NBL 355-655 Module 10 Review Q&A

1. *Functionally (based on the response they produce), NTs can be considered excitatory, inhibitory or modulatory/neuromodulatory. What are the definitions of each functional group?*

Excitatory NTs produce excitatory post-synaptic potentials (EPSPs) that are either a fast or slow depolarization of the membrane potential. They are called excitatory since they move the membrane potential closer to threshold for firing an action potential. Inhibitory NTs produce an inhibitory post-synaptic potential (IPSP) that are either a fast or slow hyperpolarization of the membrane potential. They are called inhibitory since they move the membrane potential further away from threshold for firing an action potential. Modulatory/neuromodulatory NTs don’t produce a measurable change in the membrane potential (EPSP or IPSP) but can affect some activity in the postsynaptic neuron, including metabolism, the resting membrane potential, the cytoskeleton and neuronal morphology, and/or gene expression. Through these mechanisms, NTs can modulate the responsiveness of the neuron and its synapses to their incoming NTs through regulation of synapses.

1. *What is the definition of a ligand and of a receptor? By what type of bonds do receptors interact with their ligands, and what kind of interaction is this? What is meant by an endogenous or exogenous ligand? What is an agonist; what is an antagonist?*

Ligands are molecules that form reversible non-covalent bonds with protein receptors. A receptor is a molecule in a cell that has an affinity for another molecule – a ligand. Receptors use noncovalent bonds (including ionic bonds, hydrogen bonds, Van der Waal interactions, and hydrophobic bonds) to interact with (bind their) ligands. A receptor produces a biological response after ligand binding. An endogenous ligand is a molecule produced by the neuron or cell in the body. An exogenous ligand is a naturally synthesized molecule (made by a plant or organism) or a synthetic molecule including drugs. An agonist activates the receptor and produces a response whereas an antagonist inhibits the receptor and blocks the response. When a ligand binds to its receptor that is called association. When the ligand is released from the receptor, when it unbinds, that is called dissociation.

1. *What are the main common defining characteristics that all receptors possess? Briefly, what does each property mean/describe?*

The six main common properties of a receptor are reversibility (due to noncovalent bonding between receptors and ligands), affinity (how strongly a receptor binds to the ligand, expressed as the equilibrium dissociation constant KD), specificity (how well the receptor can distinguish among different ligands), efficacy (how well the response occurs after ligand binds), desensitization (in which the receptor can’t activate a response even with the ligand is bound), and localization (where the receptor is located).

1. *What are the two main types of neurotransmitter (NT) receptors? Briefly, what are the features of each type? Which NTs function via only ionotropic receptors, only metabotropic receptors, or both types of receptors? How can some NTs mediate both fast/direct and slow/indirect synaptic transmission at the same synapse? What are the other two types of receptors found in the nervous system, and what are their ligands?*

The two main categories of NT receptors are ionotropic receptors and metabotropic receptors. Ionotropic receptors are ligand-gated ion channels. When NT binds, the receptor undergoes a conformation change and the channel opens, ions flow down their electrochemical gradient and produce a fast response, either depolarization-excitatory or hyperpolarization-inhibitory (depending on the ionotropic receptor. They mediate fast direct transmission that involves direct changes in the postsynaptic membrane potential. Metabotropic receptors are G-protein coupled receptors, which activate G proteins that can bind directly to and regulate ion channels, or regulate second messenger synthesizing enzymes. Metabotropic responses are slower, but also longer-lasting. They can be involved in slow changes in the membrane potential (producing slow EPSPs or IPSPs) and/or neuromodulation that involves changes in metabolism, cytoskeleton, and/or gene expression.

Acetylcholine, glutamate, GABA, serotonin, and ATP all have both ionotropic and metabotropic receptors. Glycine has only ionotropic receptors. Neuropeptides and all the other monoamines (dopamine, epinephrine, norepinephrine, and histamine) have only metabotropic receptors. Different types of receptors allow for different types of transmission: ionotropic for fast/direct transmission and metabotropic for slow/indirect transmission. In many synapses, both types of NT receptors are expressed, so both fast-direct and slow-indirect transmission can occur at the same synapse.

Receptor tyrosine kinases mediate responses to growth factors, trophic factors and immune modulators such as cytokines and chemokines. Intracellular receptors mediate the responses to steroids such as estrogens, androgens, cortisol, and thyroid hormones. Intracellular receptors are transcription factors. After binding their ligand (which is hydrophobic and so diffuses directly across the plasma membrane) they bind to DNA and either increase or decrease transcription of specific genes.

1. *Describe the structure of an ionotropic receptor. When ionotropic receptors are activated by NT binding, what do they do? How do ionotropic receptors produce fast EPSPs or IPSPs? What are graded potentials and what are their features? If graded potentials are so small (EPSPs or IPSPs), how does a neuron ever fire action potentials?*

Ionotropic receptors are composed of four or five subunits (with each subunit containing four transmembrane spanning domains). The receptor contains a binding site for NT on one or more subunits on the extracellular domain, and also form a channel in the membrane for ions to pass through. In the absence of NT, the channel is closed. When activated by NT binding, the channel opens, allowing ions to move down their electrochemical gradient. This produces a graded potential, **either fast EPSPs or fast IPSPs in neurons, or an end plate potential or slow wave potential in muscle cells.** Graded potentials are changes in the membrane potential produced either at a synapse (in response to NT) in a neuron or muscle, or by a sensory stimulus (for sensory neurons.) Graded potentials are short-lived, local changes in membrane potential. They are not regenerated but rather decrease in magnitude with distance and time, since they undergo passive decay. In a sensory neuron the magnitude and duration of a graded potential depends directly on the strength and duration of the stimulus. In the case of a neuron-neuron or neuron-muscle synapse, the magnitude and duration of the EPSP or IPSP depends on the amount and duration of NT released at the synapse. EPSPs and IPSPs that arrive within a short period of time can summate, and if there are enough EPSPs to depolarize the membrane beyond threshold, the neuron will generate an action potential or a train of action potentials.

1. *What are characteristics of metabotropic receptors (GPCRs)?*

Metabotropic receptors are members of the GPCRs, the largest family of receptors in the genome with over 1000 genes. They bind their ligand through extracellular domains. GPCRs have seven transmembrane alpha helical domains. Activation of the GPCR leads to activation of its associated G protein. GPCRs are the targets of over half of therapeutic drugs in clinical medicine. Metabotropic receptors are a type GPCRs that binds NTs and activate G proteins. Activated G proteins regulate ion channels and effector proteins that synthesize second messengers. One of the main targets of second messengers are protein kinases. Thus second messengers lead to changes in protein phosphorylation. The downstream targets of second messenger-regulated kinase include ion channels and transporters (that affect the membrane potential), metabolic enzymes (that affect energy and metabolic pathways), cytoskeletal proteins (that affect neuronal morphology) and gene regulatory proteins (that affect gene expression). Metabotropic receptors can modulate neuronal and synaptic function through long lasting changes. Neuromodulatory/modulatory transmission occurs through metabotropic receptors. Neuromodulators can modulate/affect synaptic transmission that occurs by fast excitatory and fast inhibitory synaptic transmission at the same or other synapses in that neuron.

1. *Before discussing acetylcholine, it’s important to think about where the cholinergic neurons are located in the CNS and PNS and how they compare in numbers and location to other NT neurons. What are the only two types of neurons located in the cerebral cortex, thalamus, amygdala, and cerebellum? Where are the monoaminergic and cholinergic cell bodies located?*

All cortical (including hippocampal), cerebellar, thalamic, and amygdala neurons, are either glutamatergic or GABAergic. In addition, the majority of subcortical neurons, brain stem neurons and spinal cord neurons are either glutamatergic, GABAergic or glycinergic. Hence glutamatergic, GABAergic and glycinergic neurons represent >90 % of all neurons in CNS. The other 10% of CNS neurons are cholinergic or monoaminergic. Some neuronal populations located in distinct regions of the brain stem, hypothalamus, basal forebrain, striatum (basal ganglia) and spinal cord are either cholinergic or monoaminergic (serotonergic, adrenergic, noradrenergic, dopaminergic or histaminergic). In contrast, many neurons in the PNS are cholinergic.

1. *How and where is acetylcholine synthesized and packaged? How is acetylcholine removed from the synaptic cleft?*

Acetylcholine is synthesized from acetylCoA and choline by the enzyme choline acetyl transferase (CAT). Acetylcholine is transported into synaptic vesicles by the vesicular acetylcholine transporter (VAT), a type of secondary active transporter that uses the proton (H+) gradient to transport acetylcholine inside the vesicle, against its concentration gradient. The proton (H+) gradient is formed by the proton ATPase, a type of primary active transporter that uses ATP hydrolysis to pump protons inside the synaptic vesicle. Acetylcholine is removed from the synaptic cleft by degradation by acetylcholine esterase. ACh is degraded into choline and acetic acid and the choline is taken back into the presynaptic neuron by the plasma membrane choline transporter.

1. *What types of neurons are somatic lower motor neurons and what target do they synapse on? What is this synapse called?*

Somatic lower motor neurons are located in the spinal cord or brainstem and are cholinergic excitatory neurons. Somatic lower motor neurons innervate skeletal muscles, forming the synapse called the neuromuscular junction. Each muscle fiber is innervated by only one motor neuron, but a motor neuron’s axon can branch and so one motor neuron can innervate several muscle fibers in the same muscle to coordinately control muscle contraction.

1. *Briefly describe synaptic transmission at the neuromuscular junction. Describe the end plate potential (what does it depend on?)*

An action potential is propagated along the somatic lower motor neuron axon and will depolarize the presynaptic terminus membrane. Depolarization activates voltage gated Ca2+ channels located in the presynaptic terminus at the active zones. Ca2+ flows into the presynaptic terminus (down its electrochemical gradient, through the VG Ca2+ channels). The Ca2+ is sensed by synaptotagmin, which stimulates the SNARE complex to induce the synaptic vesicles, which contain acetylcholine (ACh), to undergo exocytosis and release ACh into the synaptic cleft. ACh diffuses across the cleft and binds to and activates nicotinic ACh receptors (nAchRs), which are located at the muscle end plate. NAchRs are nonselective cation channels that allow Na+ and K+ to flow down their electrochemical gradients into or out of the muscle. The influx of Na+ produces the end plate potential (EPP), which is a large depolarization of the membrane potential (the membrane potential depolarizes from -90 mV, which is the muscle resting membrane potential (RMP), to about -40 mV). The Na+ current and EPP it produces, travel along the muscle membrane, to the area outside of the end plate, where voltage gated Na+ and voltage gated K+ channels are located. The EPP depolarization activates voltage gated Na+ channels (VGNa+ channels) which triggers and propagates a muscle action potential. Then the VG K+ channels open and repolarize the membrane potential in the muscle membrane.

1. *Briefly describe the structure and function of the nicotinic acetylcholine receptor. What ions does it conduct? Why does the nAChR usually produce much greater inward Na+ current than outward K+ current? What is the purpose of the end plate potential? What does curare do? What does nicotine do?*

The nAChR is composed of five subunits (with each subunit containing 4 transmembrane spanning domains). Of those five subunit, two are alpha subunits which each contain a binding site for ACh on the extracellular domain. In the absence of ACh, the channel is closed. When activated by ACh binding to the alpha subunit, the channel opens. Since it is nonselective cation channel, it facilitates the flow of Na+ and K+ down their electrochemical gradients. At the muscle end plate, initially, for the NAChRs, much more Na+ flows in than K+ flowing out, because the driving force for Na+ at the RMP is much greater than the driving force for K+ at the RMP. As the membrane depolarizes (the EPP) to about -40 mV, the driving force for K+ increases and the driving force for Na+ decreases, so at that point some K+ will flow out, helping to repolarize the membrane potential. The purpose of the EPP is to induce an AP in the muscle membrane. The AP in the muscle membrane activates muscle contraction.

Curare is a plant poison, which acts as a nicotinic acetylcholine receptor antagonist that would inhibit the EPP at the NMJ. The amount of inhibition depends on the dose of curare used. If the EPP is inhibited by at least 50% (so the EPP depolarization doesn’t reach threshold) this would prevent the activation of the muscle AP and inhibit muscle contraction. Nicotine is an agonist at the nicotinic acetylcholine receptor. However, because of the specific receptor subunit compositing in the NMJ, nicotine binds much better to the brain nicotinic receptor than the NMJ nicotinic receptor. This is discussed more below.

1. *Briefly describe how the AP in the muscle leads to an increase in Ca2+ levels in the muscle cytoplasm (the sarcoplasm). (This is the first step in excitation contraction coupling.) Include the roles of the DHPR voltage gated Ca2+ channels, sarcoplasmic reticulum, ryanodine receptor SR Ca2+ channels, and SERCA Ca2+ pump. (This Wikipedia article on muscle contraction may be helpful:* [*https://en.wikipedia.org/wiki/Muscle\_contraction#Excitation-contraction\_coupling*](https://en.wikipedia.org/wiki/Muscle_contraction#Excitation-contraction_coupling)*.)*

The AP travels along the muscle fiber plasma membrane via propagation and along and down the T-tubules, which contain DHP receptors (DHPRs) which are L-type voltage gated Ca2+ channels (VGCC) → Depolarization induces a conformational change in DHPR, activating them. The Ca2+channel opens and allows the inward flow of Ca2+. However, this influx of Ca2+ is not actually necessary→ The DHPR are bound (by protein-protein interactions) to and so they are physically coupled to the ryanodine receptor (which are a type of Ca2+ channel that are located in the SR membrane). Importantly, the voltage sensitive conformation change in the DHPR (that is there to open the Ca2+ channel region) is also transmitted to and produces a conformational change in the RyR located on the SR membrane, and opens them, allowing Ca2+ to flow out of the SR into the sarcoplasm. The SR contains a large Ca2+ store, because of the SERCA pumps that fill the SR with Ca2+ → When the coupled RyR are activated by the DHPR, they open and this allows Ca2+ to flow out of the SR and into the sarcoplasm. That released Ca2+ from the SR further promotes the release of more Ca2+ from neighboring RyR, since they are also activated by just the binding of Ca2+. This produces a feed-forward release of Ca2+ from the SR. The released Ca2+ activates the muscle contraction by the sliding filament mechanism (see below). Lastly, Ca2+ is rapidly transported inside the SR to stop muscle contraction via the SERCA pump. Ca2+ can also be transported out of the muscle cell by plasma membrane Na+/ Ca2+ exchanger and Ca2+ ATPase/pump.

1. *A category of drugs called dihyrdopyridines (DHP) are blockers of voltage gated Ca2+ channels (they block the movement of Ca2+ through the channel) and are used to treat high blood pressure (hypertension). Why don’t the DHP drugs cause paralysis or other mental problems?*

The activation of the DHPR/VGCC by depolarization of the T tubule membrane leads to an activation of the RyR in the SR membrane, since these two channel proteins are bound to each other. In response to the depolarization, the DHPR undergoes a conformation change that is transmitted to the RyR which is a Ca2+ channel and it is opened. Then Ca2+ flows out of the SR into the muscle cytoplasm where it will stimulate muscle contraction. Although the DHPR/VGCC functions as a Ca2+ channel, the movement of Ca2+ through the channel is not actually required for it to activate the RyR in the SR. So although dihydropyridines block the movement of Ca2+ through the channel, it doesn’t block the conformation change in the DHRPR that leads to activation of the RyR and release of Ca2+ from the SR. Why does it not affect mental function? Though in the CNS presynaptic NT release requires VGCCs and Ca2+ influx, the Ca2+ channels that mediate NT release are a different isoform of VGCC (they are the N and T types in the presynaptic neuron) that don’t bind and are not sensitive to DHPs. Also DHPs don’t cross the blood-brain barrier very well.

1. *Briefly describe the “sliding filament theory” (what is the mechanism whereby increased Ca2+ in the sarcoplasm (muscle cytoplasm) leads to muscle contraction.)*

A. Before contraction, when the muscle is in the relaxed but active state: Myosin binds ATP and hydrolyzes the ATP to ADP and Pi. This leads to a large conformational movement of the myosin head, and the myosin with ADP and Pi bound is now in what is called the “cocked” state. The actin filaments have troponin bound to them, which covers the myosin binding sites.

B. The muscle AP leads to Ca2+ release from the SR (described above).

C. Ca2+ binds to troponin. This releases troponin from the actin filament, and exposes the myosin binding sites on the actin filament.

D. Myosin heads bind to the actin filament.

E. After binding to actin, the myosin head immediately releases the Pi and ADP, and this induces the myosin head to move/pivot (it undergoes a large conformational change), producing the “power stroke,” and this results in the sliding of the actin filament along the myosin filament. Some models show the myosin releasing both Pi and ADP at the same time, while other models show the myosin first releasing Pi to produce the power stroke and then releasing ADP.

F. After moving/pivoting in the power stroke, the myosin head is still bound to actin. This is the “rigor state.” If there is no ATP in the cell, the myosin stays bound to the actin in the rigor state.

G. In a normal healthy muscle, the myosin head will now bind a new molecule of ATP, and when it binds ATP, that releases the myosin from the actin.

H. The myosin head then hydrolyzes the bound ATP to ADP and Pi, inducing the movement of the myosin head back into the “cocked state,” with ADP and Pi bound. When Ca2+ levels decrease, the troponin rebinds to the actin filament (blocking the myosin binding site), and the filament is back to the relaxed but active state.

1. *What features of the NMJ ensure that it is an efficient and reliable synapse (an AP in the alpha motor neuron always produces muscle contraction)? Why do you think the muscle resting membrane potential is so negative (-90 mV)?*

One muscle fiber receives inputs from only a single motor neuron, hence the activity of the single alpha motor neuron determines the activity/contraction of the muscle fiber. There is no integration of inputs at the muscle fiber. Thus the entire control of muscle contraction and relaxation occurs at the level of the control of the output of the alpha motor neuron in the spinal cord or brainstem.

The NMJ is a very large synapse (at least 10 times larger than a neuron-neuron synapse). The presynaptic region of the motor axon has many active zones and each active zone has lots of synaptic vesicles. The postsynaptic plasma membrane contains many folds (called junctional folds) that maximize the surface area, which can incorporate many nicotinic ACh receptors. Therefore when an action potential arrives, a large amount of acetylcholine is released from the presynaptic motor axon. And because of the junctional folds there are many nicotinic ACh receptors activation on the muscle membrane at the motor end plate. Activation of many nAChRs produces a large end plate potential (EPP). With the EPP, the muscle depolarizes from its resting potential (-90 mV) to about -40 mV (a change of ~ 50 mV). This. As the depolarization (the EPP) spreads/travels outside the end plate along the membrane, it is always large enough to produce threshold (-55 mV) to activate the nearby VG Na+ channels and thus an EPP will always produce an AP in the muscle membrane. Therefore, an AP in the presynaptic motor axon always produces an AP in the muscle membrane, which then always leads to muscle contraction.

Two mechanisms ensure that muscle contraction occurs only when there is a LMN AP. The resting membrane potential is very negative (-90 mV), so if there is any spurious small amount of release of ACh (in the absence of a LMN AP) it will not produce an EPP that will be large enough to trigger an AP in the muscle. Also, the presence of acetylcholine esterase (AchE) ensures that if there is any spurious small release of ACh (in the absence of an AP) from the presynaptic motor neuron, it will be degraded rapidly to prevent any unwanted muscle contraction.

1. *How is acetylcholine removed from the NMJ? What are the two categories of inhibitors?*

ACh is the only transmitter that has a dedicated degradative enzyme: acetylcholinesterase (AchE) that cleaves ACh into choline and acetate. The presence of AchE ensures that when the presynaptic motor neurons stops firing action potentials, the Ach will be rapidly degraded, stopping transmission and stopping muscle contraction. Two types of inhibitors have been developed. Therapeutic inhibitors are reversible inhibitors used to treat disorders such as myasthenia gravis and Alzheimer’s Disease, in which loss of cholinergic neurons or decreased cholinergic transmission has been observed. The idea is to block the removal/degradation of ACh to enhance the existing transmission or boost transmission in the existing neurons. Irreversible inhibitors are poisons like pesticides and nerve gas (such as Sarin gas), a chemical weapon. Exposure to these agents can produce both CNS and PNS effects, including headaches, fluid secretion, loss of consciousness, suffocation (because the muscles that control breathing can’t relax) and death.

1. *What is myasthenia gravis and how does it affect synaptic transmission at the NMJ. What are disorders involving loss of cholinergic neurons?*

Myasthenia gravis is an autoimmune disorder in which antibodies are present to the nicotinic ACh receptor. When auto-antibodies bind to the nAChR they can induce endocytosis of the receptors and degradation, or they can block nAChR function. The loss of nAChR activity will lead to a decrease in synaptic response at the motor end plate, with a decreased EPP. The decreased EPP may not be large enough to produce a muscle AP and muscle contraction, and there is decreased muscle contraction, and weakness. The first neurons that degenerate in Alzheimer’s Disease are basal forebrain cholinergic neurons (though later there is neuronal loss in many brain regions in AD). Loss of cholinergic neurons has also been observed in alcohol induced dementia and traumatic brain injury.

1. *Describe the role of acetylcholine in the autonomic nervous system.*

All preganglionic neurons of the autonomic nervous system (both parasympathetic and sympathetic), which are located in either the spinal cord or brain stem), are cholinergic. They extend their axons out of the spinal cord or brain stem, those axons form part of the spinal or cranial nerves) and they make synapses on neurons in the autonomic ganglia they control. The postganglionic neurons (in the autonomic ganglia) express nicotinic Ach receptors, so Ach acts as an excitatory transmitter to activate action potentials in the postganglionic neurons. All postganglionic parasympathetic neurons are cholinergic, and release Ach to their targets. The targets (such as the heart, vascular smooth muscles, and glands) express metabotropic receptors. Therefore, the effects of parasympathetic stimulation are mediated by the metabotropic Ach receptors, called muscarinic receptors (called M1, M2, M3, etc.) on the target cells. Muscarinic receptors activate G proteins that produce effects on ion channels and second messenger pathways in the target cells. In the heart, Ach decreases the rate and force of the heart beat through the activation of M2 muscarinic receptors which activate/open a K+ channel leading to hyperpolarization of the membrane potential, and by inhibition/closing of a Ca+2 channel that decreases the Ca2+ influx and reduces the force of contraction. These effects occur in different cardiac cells that control different aspects of the heart beat.

In the sympathetic nervous system, a small number of postganglionic sympathetic neurons (those that innervate sweat glands) are cholinergic and the target sweat glands express muscarinic receptors. (The majority of postganglionic sympathetic neurons are noradrenergic –they use norepinephrine as their NT.)

1. *What are the functions for acetylcholine in the brain? Where are nAChRs located in the brain? Nicotine is a nAChR agonist. How does nicotine cross the BBB? Why doesn’t nicotine produce spasticity (unwanted muscle contractions)? and what does nicotine do in the CNS?*

In the CNS, ACh has a variety of effects on plasticity, arousal and reward. ACh has an important role in the enhancement of alertness when we are awake, in sustaining attention, in motivation and reward, and in learning and memory. Both nicotinic and muscarinic receptors are expressed in the CNS. The neuronal type nAChR bind are activated by nicotine with about a 50 fold greater potency that the muscle nAChRs. Nicotine is hydrophobic and can easily cross the BBB. Nicotinic ACh receptors are expressed by neurons in the brainstem, hippocampus, amygdala, nucleus accumbens (NA) (which is the ventral striatum), and prefrontal cortex. Nicotine enhances attention, concentration and memory. It can produce a calming, relaxing effect and is an appetite suppressant. Muscarinic receptors are also involved in many of the effects of ACh in the brain.