NBL 355-655 Module 12 Review Q&A

1. *What are the three categories of monoamines? Where are monoamine NTs synthesized and how are synaptic vesicles packaged/filled with monoamines? What types of receptors do monoamines activate? How are monoamines removed from the synapse?*

The three categories of monamines are catecholamines (dopamine, norepinephrine and epinephrine), indolamines (serotonin), and histamine. All monoamines are transported into synaptic vesicles by the vesicular monoamine transporter (VMAT). Each monoamine activates its specific metabotropic receptors. Only serotonin has both an ionotropic and metabotropic receptor. Monoamines are removed from the synapse by specific plasma membrane monoamine transporters, which are specific for each monoamine (see below).

1. *What is the first substrate in dopamine biosynthesis? What two main areas are dopaminergic cell bodies located in the brain, where do they project, and what functions is dopamine involved in? What is dysfunction of dopamine pathways implicated in?*

The amino acid tyrosine is the precursor in all catecholamine NT biosynthesis. Dopaminergic neuron cell bodies are located in the substantia nigra and the ventral tegmental area. These two areas are located in the midbrain, which is part of the brainstem. Dopaminergic neurons project to the striatum, the cortex and the spinal cord. Dopamine is involved in motivation, pleasure and reward, motor control, (including initiation, selection, control and fine-tuning of motor function), euphoria (mood), reward-driven learning, and cognitive functions. Dysfunction of dopamine pathways has been implicated in Parkinson’s disease, schizophrenia, anhedonia (inability to enjoy pleasure) and bipolar disorder. Most monoamines have roles of mood and cognition (including memory). The four functions that distinguish dopamine are motivation, pleasure, reward and motor control.

1. *What main areas are noradrenergic (norepinephrine) and adrenergic (epinephrine) neuron cell bodies located. What is the main function for norepinephrine within the CNS?*

Noradrenergic cell bodies are located in the locus coeruleus (in the pons) while adrenergic neurons are located in the lateral tegmental system and medulla. These regions are located in the brainstem. The general function of norepinephrine is to mobilize the brain and body for action. Norepinephrine regulates attention, arousal and alertness, is involved in sleep and wake cycles, feeding, pleasure and anxiety (mood), enhancing sensory processing, and learning and memory. Most monoamines have roles of mood and cognition (including memory). The four functions that distinguish norepinephrine are attention, arousal, alertness, and sleep-wake cycles.

The functions of epinephrine in CNS are not well characterized.

The name "noradrenaline," derived from Latin roots meaning "at/alongside the kidneys", is more commonly used in the United Kingdom; in the United States, "norepinephrine", derived from Greek roots having that same meaning, is used and is the international nonproprietary name. Parts of the body that produce or are affected by it are referred to as noradrenergic.

1. *What is the first substrate in serotonin biosynthesis? Why is it also called 5HT? What is the main area where serotonergic cell bodies are located in the brain, where do they project, what functions is serotonin involved in? What is dysfunction of serotonin pathways implicated in?*

The first substrate in serotonin biosynthesis is the amino acid tryptophan. Serotonin is 5’ hydroxytryptamine (5HT). Serotonergic cell bodies are primarily located in the dorsal Raphe nuclei (nine nuclei in the midbrain and pons in the brainstem) and project throughout the brain, including the cerebellum and spinal cord. In the nervous system, serotonin is involved in mood, arousal, memory processing, sleep/wake cycle, circadian rhythms, appetite/eating and digestion, cognition and motor behaviors. The dysfunction of serotonin pathways has been implicated in depression and obsessive-compulsive disorder. Most monoamines have roles of mood and cognition (including memory). The four functions that distinguish serotonin are mood (happiness and well-being), sleep-wake cycle, and appetite/eating and digestion.

1. *Where are histaminergic neuron cell bodies located, where do they project, and what functions is histamine involved in?*

Histaminergic neuron cell bodies are located in the hypothalamus, they project throughout the forebrain and they function in wakefulness and sleep, and modulation of the circadian rhythm. The ability of antihistamine drugs to treat nausea/emesis (working as antiemetics) and vomiting suggest that histamine may be involved in controlling nausea. (Note that acetylcholine and norepinephrine are also implicated in nausea, especially in motion sickness.)

1. *How are monoamines removed from the synaptic cleft? Which therapeutic and recreational drugs of abuse target this mechanism?*

Monoamines are transported back to the presynaptic neuron by specific plasma membrane monoamine transporters. There is a specific dopamine transporter, serotonin transporter, norepinephrine transporter, and presumed to be a histamine transporter.

Antidepressants include the selective serotonin reuptake inhibitor (SSRIs such as Prozac, Paxil and Zoloft), serotonin-norepinephrine reuptake inhibitor (SNRI such as Cymbalta and Myridia used to treat depression and fibromyalgia) and traditional tricyclic antidepressants (nonselective monoamine uptake inhibitors). Monoamine oxidase inhibitors are also used to treat depression.

The therapeutic drugs Ritalin (methylphenidate) and Adderall, which are used to treat ADHD, are stimulants that function by acting on the dopamine transporters, though by different mechanisms. Ritalin blocks the plasma membrane dopamine transporter (PMDT) and blocks dopamine reuptake. Adderall blocks the vesicular monoamine transport (VMAT) and so blocks uptake of dopamine into synaptic vesicles and this increases the cytosolic dopamine levels at the presynaptic region. Adderall also binds to the PMDT and reverses its direction, producing dopamine transport out of the presynaptic neuron and into the synapse. Both drugs lead to an increase in dopamine levels at the synapse. Recreational drugs of abuse work by similar mechanisms. Amphetamine and methamphetamine act similar to Adderall, while cocaine acts similar to Ritalin.

1. *Describe four similar properties/characteristics of monoamine neurons and systems. What two functions do dopamine, norepinephrine and serotonin share?*

The majority of monoamine cell bodies are located in the brainstem.

Their axons project diffusely to many brain regions, including the cerebral cortex and subcortical regions.

Monoamines are packaged in the presynaptic terminus into synaptic vesicles by the vesicular monoamine transporter (VAT).

Monoamines are removed from the synapse by specific plasma membrane NT transporters.

Monoamines function in mood, reward, motivation, feeding behaviors, attention, arousal and alertness, sleep, motor function, and cognition, including memory.

Dopamine, NE and serotonin share roles in cognition and mood.

1. *Which neurons in the sympathetic nervous system use dopamine, norepinephrine and acetylcholine?*

In the sympathetic nervous system, postganglionic sympathetic neurons use either norepinephrine (the majority including those that innervate the heart and blood vessels), acetylcholine (innervating sweat glands), or dopamine (innervating renal blood vessels.) The adrenal gland functions like a postganglionic sympathetic neuron. It is innervated by a preganglionic sympathetic neuron (which releases acetylcholine onto the adrenal chromaffin cells, which when stimulated, release epinephrine and norepinephrine into the bloodstream). (From a previous lecture: all postganglionic parasympathetic neurons are cholinergic.)

1. *What about other monoamines? What is melatonin? What are phenethylamines?*

Melatonin is a neurohormone primarily released by the pineal gland that regulates the sleep–wake cycle. As a dietary supplement, it is often used for the short-term treatment of insomnia, such as from jet lag or shift work, and is typically taken by mouth. In animals (including humans), melatonin is involved in synchronizing the circadian rhythm, including sleep–wake timing, blood pressure regulation, and seasonal reproduction. Many of its effects are through activation of the melatonin receptors, while others are due to its role as an antioxidant.

Phenethylamine is a trace amine, which acts as a central nervous system stimulant in humans. In the brain, phenethylamine regulates monoamine neurotransmission by binding to trace amine-associated receptor 1 (TAAR1) and inhibiting vesicular monoamine transporter 2 (VMAT2) in monoamine neurons. To a lesser extent, it also acts as a neurotransmitter in the human central nervous system. In mammals, phenethylamine is produced from the amino acid L-phenylalanine by the enzyme aromatic L-amino acid decarboxylase via enzymatic decarboxylation. In addition to its presence in mammals, phenethylamine is found in many other organisms and foods, such as chocolate, especially after microbial fermentation. Studies have found abnormally low urinary phenethylamine concentrations in ADHD individuals when compared with controls

1. *What brain functions have the purines, adenosine and ATP been implicated in?*

Adenosine has been implicated in promoting sleep and suppressing arousal. Adenosine levels rise during the day and then decrease as sleep progresses. Adenosine functions to increase inhibition (by reducing glutamate levels from glutamatergic neurons and by increasing activity of GABAergic neurons).

ATP acts as a neurotransmitter in both peripheral and central nervous systems. In the peripheral nervous system, ATP is involved in chemical transmission in sensory and autonomic ganglia. In the central nervous system, ATP, released from synaptic terminals, induces fast excitatory postsynaptic currents. Postsynaptic action of ATP is mediated by a plethora of ionotropic and metabotropic receptors. ATP functions as a peripheral pain mediator. Furthermore, ATP also acts as an important mediator in neuronal-glial and glial-glial signaling. All types of glial cells are endowed with diverse ATP receptors, which trigger Ca2+ signaling events and membrane currents. ATP may also act as a gliotransmitter released from astrocytes via regulated exocytosis or through regulation of ion channels.

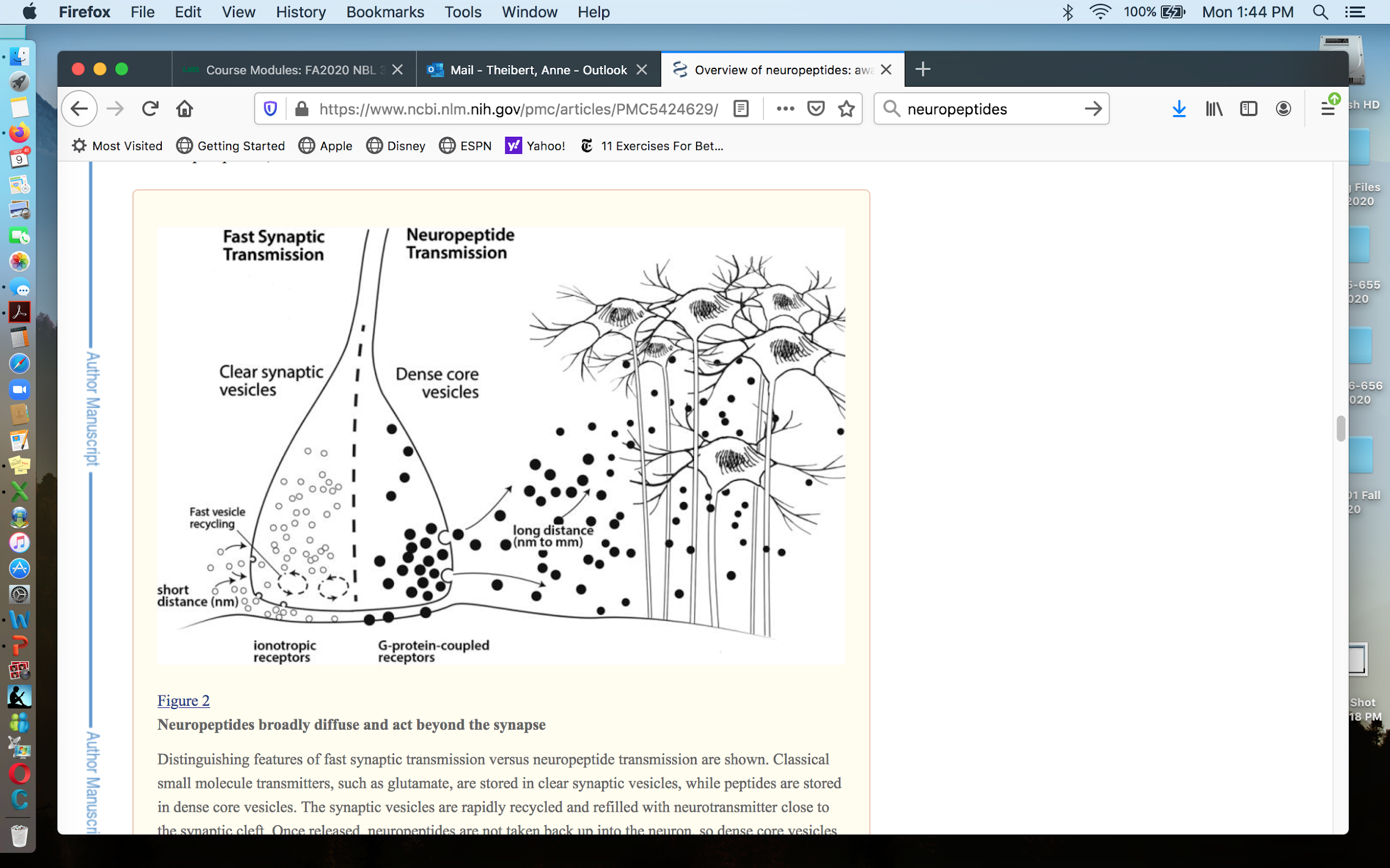
There are two types of purinergic receptors, called P2X, and P1 and P2Y receptors. P2X receptors are ligand-gated ion channels, whereas the P1 and P2Y receptors are G protein-coupled receptors. P1 receptors are preferentially activated by adenosine and P2Y receptors are preferentially more activated by ATP. P1 and P2Y receptors are known to be widely distributed in the brain, heart, kidneys, and adipose tissue.

The adenosine receptors (P1 receptors) are a class of purinergic G protein-coupled receptors with adenosine as the endogenous ligand. There are four known types of adenosine receptors in humans: A1, A2A, A2B and A3; each is encoded by a different gene. The adenosine receptors are commonly known for their antagonists caffeine and theophylline, whose action on the receptors produces the stimulating effects of coffee, tea and chocolate that reduce drowsiness.

ATP receptors (P2 receptors) consist of P2x, P2y, P2z, P2t, and P2u. The P2x and P2z subtypes are ionotropic receptors. P2X receptors are greatly distributed in neurons and glial cells throughout the central and peripheral nervous systems. P2X receptors mediate a large variety of responses including fast transmission at central synapses, contraction of smooth muscle cells, platelet aggregation, macrophage activation, and apoptosis. Moreover, P2X receptors have been implicated in integrating functional activity between neurons, glial, and vascular cells in the central nervous system, thereby mediating the effects of neural activity during development, neurodegeneration, inflammation, and cancer. The P2Y receptors) are GPCRs that preferentially bind ATP.

1. *How and where are neuropeptides synthesized and packaged? How is presynaptic release of small molecule NTs and neuropeptide NTs different? How do neuropeptides function?*

Neuropeptides are synthesized in the cell soma by the RER and packaged at the trans Golgi network into secretory vesicles (sometimes called dense core vesicles/granules), and transported to the presynaptic terminus by fast axonal transport (FAT). Many NPs are cleaved by proteases inside their secretory vesicles into smaller peptides as the vesicles mature during FAT. The release of small molecule NTs occurs at the active zone while neuropeptide NTs are located further away from the active zone and are often released outside of the synapse into the ECF. The exocytosis of secretory vesicles containing NPs involves synaptotagmin and the SNARE complex. Because they are located further from the active zones where the voltage gated Ca2+ channels are localized, the secretory vesicles containing neuropeptide NTs require much higher presynaptic AP frequency for exocytosis than is required for synaptic vesicles containing small molecule. The reason for this is that once the Ca2+ flows into the presynaptic neuron through VG Ca2+ channels, it is rapidly pumped back out by the plasma membrane Ca2+ transporters, or sequestered inside the ER. So to get a large enough Ca2+ signal all the way to the secretory vesicles to induce exocytosis, it takes the opening of many more VG Ca2+ channels for a longer time. NPs function as neuromodulators by activating metabotropic receptors. Since NPs can be released outside the synapse and into the ECF, they can function on perisynaptic and extrasynaptic receptors as well.



1. *How can neuropeptides function? What are oxytocin, NPY, CGRP and endogenous opioid peptides involved in?*

Neuropeptides (NPs) are small proteins (peptides) that neurons use to communicate with their targets. Neuropeptides function as neurotransmitters (typically neuromodulators) or as neurohormones, depending on whether they are released into the synapse/ECF or blood, respectively. “Humans have a diverse collection of neuropeptides that can influence a multitude of activities. There are now over 100 known neuropeptides and probably many more yet to be identified from the over 1000 predicted peptides encoded by the genome. While diverse, peptides generally share three common characteristics: (1) post-translational processing and release from vesicles, (2) activation of cell-surface receptors over a relatively large distance, and (3) modulation of target cells that are often in the brain and periphery.

In the CNS, oxytocin has been implicated in mate-pair bonding and romantic attachment, parent-child bonding, pro-social behaviors and empathy. In the CNS, neuropeptide Y (NPY) has been implicated in food intake, fat storage and metabolism; in the PNS NPY is a vasoconstrictor and involved in growth of adipose tissue. Endogenous opioids are involved in analgesia (inhibition of pain) and euphoria (the experience (or affect) of pleasure or excitement and intense feelings of well-being and happiness). A number of neuropeptides could potentially play roles in migraine, with CGRP (calcitonin gene related peptide) being the best characterized. CGRP antagonists are now available for migraine treatment.

From Wikipedia: “Many populations of neurons have distinctive biochemical phenotypes. For example, in one subpopulation of about 3000 neurons in the arcuate nucleus of the hypothalamus, three anorectic peptides are co-expressed: α-melanocyte-stimulating hormone (α-MSH), galanin-like peptide, and cocaine-and-amphetamine-regulated transcript (CART), and in another subpopulation two orexigenic peptides are co-expressed, neuropeptide Y and agouti-related peptide (AGRP). These are not the only peptides in the arcuate nucleus; β-endorphin, dynorphin, enkephalin, galanin, ghrelin, growth-hormone releasing hormone, neurotensin, neuromedin U, and somatostatin are also expressed in subpopulations of arcuate neurons. These peptides are all released centrally [meaning they are released at synapses to target neurons] and act on other neurons at specific receptors. The neuropeptide Y neurons also make the classical inhibitory neurotransmitter GABA.

Peptide signals play a role in information processing that is different from that of conventional neurotransmitters, and many appear to be particularly associated with specific behaviors. For example, oxytocin and vasopressin have striking and specific effects on social behaviors, including maternal behavior and pair bonding. The following is a list of neuropeptides that are co-expressed with small molecule neurotransmitters. You don’t need to memorize these for the quiz, the list is to demonstrate examples of and how widespread co-expression is.

Norepinephrine (noradrenaline). co-expressed with: Galanin, Enkephalin or Neuropeptide Y

GABA co-expressed with Somatostatin (in the hippocampus), Cholecystokinin or Neuropeptide Y (in the arcuate nucleus)

Acetylcholine co-expressed with VIP or Substance P

Dopamine co-expressed with Cholecystokinin, Neurotensin or Glucagon-like peptide-1 (in the nucleus accumbens)

Epinephrine (adrenaline) co-expressed with Neuropeptide Y or Neurotensin

Serotonin (5-HT) co-expressed with Substance P, TRH or Enkephalin

1. *How do metabotropic receptors work?*

Metabotropic receptors are G protein coupled receptors that are neuromodulatory NT receptors. Neuromodulatory NTs act through metabotropic receptors to modulate the overall activities and physiology of the neuron and synaptic transmission. Metabotropic receptors are found on the postsynaptic neuron in the synaptic, perisynaptic and extrasynaptic regions, as well as the presynaptic neuron, and also on nearby neurons. Neuromodulation is slow and long lasting and can be involved in point-to-point wiring transmission and volume transmission. It doesn’t directly or rapidly change the postsynaptic potential, so it doesn’t produce fast PSPs and contribute directly to AP firing. Instead, it affects other activities in the neuron, such as metabolic activities, the resting membrane potential, ion gradients and Nernst/equilibrium potentials, neurotransmitter synthesis and release, cytoskeletal proteins, membrane trafficking, and gene expression. In these ways, neuromodulators can influence synaptic transmission that occurs by fast excitatory and fast inhibitory synaptic transmission at the same synapse (where the metabotropic receptors are located) or other synapses in that neuron.

1. *Describe the two main metabotropic second messenger pathways (adenylyl cyclase and phospholipase C), what second messengers each produces, what the main targets of these second messengers are, and the function of the second messenger targets?*

Adenylyl (also called adenylate) cyclase is activated by Gs. It synthesizes the second messenger cAMP from ATP. cAMP has several targets, including the cyclic nucleotide gated channel and PKA. PKA is cAMP dependent protein kinase. PKA is a serine/threonine protein kinase that phosphorylates and regulates many neuronal proteins, including ion channels and transporters, NT receptors, cytoskeletal proteins and transcription factors. One of the PKA targets is the transcription factor CREB, which regulates gene expression. PKA phosphorylated CREB and that induces it to bind CREB binding protein (CBP). CBP is a histone acetylase which phosphorylates histones and enhances transcription.

Phospholipase C (PLC) is activated by Gq. PLC produces two second messengers, DAG and IP3. DAG activates a protein kinase called PKC. IP3 binds to the IP3 receptor which is a Ca2+ channel, and when open it opens, Ca2+ flows out of the ER, and increase the cytoplasmic Ca2+ concentration. Ca2+ binds to an adaptor protein called calmodulin. Ca2+/calmodulin has numerous targets, one of which is the calmodulin dependent protein kinase CaMKII. Similar to PKA, CamKII and PKC are serine/threonine protein kinases that have hundreds of substrates in neurons, including metabolic enzymes, channels, receptors, transporters, and cytoskeletal, and gene regulatory proteins.

Here is a summary of how metabotropic receptors function: Metabotropic receptors are GPCRs that activate G proteins. Activated G proteins regulate effector proteins that include ion channels, and enzymes 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 kinases include channels and transporters (that affect the membrane potential), metabolic enzymes (that affect energy and metabolic pathways), gene regulatory proteins (that affect gene expression), and cytoskeletal proteins (that affect neuronal morphology.) Thus metabotropic receptors can modulate neuronal and synaptic function, through long lasting changes.

1. *Which categories of proteins are phosphorylated (and regulated) by protein kinases? How can phosphorylation affect a protein?*

The downstream targets of second messenger-regulated kinases include channels and transporters, metabolic enzymes, gene regulatory proteins, and cytoskeletal proteins. Phosphorylation can affect the activity of a protein (if the protein has an activity such as being an enzyme, an ion channel or transporter, a motor protein or a protein that affects the cytoskeleton.) Phosphorylation can also affect the localization of a protein, its ability to bind to other proteins through protein-protein interactions, and the stability of a protein (how it is degraded by proteases.)

1. *Metabotropic receptors are GPCRs that activate G proteins. Activated G proteins can directly regulate ion channels, and also regulate enzymes that synthesize second messengers. What are the main targets of these second messengers, and the function of the second messenger targets? Which categories of proteins are phosphorylated (and regulated) by protein kinases? How can phosphorylation affect a protein?*

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 kinases include channels and transporters (that affect the membrane potential), metabolic enzymes (that affect energy and metabolic pathways), gene regulatory proteins (that affect gene expression), and cytoskeletal proteins (that affect neuronal morphology.) Thus metabotropic receptors can modulate neuronal and synaptic function, through long lasting changes. The downstream targets of second messenger-regulated kinases include channels and transporters, metabolic enzymes, gene regulatory proteins, and cytoskeletal proteins. Phosphorylation can affect the activity of a protein (if the protein has an activity such as being an enzyme, an ion channel or transporter, a motor protein or a protein that affects the cytoskeleton.) Phosphorylation can also affect the localization of a protein, its ability to bind to other proteins through protein-protein interactions, and the stability of a protein (how it is degraded by proteases.)