NBL 355-610 Module 8 Review Q&A

1. *Where are glutamatergic cell bodies found in the brain, where do they project and what are glutamatergic neurons involved in? What are some important types of glutamatergic neurons?*

Glutamatergic cell bodies are found in every region in the CNS, including the cerebral cortex, subcortical structures including the basal ganglia, amygdala, thalamus, hypothalamus, retina, brainstem, cerebellum and spinal cord. Hence, glutamatergic neurons are located in the CNS. Glutamatergic neurons are also located in the PNS. Somatosensory neurons in sensory ganglia (dorsal root ganglia or cranial ganglia) are glutamatergic. Also several special sense neurons (photoreceptor cells, hair cells, and olfactory neurons) are also glutamatergic. It has been suggested that some enteric neurons in the GI tract may be glutamatergic. Approximately 60% of all neurons in the CNS are glutamatergic. Glutamatergic neurons project to other brain regions in the CNS or work locally. They are mainly involved in fast, excitatory synaptic transmission. Approximately 70% of all cerebral cortical neurons are glutamatergic, and the other 30% are GABAergic. In the cerebral cortex, glutamatergic neurons include cortical pyramidal neurons, which are projection/principal neurons, and cortical excitatory stellate neurons, which are local circuit neurons. (Note that although the cerebral cortex contains the cell bodies of only glutamatergic or GABAergic neurons, those neurons receive synaptic inputs from all types of neurons, including other glutamatergic and GABAergic neurons, and cholinergic, dopaminergic, noradrenergic, and serotonergic neurons.)

1. *What are two mechanisms by which glutamate is produced? How is glutamate pumped into synaptic vesicles? How and by what cells is glutamate removed from the synaptic cleft?*

L-glutamate is an amino acid used in both protein synthesis and as the major excitatory neurotransmitter in the CNS. Glutamate is obtained from the diet, and can also be synthesized in the Kreb’s cycle in mitochondria, and produced from glutamine in the cytosol. Glutamate is pumped/transported into synaptic vesicles by the vesicular glutamate transporter, which is a secondary active transporter that uses the proton gradient established by the proton ATPase, to transport glutamate inside synaptic vesicles. To remove glutamate from the synaptic cleft, glutamate is taken back into the presynaptic terminus, and into nearby astrocytes by plasma membrane glutamate transporters called EAATs (excitatory amino acid transporters). Note that the vesicular and plasma membrane glutamate transporters are different proteins encoded by different genes. Astrocytes can convert glutamate to glutamine, transport the glutamine into the extracellular fluid whereby it is taken up by the presynaptic neuron and gets converted back to glutamate by the enzyme glutaminase.

1. *The two main types of ionotropic glutamate receptors are AMPA/kainate receptors and NMDA receptors? How is each type activated? What is each type selective for (ions)? How are the two types of receptors similar and different? Why is the PSD important?*

Ionotropic glutamate receptors are composed of four subunits, where each subunit has four membrane spanning domains. AMPA/kainate receptors require only glutamate binding to activate and open the channel region. In the absence of NT, the channel region is closed. When glutamate binds to the extracellular domain, it induces a conformation change that opens the channel. AMPA/kainate receptors are nonselective cation channels. They allow both Na+ and K+ to move down their electrochemical gradient. (A few minor types of AMPA receptors are also permeable to Ca2+, but the majority are not.)

In contrast, the NMDA receptors require glutamate binding and membrane depolarization, as well as a co-agonist (glycine or D-serine) to be activated. At the resting membrane potential, a Mg2+ ion sits in the pore region and blocks the NMDA receptor channel. When the membrane is depolarized, this expels the Mg2+ ion and removes the Mg2+ block, so when glutamate and the co-agonist bind, the NMDA receptor channel will open and allow ion to flow through. NMDA receptors are also nonselective cation channels. In addition to being permeable to both Na+ and K+, the NMDA receptors are also permeable to Ca2+. This is a critically important feature of the NMDA receptor and underlies its role in synaptic plasticity.

Similar: both types are ionotropic glutamate receptors that mediate fast EPSPs. They are both composed of 4 subunits that each have 4 membrane spanning domains. Both types allow Na+ and K+ to flow through the pore, down their electrochemical gradients. Both types bind to scaffolding proteins in the postsynaptic density (PSD) that tether them to the PSD and hence synapse which affects their localization. (See below.) Both are synthesized and trafficked via the secretory pathway, and their levels at the plasma membrane are affected by endocytosis and exocytosis.

Different: NMDA receptors require membrane depolarization (to remove the Mg2+ block) and a co-agonist (either glycine or D-serine), together with glutamate binding, to be opened. NMDA receptors also allow Ca2+ to flow through the channel and are responsible for increasing intracellular Ca2+ levels during glutamate synaptic transmission.

The PSD is a region of the postsynaptic membrane where many receptors are localized. Scaffolding proteins bind to the intracellular domains and help to tether the receptors at the PSD. That ensures they are right across the synapse from the active zone where NT is released and therefore will be exposed to the highest concentration of NT following release. The PSD is important because the binding of receptors to those scaffolds prevents the lateral movement of the receptors outside of the synapse to the peri-synaptic and extra-synaptic regions. Also, the tethering of receptors affects their rate of endocytosis. The number and localization of receptors in the synaptic membrane region is one of the factors that determines the magnitude of the postsynaptic response to glutamate.

1. *In glutamate transmission at dendritic spines, which subtype of glutamate receptor is activated first, and which subtype of receptor does it then activate? What are the immediate responses in the postsynaptic neuron/spine? Glutamatergic synapses often involve the release of one or only a few synaptic vesicles. Why is that not an issue for neurons in the CNS?*

Most dendritic spines express both AMPA and NMDA glutamate receptors. At the resting membrane potential, immediately after glutamate binding, only the AMPA receptors will be opened. The reason for this is that at the RMP, the NMDA receptors are blocked by Mg2+. After the AMPARs open, Na+ flows through the channel, down its electrochemical gradient, into the spine (producing a Na+ current and an excitatory postsynaptic potential-EPSP) and this depolarizes the membrane potential. That depolarization removes the Mg2+ block from the NMDA receptors, and together with glutamate and co-agonist binding, will then open the NMDA receptors. Usually, AMPA receptors provide the initial EPSP, followed by NMDA receptors augmenting the EPSP and increasing the dendritic Ca2+ levels.

Glutamate synapses are fairly small, and usually contain only a few (1-5) docked synaptic vesicles. A single presynaptic AP may result in no response or only a small (<1mV) EPSP, and a single presynaptic AP would never produce a response large enough to lead to the generation of an AP in the postsynaptic neuron. However, since neurons receive many tens of thousands of synapses with trains of APs at each synapse, and all the responses are constantly summating with each other (integration), the neuron doesn’t have to rely on an individual synaptic response to produce enough depolarization to generate an action potential. Instead it relies on tens of thousands of inputs to summate to produce enough depolarization to produce an AP.

1. *Where are GABAergic and glycinergic cell bodies found? What do they do? Where are some important GABAergic neurons?*

GABA (Gamma Amino Buteric Acid) and glycine are the major inhibitory neurotransmitters and are involved in fast inhibitory synaptic transmission. Approximately 30% of all neurons in the CNS are inhibitory (GABAergic or glycinergic.) GABAergic neurons are found throughout the brain and spinal cord, and they often function as local circuit neurons. There are a few populations of GABAergic principal/projection neurons, including the Purkinje neurons in the cerebellum and medium spiny neurons in the striatum, that project to other regions. Glycinergic neurons are found in the caudal brain stem and spinal cord where the also work mainly locally. Interestingly, GABA and glycine function as co-transmitters in the spinal cord. GABA and glycine are co-released from inhibitory interneurons in the spinal cord.

1. *How are GABA and glycine produced and pumped into synaptic vesicles? How are GABA and glycine removed from the synaptic cleft?*

GABA is synthesized from glutamate by GAD (glutamate decarboxylase). Glycine is an amino acid that is obtained from the diet or synthesized from another amino acid, serine. GABA and glycine are pumped/transported into synaptic vesicles by the vesicular GABA/glycine transporter (VGAT). GABA and glycine are removed from the synaptic cleft by plasma membrane transporters called the GABA transporter (GAT) or IAAT (inhibitory amino acid transporter) located on both neurons and astrocytes.

1. *What are the ionotropic GABA and Glycine receptor channels selective for (which ion)? In a typical neuron, when activated, what effect would the GABA or Glycine receptors have on a neuron? What types of responses do GABA and Glycine (working via their ionotropic receptors) produce?*

Ionotropic GABA and glycine receptors are ionotropic receptors that are selective for Cl- (this is called the chloride ion, not chlorine; chlorine is poisonous gas). GABA and glycine produce inhibition since the influx of Cl- hyperpolarizes the membrane potential and moves the membrane potential further away from generating an action potential.

1. *Describe the ionotropic GABA and glycine receptors. What is the channel selective for? What responses do they produce when they are activated? How are GABA and glycine removed from the synaptic cleft?*

Ionotropic GABA and glycine receptors are composed of five subunits and each subunit has four transmembrane spanning domains. The channel is selective for Cl-. GABA and glycine produce inhibition, by producing an inhibitory post-synaptic potential (IPSP), which is usually a small (~ 0.5 - 1 mV hyperpolarization). GABA is transported back into the presynaptic neuron, or into nearby astrocytes by the plasma membrane GABA transporter (GAT) and glycine is transported into the presynaptic neuron or nearby astrocytes by the plasma membrane glycine transporter (GLYT).

1. *What is significant about the fact that the Nernst potential for Cl- is very close to the RMP? GABA and glycine can produce IPSPs. How could it be possible that GABA and glycine function in inhibition even when a measurable IPSP is not produced? What is shunting inhibition?*

Since the Nernst potential for Cl- is close to the RMP, this means that the driving force for Cl- at the RMP is small. At the RMP, if a GABA or glycine receptor is activated, it will produce only a small amount of Cl- current, a very small IPSP (<0.1 mV) and not much inhibition. However, if the membrane potential has become depolarized by nearby excitatory synapses and summating EPSPs, which depolarize the membrane potential, then that will then increase the driving force for Cl- (DF= Vm –Ex). If GABA or glycine synapses are activated when the nearby membrane is depolarized by EPSPs, this will produce much larger IPSPs, that will then bring the membrane potential back to the RMP. Therefore, inhibitory effects are much greater when the neuron is being excited at the same time.

Because the typical reversal/Nernst potential for Cl- is between -60-70 mV, which is close to the resting membrane potential, activation of GABAARs produces only a very small IPSP, which may not even be a detectable IPSP. Instead GABA can mediate a type of inhibition called shunting inhibition. If a neuron receives excitatory inputs that depolarize the neuron, coincident activation of GABAARs at GABAergic synapses produces Cl- influx that can reduce or reverse the depolarization response produced at excitatory synapses. Consequently, shunting inhibition can override the excitatory effect of depolarizing glutamatergic inputs, resulting in overall inhibition, even if the membrane potential remains the same or becomes slightly less negative. Importantly, GABAergic transmission can prevent the neuron from firing an AP.

1. *Where are glutamate synapses located and what does glutamate binding to AMPA and NMDA receptors produce? Where are the majority of GABA synapses located? How are GABA and glycine receptors localized to the synapse? What do IPSPs affect/regulate in a neuron?*

Glutamate synapses are typically located on dendritic spines and dendritic shafts. They typically produce small (~0.5-1 mV) EPSPs. The majority of GABA (and glycine) synapses are located on the cell body and dendrites (with a few found on dendritic spines and the axon.) Similar to glutamate receptors, GABA and glycine receptors are localized at the synapse by scaffolds. IPSPs are inhibitory/hyperpolarizations of the membrane potential. They move the membrane potential away from firing an action potential, and therefore they can affect the ability of a neuron to fire an action potential and/or by reducing the overall total depolarization of a neuron, it can also affect the rate of action potential firing of that neuron. (Remember that the AP rate is determined by the summed depolarization detected at the initial segment.)

1. *What is the PSD and what is its function? How else are receptors localized at the postsynaptic region? Describe postsynaptic transmission at a typical glutamate dendritic spine synapse.*

The PSD is the postsynaptic density, a region of excitatory/glutamate postsynaptic region directly across from the presynaptic active zone (where NTs are released into the cleft). It is dense because it contains a lot of proteins, including the NT receptors and scaffolding proteins such as PSD-95 or GPIP-1 that bind the receptor cytoplasmic regions and help to tether them at this region. Receptors are also localized by binding to a protein called pentraxin located in the synapse. Most dendritic spines express both AMPA and NMDA types of ionotropic glutamate receptors.

This is repeated from the previous answer:

At the resting membrane potential, after glutamate binding, only the AMPA receptors will be opened. The reason for this is that at the RMP, the NMDA receptors are blocked by Mg2+. After the AMPARs open, Na+ flows through the channel, down its electrochemical gradient, into the spine (producing an excitatory postsynaptic potential-EPSP) and this depolarizes the membrane potential. That depolarization removes the Mg2+ block from the NMDA receptors, and together with glutamate binding, will then open the NMDA receptors, which are permeable to Na+, K+ and Ca2+. So usually, the AMPA receptors provide the initial synaptic transmission, followed by NMDA receptors augmenting that membrane depolarization (longer EPSPs), and also increasing the dendritic Ca2+ levels. Glutamate synapses are fairly small, and usually contain only a few (1-5) docked/primed synaptic vesicles. That means that in response to a presynaptic AP, there may be no transmission (failures) or just one or two vesicles released. Thus, a presynaptic AP may result in only a small (<1mV) EPSP, and a single presynaptic AP would never produce a response large enough to lead to the generation of an AP in the postsynaptic neuron. However, since neurons receive many (thousands) of synapses, and all the responses are constantly summating with each other (integration), the neuron doesn’t have to rely on an individual synaptic response to produce enough depolarization to generate an action potential. Instead it relies on tens of thousands of inputs to summate to produce enough depolarization to produce an AP.

1. *What is summed in “summation?” Describe the two types of “summation.” In a typical neuron, what is the region (of the neuron) where this summation is most important and why?*

Summation involves the arithmetic addition and subtraction of all the changes in membrane potentials (all the EPSPs and IPSPs) all over the neuron. There is temporal summation and spatial summation. In temporal summation, EPSPs or IPSPs produced at a single synapse that occur in response to high frequency presynaptic action potentials, will summate with each other. In spatial summation, different synapses that produce EPSPs or IPSPs in close proximity to each other and that occur within a few msecs of each other, will summate. Summation occurs everywhere in every neuronal membrane in the neuron. The sum of the membrane potentials (through summation) is most important at the initial segment of the axon, since that is where the AP is triggered/produced, because that is the place where the VG Na+ channels are first concentrated in the axon.

1. *What can happen if there is too little inhibition or too much excitation of neurons? What can happen if there is too much inhibition or too little excitation of neurons? What is epilepsy?*

Too little inhibition or too much excitation can produce seizures, which involves synchronous and persistent activation and firing of glutamatergic neurons that can be localized (focal) or generalized. Epilepsy is “a neurological disorder marked by sudden recurrent episodes of sensory disturbance, loss of consciousness, or convulsions, associated with abnormal electrical activity in the brain.” Untreated, generalized seizures can lead to brain damage and death. Too much inhibition of too little excitation can lead to loss of consciousness, coma and death.

From Wikipedia: Epilepsy is a group of neurological disorders characterized by recurrent epileptic seizures. Epileptic seizures are episodes that can vary from brief and nearly undetectable periods to long periods of vigorous shaking. These episodes can result in physical injuries, including occasionally broken bones. In epilepsy, seizures have a tendency to recur and, as a rule, have no immediate underlying cause. Isolated seizures that are provoked by a specific cause such as poisoning are not deemed to represent epilepsy. People with epilepsy may be treated differently in various areas of the world and experience varying degrees of social stigma due to their condition. The underlying mechanism of epileptic seizures is excessive and abnormal neuronal activity in the cortex of the brain. The reason this occurs in most cases of epilepsy is unknown. Some cases occur as the result of brain injury, stroke, brain tumors, infections of the brain, or birth defects through a process known as epileptogenesis. Known genetic mutations are directly linked to a small proportion of cases. The diagnosis involves ruling out other conditions that might cause similar symptoms, such as fainting, and determining if another cause of seizures is present, such as alcohol withdrawal or electrolyte problems. This may be partly done by imaging the brain and performing blood tests. Epilepsy can often be confirmed with an electroencephalogram (EEG), but a normal test does not rule out the condition.

1. *What is glutamate excitotoxicity? What pathological conditions can lead to glutamate excitotoxicity? How could glutamate be toxic to a neuron or cause neuronal death?*

From Wikipedia: “Excitotoxicity is the pathological process by which nerve cells are damaged or killed by excessive stimulation by neurotransmitters such as glutamate and similar substances. This occurs when receptors for the excitatory neurotransmitter glutamate (glutamate receptors) such as the NMDA receptor and AMPA receptor are over-activated by glutamatergic storm.” In epilepsy, excitotoxicity is caused by release of too much glutamate. The membrane potential depolarizes → activation of voltage gated Ca2+ channels → increased presynaptic Ca2+ → release of glutamate → activation of AMPA and NMDA receptors → depolarization of the postsynaptic neuron and Ca2+ increases (the postsynaptic dendrites and spines also express VG Ca2+ channels which contribute to the Ca2+ increases) → activation of toxic Ca2+ dependent processes such as formation of free radicals, degradation of membrane lipids and proteins → neuronal degeneration and cell death. In summary, glutamate excitotoxicity is a result of release of glutamate, which leads to hyper activation at glutamatergic synapses, and excess release of glutamate. It is the activation of the NMDA receptor and the influx of Ca2+ that triggers a series of reactions leading to neuronal damage or death.

1. *What is the Excitation/Inhibition (E/I) ratio and what is E/I balance? In what brain region has the E/I balance been well studied? What disorders are imbalances in E/I ratio thought to contribute to?*

The E/I balance is the excitation/inhibition balance or ratio. It is the balance/ratio of excitatory (glutamatergic) and inhibitory (GABAergic) synaptic inputs. The E/I ratio has been studied extensively in the cerebral cortex (neocortex). In the cerebral cortex, circuit activity (involving excitatory and inhibitory inputs) is believed to be the basis of information processing for sensory, motor and cognitive functions. Imbalances in the E/I ratio are thought to underlie some neurodevelopmental disorders such as autism, and some psychiatric disorders such as schizophrenia.

From Scholarpedia: “In the context of neurophysiology, balance of excitation and inhibition (E/I balance) refers to the relative contributions of excitatory and inhibitory synaptic inputs corresponding to some neuronal event, such as oscillation or response evoked by sensory stimulation. In the current literature, owing to the extremely wide range of conditions in which the term is applied, it has several different, albeit related, meanings. As described in more detail below, the precise meaning depends on various considerations, such as averaging across time or population of neurons that is involved; the relevant timescale; whether the synaptic activity is sustained or transient, spontaneous or evoked. In general, excitatory and inhibitory inputs of a neuron are said to be balanced if across a range of conditions of interest the ratio between the two inputs is constant.

In the cortex, interneurons responsible for inhibition comprise just a small fraction of the neurons, yet they have an important function in regulating activity of principal cells. When inhibition is blocked pharmacologically, cortical activity becomes epileptic, and neurons may lose their selectivity to different stimulus features. These and other data indicate that the interplay between excitation and inhibition has an important role in determining the cortical computation. Our understanding of the relationships between these two opposing forces has advanced significantly during the recent years, mainly due to the growing use of in-vivo intracellular recording techniques.”

1. *What is a therapeutic NMDA receptor antagonist and what is it prescribed for? Ketamine is used as an anesthetic and is also an illegal drug of abuse. Why are pharmaceutical drug companies developing and testing ketamine derivatives?*

Memantine is a therapeutic NMDA receptor antagonist used in the treatment of Alzheimer’s Disease. (AD has been proposed to involve glutamate toxicity that underlies neuronal degeneration.) Ketamine is another NMDA receptor antagonist that is used both clinically and is an illicit drug of abuse, which produces euphoria, analgesia, hallucinations, and paralysis. It is used clinically (therapeutically) as an anesthetic and for pain management in palliative care. It has also shown to be a rapidly reduce or reverse severe depression. Esketamine recently received FDA approval for the treatment of depression, administered as a nasal spray in a doctor’s office or clinic, and only after two other antidepressants have been tried. Pharmaceutical companies are currently developing selective ketamine derivatives to treat depression with fewer side effects.

1. *What are two main categories of drugs that are GABA receptor agonists? What do they do (how do they work)? What are they used for clinically?*

The two main GABA receptor agonist drugs are benzodiazepines and barbiturates. These drugs are allosteric modulators that work by increasing the activity of the GABA receptor when GABA is bound. This leads to an increase in GABA-mediated inhibition. They are used to treat/decrease seizures (anticonvulsants/antiepilepsy drugs), as sedatives, to treat anxiety (anxiolytics), and as centrally acting muscle relaxants. Propofol is a more recently developed GABA receptor modulator drug used as a general anesthetic but is also a drug of abuse.

1. *Where are NT receptors localized?*

Ionotropic and metabotropic receptors are localized at postsynaptic, presynaptic, perisynaptic and extrasynaptic regions.

1. *What is synaptic plasticity and long term potentiation (LTP)? What are the roles of ionotropic glutamate receptors in LTP? What is one important physiological function for synaptic plasticity in the adult brain?*

From Wikipedia: “Synaptic plasticity is the ability of synapses to strengthen or weaken over time, in response to increases or decreases in their activity.” “Long-term potentiation (LTP) is a persistent strengthening of synapses based on recent patterns of activity. These are patterns of synaptic activity that produce a long-lasting increase in signal transmission between two neurons.” “LTP is widely considered one of the major cellular mechanisms that underlies learning and memory.”

AMPA receptor activation leads to EPSPs and depolarization of the membrane potential that relieves the Mg2+ block of the NMDA receptor. NMDA receptors must be activated (and allow influx of Ca2+) for many types of LTP. The increase in postsynaptic Ca2+ activates protein kinases that phosphorylate AMPA receptors, and AMPA receptor trafficking and tethering proteins, which together lead to an increase in AMPA receptor number and activity at the synapse. Protein kinases also lead to changes in the gene expression of many plasticity proteins that regulate synaptic transmission and the morphology of the synapse. It is the increase in AMPA receptor number and activity that produces a larger response the next time synaptic transmission occurs at the synapse. And the plasticity proteins maintain this enhanced AMPA receptor change in the synapse. This is a reversible process, and when there is very little activity at a synapse, it can be weakened through long term depression (LTD) by processes that decrease AMPA receptor number and activity.