak of the action potential, when the membrane potential is near ENa, there is less current flow. However, there is now a large driving force on potassium – V_m-E_K = +151 mV. So when gK is active, it will cause a large outward current that will bring the membrane potential back down towards EK.

Slide 11

1) These terms – I,g, and V, change dynamically during an action potential.
2) At the resting potential, gK exceeds gNa; therefore Vm is near EK (Goldman equation). The driving force (Vm-ENa) is large.
3) During the rising phase of the action potential, gNa suddenly exceeds gK. Becaue (Vm-ENa) is large, INa is large. As a result, Vm approaches ENa.
4) During the falling phase of the action potential, gNa drops to near zero because of a molecular mechanism called inactivation – a gate in the Na channel itself causes the Na channel to become nonconducting. Meanwhile gK becomes large and (Vm-EK) is large, causing a large IK which forces Vm back towards EK.
5) During the undershoot, it takes a while for gK to turn off, so Vm hovers near EK before eventually the K+ channels close and equilibrate around Vm.
6) The key to the action potential is that really the intrinsic properties of the Na and K channels themselves. The inactivation of sodium channels is an important mechanism that makes the sodium channel current transient – this is shown here in one of Dr. Blanton's figures. Likewise, the delayed, non-inactivating response of K channels to depolarization is also important in providing the repolarizing force during an action potential.

Slide 12

(Turning Point Question)

Slide 13

1) Now that you have had action potentials, it's important that you know how they propagate down an axon.
2) This is shown in this schematic here. First, upon injection of a current at Point A, an action potential is generated locally.

3) Some of the depolarizing current passively flows down the axon to Point B.
4) Local depolarization at Point B generates an action potential. Meanwhile, at Point A, Na channels inactivate and potassium channels open.
5) Some of the depolarizing current generated in Point B passively flows down the axon to Point C, causing generation of an action potential at Point C.
6) Note that backward propagation of the action potential is prevented by sodium channel inactivation, which causes a refractoriness immediately after the action potential is generated. This mechanism allows action potentials to propagate in one direction down an axon.

Slide 14

1) Now we'll cover action potential propagation down a myelinated axon.
2) In a myelinated axon, oligodendrocytes form myelin sheaths. Located periodically along the axon are nodes of Ranvier, which is where sodium channels are concentrated.
3) When an action potential is initiated in the axon, it occurs in the Nodes of Ranvier. The passive current then passes into the myelinated region of the axon. Because the axon has very few if any channels in this region, the resistance in this part of the axon is very high, which prevents loss of current and speeds propagation.
4) The passive current then arrives at Point B, and an action potential is initiated in the Node of Ranvier.
5) The right panel compares the conduction velocity of unmyelinated to myelinated axons.

Slide 15

(Turning Point Question)

Slide 16

1) So we are on to Chapter 5 now, Synaptic Transmission.
2) The first learning objective is to understand fundamental mechanisms in synaptic transmission.
3) The second learning objective is to understand postsynaptic membrane permeability changes during synaptic transmission.

Slide 17

1) There are two fundamental mechanisms of synaptic transmission.
2) The first is electrical synapses. These synapses contain gap junctions within the synapse. The gap junctions are formed from connexon channels.
3) The other type is chemical synapses. At chemical synapses, the action potential goes down the axon, arrives at the presynaptic terminal, causes neurotransmission through the fusion of synaptic vesicles containing

neurotransmitter, and then is converted back to an electrical signal once it crosses the synapse through the action of neurotransmitter receptors.

Slide 18

There is a sequence of steps for chemical transmission.

1) Neurotransmitter is synthesized and stored in synaptic vesicles.
2) An action potential arrives at the presynaptic terminal.
3) The action potential induces a depolarization which causes the opening of voltage-gated calcium channels.
4) There is influx of calcium through the calcium channels.
5) Calcium binds to special calcium sensors on the synaptic vesicles called synaptotagmins, which then causes the association of proteins on both the pre- and postsynaptic membrane. This draws the synaptic vesicle towards the presynaptic membrane and eventually causes fusion, or exocytosis.
6) Neurotransmitter is then released into the synaptic cleft.
7) Neurotransmitter binds to neurotransmitter receptors on the postsynaptic membrane.
8) The neurotransmitter receptor then activates, causes a change in ionic conductance.
9) There is then a change in cellular excitability.
10) The neurotransmitter is then removed from the synaptic cleft through uptake mechanisms or degradation.
11) Finally, there is endocytosis, or retrieval of the synaptic vesicle from the plasma membrane and refilling of the synaptic vesicle with neurotransmitter.

Slide 19

1) What is happening postsynaptically when neurotransmitter bings neurotransmitter receptors?
2) This is best explained by revisiting the pioneering work of Bernard Katz at the frog neuromuscular junction.
3) Katz stimulated a presynaptic motor nerve that innervated a muscle while recording from the postsynaptic membrane potential in a muscle cell.
4) He observed an end plate potential (EPP) that drove the postsynaptic action potential in induced a twitch in the muscle fiber (shown in red).
5) Katz also observed small, spontaneous potentials that he called miniature end-plate potentials or MEPPs.
6) When he lowered calcium, the amplitude of the EPP was reduced and a subset of the EPPs had the same amplitude as the MEPP, which is shown in amplitude histograms on the right.
7) He called the MEPP a quantal response, and proposed that the EPP was composed of multiple MEPPs. Before even the discovery of synaptic vesicles, Katz proposed that neurotransmitter was released in packets, or quanta.

Slide 20

1) How do the postsynaptic receptors generate electrical signals? At the neuromuscular junction, the receptors are nicotinic acetylcholine receptors (nAChRs). Here is a picture of the flow of ions through a single nAChR.
2) The nAChRs are permeable to both sodium and potassium, so the reversal potential for these channels is between ENa and EK.
3) Current flow through nAChRs follows Ohm's law. $I_{ACh} = g_{ACh}(V_m - E_{ACh})$.
4) So if you start with a single channel and add more, you eventually build to an end plate current or EPC.
5) At the resting potential, opening of the nAChRs causes a depolarization, which moves the resting membrane potential towards E_{ACh}, causing the EPP.

Slide 21

1) Now we move these principles of synaptic transmission from the NMJ to central synapses. There are more neurotransmitters, but the principles remain the same.
2) In the CNS, there are gluamatergic synapses – they release then neurotransmitter glutamate and bind two different types of glutamate receptors. AMPA receptors are cationic channels like nAChRs have an equilibrium potential near zero.
3) NMDA receptors are also cationic channels, but they are slower to activate and slower to decay, as visualized by superimposing the AMPA and NMDA receptor mediated excitatory postsynaptic currents.
4) OK suppose there are two glutamate synapses, E1 and E2. Both have AMPA and NMDA receptors.
5) If either E1 or E2 are stimulated, this typically results in a subthreshold excitatory postsynaptic potential (EPSP). Release of glutamate causes the activation of AMPA and NMDA receptors, which open to allow influx of cations, causing the resting membrane potential to move towards EGlu.
6) If multiple EPSPs are activated in a short time window, they will summate. In this example, E1 and E2 are activated together, which cause a larger depolarization and allow the voltage to reach threshold. An action potential is triggered when the voltage reaches threshold.
7) In addition to multiple glutamatergic synapses, there are also inhibitory synapses that release the neurotransmitter GABA. We will call that synapse I.
8) GABAA receptors are GABA gated chloride channels. ECl can vary from cell to cell and during development, but typically EGABA has a hyperpolarized reversal potential – let's say -80 mV.
9) Since EGABA is more negative than the resting membrane potential, activation of GABAA receptors will result in a hyperpolarizing inhibitory postsynaptic potential, or IPSP.
10) The summation of E1 and I will essentially reduce the effect of E1, and not allow E1 to depolarize the cell as much. In other words, the amplitude of the EPSP will be reduced by the simultaneous activation of the IPSP.

11) In the case of the activation of E1 and E2 summing to cause a suprathreshold EPSP, in the presence of the IPSP, the EPSP falls subthreshold. This shows how powerful the activation of an IPSP can be on influencing excitatory synaptic transmission.
12) The summation of numerous EPSPs with IPSPs enables neurons to make computations. The bombardment of EPSPs and IPSPs and their summation determines when and if an action potential occurs.

Slide 22

(Turning Point Question)

Slide 23

(Turning Point Question)

Slide 24

1) Finally, I'll cover Chapter 8 Synaptic Plasticity.
2) The first learning objective is to describe forms of short-term synaptic plasticity.
3) The second learning objective is to describe long-term synaptic plasticity.

Slide 25

1) There are many forms of short-term synaptic plasticity. Repetitive activation of a synapse causes the second EPSP to be larger than the first. This phenomenon is called synaptic facilitation. Note that this phenomena should not be mistaken for summation – the second EPSP is larger even though the first EPSP decays fully back to baseline.
2) Augmentation and postsynaptic potentiation are similar but occur at different time scales to facilitation.
3) Synaptic depression is an activity-dependent weakening of synaptic strength.
4) The mechanism of action of facilitation/augmentation/post-tetanic potentiation is a that there is a memory of a previous event – this is thought to involve calcium or calcium binding proteins that alter the nature of the presynaptic terminal.
5) The mechanism of depression is likely to involve depletion of vesicles.
6) These mechanisms are not mutually exclusive and can occur at the same synapses, with each mechanism happening at a different time scale.
7) This type of short-term plasticity enables synapses to perform complex activity-dependent computations which essentially act as a filter during transmission.

Slide 26

(Turning Point Question)

Slide 27

1) Finally, we're on to long-term synaptic plasticity.
2) This phenomenon involves a long-term increase in synaptic transmission.
3) The recording configurations involves recording from a CA1 pyramidal cell in the hippocampus.
4) Two stimulus electrodes, Stimulus 1 and Stimulus 2, stimulate the Schaffer collaterals, which are the axons of the CA3 pyramidal cells. This way, EPSPs that are associated with different glutamate synapses can be generated onto the same CA1 pyramidal cell.
5) The EPSP amplitude is monitored at low stimulation. At time zero, a high frequency train of stimuli are applied to Pathway 1. There is a post-tetanic potentiation but it is maintained for up to an hour after tetanus was applied.
6) Meanwhile, EPSPs that are associated with Stimulus 2, which did not receive the tetanus, were unaltered.
7) This phenomenon is referred to as long-term potentiation, or LTP. It is a persistent increase in the strength, or efficacy of excitatory synaptic transmission. When monitored in vivo, it can last for up to 6 months!

Slide 28

1) The mechanism of LTP is shown on the next slide.
2) The two glutamate receptors, the AMPA and NMDA receptors, are involved.
3) LTP has an initial induction and expression phases. At the resting potential, glutamate transmission proceeds mostly through AMPA receptors because at the resting membrane potential, NMDA receptors are blocked by magnesium ions that occlude to the pore of the NMDA receptor channel.
4) During induction of LTP, there is a sufficient depolarization to expel the Mg^{2+} from the NMDA receptor, allowing calcium to flow into the postsynaptic neuron. The calcium influx triggers the activation of calmodulin and calmodulin-dependent protein kinase (CaMKII).
5) During the expression phase, activation of CaMKII results in increased function of existing AMPA receptors and insertion of AMPA receptors in the postsynaptic neuron, which contributes to the increased amplitude of the EPSP observed after LTP induction.
6) The late phase involves the activation of protein kinase A, which activates the transcription factor CREB signaling (cAMP response element binding protein). CREB induces the transcription of proteins that stabilize and enhance dendritic spine growth.

Slide 29

1) In addition to a mechanism by of long-term plasticity that leads to an long-term enhancement of synaptic transmission, there is a mechanism that leads to long-term weakening of synaptic transmission. This is called **long-term depression.**
2) This phenomenon is demonstrated the same recording and stimulating configuration as for LTP.

3) Upon monitoring the EPSP amplitude, one can apply a lower frequency stimulus (1 Hz) to the Schaffer collateral pathway. There is an initial augmentation that declines over the course of the 1 Hz stimulus. Upon resumption of the EPSP, the EPSP amplitude is observed to be reduced, and this reduced amplitude persists for the duration of the recording.
4) The mechanism is similar to LTP in the sense that NMDA receptors are involved. The influx of calcium through the NMDA receptor instead activates protein phosphatases, which lead the internalization of AMPA receptors in the postsynaptic membrane.

Slide 30

(Turning Point Question)