**Mod9 Study Guide**

1. *In the history of neuroscience, what contributions did anatomists make in the early to mid-1800s?*

Using the microscopes and tissue stains available in the 1830s-1840s, anatomists observed and documented the presence of cells (which they called neurons) and neuronal processes in the brain. Their observations supported the concept that the brain is composed of cells and that communication (proposed to involve electrical signals) between brain cells could involve the neuronal processes.

1. *What is the reticular theory and who proposed it? What is the neuron doctrine? Who proposed it? Which theory turned out to be correct? Why were these concepts important for neuroscience research?*

Camillo Golgi proposed the reticular theory, that cells in the nervous system form a continuous network of interconnected processes, and electrical signals can flow unimpeded through this continuous network. In contrast, the neuron doctrine is the concept that the nervous system is composed of individual cells (each with its own processes) that communicate at the gaps between them, later called synapses. It was proposed by Santigo Ramon y Cajal, Foster and others. Cajal was correct in describing chemical synapses; Golgi was somewhat correct in describing electrical synapses. These concepts are important because it raised questions about the mechanisms whereby neurons could communicate at synapses, which led to the discoveries of chemical synaptic transmission, neurotransmitters, receptors, membrane potentials, electrical signaling via the action potential, and GAP junctions.

1. *Describe an electrical synapse. What are the features, mechanism, advantage and limitations of transmission at an electrical synapse? Why is the term “electrical synapse” confusing?*

At an electrical synapse, ions (and small molecules) from the pre-synaptic region flow directly to the post-synaptic terminus via gap junction channels known as connexons. A change in membrane potential in the pre-synaptic neuron leads directly to a change in membrane potential in the post-synaptic neuron by the flow of current through the connexon. Electrical synapses are thought to be much less abundant than chemical synapses in the mammalian CNS. An advantage of an electrical synapse is that it is very fast. A limitation is that communication can only occur only at the specific regions of the membranes that are physically connected (forming the gap junction), and there is no adaptability. In electrical synapses, the pre-synaptic and post-synaptic neurons always have the same response, and there is no mechanism to boost the signal (there is no gain). The term “electrical synapse” is confusing because at chemical synapses, the response often involves an electrical response in the target cell.

1. *Describe a chemical synapse. What are the features, mechanism, advantages and limitation of transmission at a chemical synapse?*

At a chemical synapse, the electrical signal from the presynaptic neuron (the AP) is converted to chemical information through the release of neurotransmitters into the synaptic cleft. The neurotransmitter binds to receptors on the target cell leading to a response in the target. There is often an interconversion of electrical and chemical information. Chemical synapses make up the majority of synapses in the CNS and PNS. An advantage is that this type of synapse is very adaptable. The postsynaptic response can be excitatory, inhibitory, or modulatory. Transmission can be fast and brief or slow and long-lasting. One presynaptic neuron can communicate with numerous postsynaptic target regions (in volume transmission). A limitation is that it involves multiple biochemical steps that results in a brief time delay, so transmission at a chemical synapse is slower than at an electrical synapse.

1. *In a way, both Golgi (reticular theory) and Cajal (neuron doctrine) were correct about synaptic transmission. Explain.*

Golgi’s reticular theory was the idea that neuronal processes (axons and dendrites) form a continuous reticular network, and electrical signals can flow directly through this continuous network. At an electrical synapse, the depolarization of the presynaptic membrane is transmitted directly to the postsynaptic neuron by current flow via GAP junction channels. Consequently, the pre-synaptic and post-synaptic neurons are in an electrical continuum with each other (similar to what Golgi proposed, though only a few synapses function this way). In Cajal’s neuron doctrine, the idea is that neurons are separate cells that communicate at the spaces between them, the synapse, which would be similar to what occurs at chemical synapses where neurotransmitter is released and binds to postsynaptic receptors. This is how the majority of synapses in the mammalian CNS function.

1. *Describe an en passant synapse. Where are they proposed to be located? Why could they be important?*

From Wiki: “Some synaptic junctions appear along the length of an axon as it extends—these are called en passant ("in passing") synapses and can be in the hundreds or even the thousands along one axon. Other synapses appear as terminals at the ends of axonal branches.” Swellings termed axonal varicosities or axonal boutons are typically the sites where synapses occur. Boutons form as terminal bulbs at the end of an axon, and/or along the length of individual axons as boutons *en passant.*

Many en passant synapses are thought to occur between an axon and multiple dendritic spines located along a dendrite and/or many dendrites. Spine synapses are usually glutamate/excitatory synapses. This may be important to coordinate synaptic transmission between one axon and multiple spines along a dendrite, to produce a large response in the target dendrite.

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1. *There are about 86 billion (86 x 109) neurons in the human brain. Each neuron receives at least 1,000 synapses, hence there are at least 86 trillion (86 x 1012) synapses in the brain (and probably many many more than that). Explain how so many synapses are possible if each neuron has only one axon (86 million axons), and each synapse has one presynaptic axon terminus.*

It is possible because each axon can form multiple branches, and an axon branch can form multiple en passant synapses (see #6). If each axon formed between 10-100 branches, and each branch formed 10-100 en passant synapses, then each axon could form between 100-10,000 total synapses.

1. *What types of cells do neurons makes synapses on and communicate with?*

In the CNS, neurons communicate with other neurons. In the PNS, neurons communicate with other neurons, skeletal muscles, cardiac (heart) muscles, smooth muscles, and glands.

1. *Describe the two types of chemical neurotransmission, synaptic (wiring/point-to-point) transmission and volume transmission. How are they similar and how are they different? What is the role of astrocytes and extracellular matrix in determining which type of transmission occurs?*

Wiring transmission is point-to-point neurotransmission that occurs at a synapse between a presynaptic neuron and its postsynaptic target. Neurotransmitter is released from synaptic vesicles at the active zone. Communication is localized to the synapse, and is usually fairly short lived (on the order of msecs to secs) and localized since the neurotransmitter is rapidly removed from the synapse. Wiring transmission often involves electrical responses in the postsynaptic cell, in the form of EPSPs and IPSPs. Nearby astrocytes or extracellular matrix (ECM) proteins could help to enclose the synapse and prevent the NT from diffusing to nearby regions.

Volume transmission is not localized to the synapse. Neurotransmitters are released into the synapse where they can spill out, or be released outside the synapse, and diffuse into the extracellular fluid. In volume transmission, NTs can act on receptors found at the synapse, outside the synapse (perisynaptic or extrasynaptic receptors) and on receptors in the cell body and neighboring neurons. Communication can occur in a volume around the synapse, and is often longer lived (secs to mins) and not localized since the NT diffuses a long distance and is only slowly removed. Volume transmission often involves receptors that mediate neuromodulation.

1. *What are the different types of chemical synapses that can occur between two neurons in the CNS? (Think function, location and axons.)*

The three functional types of synapses are excitatory, inhibitory and modulatory/neuromodulatory. The four types of anatomical synapses (defined by the target) are axo-dendritic (on dendritic spines or dendritic shafts), axo-somatic (on the cell body), and axo-axonic (on another axon). Two additional types of synapses (defined by the axon) are terminal synapses (where the presynaptic axon ends where it forms a synapse on its target) and en passant synapses (where the axon forms a synapse by a bouton that blebs out from the axon, but does not end there but rather it continues on to form additional synapses).

1. *How are synapses physically connected across the synaptic cleft? Why is this important?*

From PMID: 17299456 “Many cell adhesion molecules are localized at synaptic sites in neuronal axons and dendrites. These molecules bridge presynaptic and postsynaptic specializations but do far more than simply provide a mechanical link between cells… Synaptic adhesion proteins participate in the formation, maturation, function and plasticity of synaptic connections. Together with conventional synaptic transmission mechanisms, these molecules are an important element in the trans-cellular communication mediated by synapses.”

1. *Why and how are synapses modified during development? What is one role for synaptic plasticity in the adult brain?*

Following synaptogenesis, in early infancy and childhood, synapses can be strengthened, weakened or removed, in an activity dependent manner in a process called synaptic refinement (the removal of synapses is often called synapse elimination). Microglia are proposed to function in the removal of synapses. Similar types of activity dependent plasticity have been documented in the adult brain, in the hippocampus and cerebral cortex, where long term potentiation (LTP) and long term depression (LTD) are proposed to be mechanisms involved in learning and memory.

1. *What was the first neurotransmitter discovered and by whom and in what system? What was the argument against a chemical mechanism of neurotransmission (the “soups”)? What was the argument against an electrical mechanism of neurotransmission (the “sparks”)? Who was correct?*

Acetylcholine was the first neurotransmitter discovered by Dr. Otto Loewi in the 1920s in the PNS in the synapse between the vagus nerve and the heart. Loewi was one of the “soups” a group of scientists who proposed that communication between neurons occurs by chemicals. The criticism of this idea was the use of chemical signaling would be much too slow for the fast communication that occurs in the brain. The other group, the “sparks” proposed that communication occurred by electrical communication, but one criticism of that idea is that since there are synaptic gaps between neurons (the synaptic cleft), the mechanism for electrical communication was not apparent or obvious. Both were correct. The action potential is an electrical signal used within neurons (to send a signal from the cell body to the presynaptic terminus). At chemical synapses (the majority of synapses), the action potential is converted into release of neurotransmitter at the synapse. Then in many synapses the chemical signal is converted into an electrical signal in the postsynaptic membrane. Postsynaptic potentials can summate to trigger an action potential.

1. *The two main types of conventional neurotransmitters (NT) are small molecule NTs and neuropeptide NT. What are the characteristics of conventional NTs?*

The two types of conventional NTs are small molecule NTs and neuropeptide NTs. Small molecule NTs have a low molecular weight (between 75-500 daltons). They are synthesized in the presynaptic region and packaged/transported into synaptic vesicles by vesicular NT transporters. Neuropeptide NTs are synthesized in the cell soma by the RER and packaged at the trans Golgi network (TGN) into secretory vesicles (sometimes called dense core vesicles/granules), and transported to the presynaptic terminus by fast axonal transport. Many neuropeptides are synthesized as larger precursors and undergo proteolytic cleavage during maturation as the vesicles are transported by fast axonal transport from the cell body to the presynaptic region.

1. *What are the three main categories of small molecule neurotransmitters and what are the names of the neurotransmitters in each category? What does Dr. Theibert find surprising about this list?*

A. Amines: acetylcholine and the monoamines: dopamine, norepinephrine, epinephrine, serotonin, and histamine

B. Amino Acids: glutamate, aspartate, GABA, and glycine

C. Purines: ATP and adenosine

One thing that is surprising about this list is that with all the variety of basic survival, behavioral, motor, sensory and cognitive functions that the nervous system is involved in and controls, there are actually so few neurotransmitters used. For many of these neurotransmitters there are different types of receptors and receptor subunit genes, which may help provide the diversity required for so many different types of functions of the brain.

1. *Where are synaptic vesicles generated? Where are small molecule NTs synthesized and by what mechanism are they transported into synaptic vesicles?*

Synaptic vesicles are generated by budding off from the early endosome (EE) found at the presynaptic area/terminus. This specific EE is often called a synaptic endosome. Small molecule NTs are synthesized at the presynaptic region and transported into synaptic vesicles by NT transporters. NT transporters are a type of secondary active transporter that use the proton (H+) gradient established by the H+ ATPase, to transport NTs inside synaptic vesicles, up their concentration gradient. A single synaptic vesicle contains from 3,000 to 8,000 molecules of NT, depending on the NT and the synapse.

1. *In terms of neurotransmitters and synapses, what is a neuron often named for?*

Neurons are often named for the small molecule NT they synthesize and release. For example: cholinergic, glutamatergic, GABAergic, glycinergic, dopaminergic, noradrenergic (norepinephrine releasing neurons), adrenergic (epinephrine releasing neurons), serotonergic, and histaminergic neurons. Most neurons release one small molecule NT and one neuropeptide NT from the same presynaptic terminus. Hence some neurons, such as GABAergic inhibitory interneurons located in the neocortex and other areas, are identified by the neuropeptide they co-release. For example, somatostatin or VIP expressing GABAergic interneurons. Neurons receive thousands of inputs, and those inputs can be from all the different types of neurons, for example, GABAergic, glutamatergic, dopaminergic etc, so a neuron will express lots and lots of different types of NT receptors, which are specific for the NT released at those synapses.

1. *What is the Ca2+ gradient (extracellular/intracellular) in a neuron? What specific mechanisms keep the intracellular Ca2+ levels low in a neuron (there are five)? What is meant by an intracellular Ca2+ pool? Why do neurons and other cells need to keep their intracellular Ca2+ levels so low?*

Ca2+ is a divalent cation. The Ca2+ gradient is a 10,000:1 concentration ratio between the outside and the inside. Intracellular Ca2+ is kept low by a Na+-dependent Ca2+ exchanger (secondary active transport) and a Ca2+ pump, also called the Ca2+ ATPase (primary active transporter), both located on the plasma membrane. The intracellular Ca2+ pool is the Ca2+ stored within the endoplasmic reticulum (ER) of a cell. There is a Ca2+ ATPase on the ER membrane, which pumps Ca2+ inside the ER. (It is called the SERCA pump for sarcoplasmic/endoplasmic reticulum Ca2+ ATPase, also expressed in skeletal muscle). There are also Ca2+ binding proteins in the cytosol, and mitochondria also take up Ca2+. Neurons need to keep their intracellular Ca2+ levels low because Ca2+ acts as a secondary messenger, involved in NT and NP release, and regulates gene expression. In addition, an excess of Ca2+ would lead to apoptosis, a type of cell death.

1. *Ca2+ is not required for the action potential, but Ca2+ is necessary and sufficient to induce synaptic transmission. Explain.*

The action potential (AP) requires activation of voltage gated (VG) Na+ and VG K+ channels. The AP is conducted along the length of the axon and causes depolarization of the presynaptic membrane and this depolarization causes activation of VG Ca2+ channels. Blocking VG Ca2+ channels, or buffering/removing Ca2+ in the presynaptic neuron blocks the postsynaptic response (but not the AP). Hence Ca2+ is required for synaptic transmission. An increase in presynaptic intracellular Ca2+ is the signal that is required to trigger exocytosis of synaptic vesicles and NT release. In fact, Ca2+ is likely to be the only signal that is required for this. Injection of Ca2+ into the presynaptic neuron (in the absence of an AP) is sufficient to induce synaptic vesicle fusion/exocytosis and NT release. That said, presynaptic Ca2+ does also have a number of other effects in the presynaptic neuron, including the trafficking/mobilization of synaptic vesicles among the three pools of synaptic vesicles.

1. *Describe the general structure of a voltage gated Ca2+ channel. What activates-opens VG Ca2+ channels? Where are they localized? What is the function of the non-channel auxiliary subunits (α2, β, γ and δ)?*

A voltage gated Ca2+ channel is composed of 24 transmembrane-spanning domains (including four voltage-sensing S4 regions). The voltage sensor (S4) domains contain positively charged amino acids along one side. The VG Ca2+ channel is generally similar to the VG Na+ channel but lacks the inactivation segment/gate. The VG Ca2+ channels are activated by depolarization, but they have a slightly higher threshold than the VG Na+ channel, requiring a depolarization to about -45 to -40 mV to be activated (compared with -55 mV for the VG Na+ channels). When activated, the VG Ca2+ channel changes conformation and opens, allowing Ca2+ to move through the channel as Ca2+ flows down its electrochemical gradient. VG Ca2+ channels are localized at the presynaptic plasma membrane at the active zone, very close to where the docked synaptic vesicles are located. The localization to the active zone is one of the functions of the non-channel auxiliary α2, β, γ and δ subunits. It is important the VG Ca2+ channels do not inactivate so they can accurately translate the frequency, pattern and number of presynaptic action potentials into the concentration of Ca2+ present at the presynaptic terminus, and thus, the amount and duration of NT released.

1. *How are NTs released from synaptic vesicles? What is the Ca2+ sensor for NT release and where is it found? What are SNARE proteins, where are they found and what do they mediate?*

NTs are released from synaptic vesicles after Ca2+ flows into the presynaptic terminus through the VG Ca2+ channels. The active zone is the region where the VG Ca2+ channels are localized and this is right next to where the docked and primed synaptic vesicles are located. Depolarization of the presynaptic terminus by the AP causes opening of VG Ca2+ channels and presynaptic Ca2+ levels to increase, and the Ca2+ causes fusion of the synaptic vesicles (exocytosis) and the release of NT. The Ca2+ sensor is located on the synaptic vesicle membrane and is called synaptotagmin. (Synaptotagmin has multiple transmembrane spanning domains and the Ca2+ binding domain faces the cytoplasm.) V-SNARE proteins (synaptobrevin/VAMP) are also found on the synaptic vesicle membrane and interact with the t-SNAREs (syntaxin and SNAP25) on the presynaptic plasma membrane and cytoplasm to form the SNARE complex. When synaptotagmin binds to Ca2+, it regulates the SNARE complex and this induces fusion of the vesicle with the presynaptic membrane (exocytosis) and release of contents of the vesicle, the NT, into the synaptic cleft.

1. *What happens to the synaptic vesicle after partial fusion (kiss-and-run) and what happens to the synaptic vesicle after full fusion? What does membrane recycling involve? How are new synaptic vesicles generated/produced?*

After exocytosis, the membrane is retrieved by endocytosis. After partial fusion (kiss and run), the synaptic vesicle undergoes very rapidly endocytosis before the synaptic vesicle transmembrane proteins and lipids can mix with the presynaptic plasma membrane. Thus after partial fusion, the endocytosed synaptic vesicle can be immediately refilled with NT. In the case of full fusion, following exocytosis, the synaptic vesicle transmembrane proteins and lipids have time to diffuse and comingle with the presynaptic plasma membrane proteins and lipids, and when an endocytic vesicle is pinched off from the presynaptic plasma membrane it will contain synaptic vesicle proteins and presynaptic plasma membrane proteins. Those endocytic vesicles need to traffic to and fuse with the synaptic/early endosome, and new synaptic vesicles are produced by budding off, and are then refilled with NT by the vesicular NT transporters. Membrane recycling involves the recycling of membrane proteins and lipids following exocytosis and endocytosis.

1. *In response to presynaptic action potentials, the presynaptic Ca2+ signal is usually transient and localized to the active zone. Why is this? (Hint: see question #17).*

All those mechanisms that keep intracellular Ca2+ levels low are present in the presynaptic region/terminus. Therefore, after Ca2+ flows into the presynaptic neuron through VG Ca2+channels, that Ca2+ will be rapidly pumped or exchanged out, bound to cytoplasmic Ca2+ binding proteins and sequestered into ER compartments and mitochondria. This means that the increase in presynaptic Ca2+ is transient and localized to the area around the active zone. Since the docked synaptic vesicles are close to the active zone, only those docked vesicles will fuse and this produces a small amount of NT release. However, if there is a train of APs, then the VG Ca2+ channels will open again and again, the Ca2+ increase will be much longer lasting and the Ca2+ will diffuse further into the presynaptic neuron. This will lead to the fusion of more synaptic vesicles and more NT will be released into the synaptic cleft.

1. *Describe the mechanism whereby the AP (an electrical signal) is converted to a presynaptic chemical signal (Ca2+) and then to another chemical signal (NT) at the synapse.*

When the AP arrives at the presynaptic active zone, the large depolarization of the membrane potential activates voltage gated Ca2+ channels. The Ca2+ flows into the presynaptic neuron (This is the Ca2+ signal, a chemical signal.) The Ca2+ activates synaptotagmin and the SNARE complex, causing the fusion of synaptic vesicles, exocytosis and release of NT into the cleft. (This is the NT signal, a chemical signal at the cleft.) The AP involves repolarization of the membrane potential back to the resting membrane potential, which leads to closing of the VG Ca2+ channels, and termination of synaptic vesicle fusion.

1. *What determines the amount and location of neurotransmitter release? What determines the magnitude, area and duration of the presynaptic Ca2+ signal? The voltage gated Ca2+ channels “decode” the information encoded in the AP neural code. Explain. Why is important that VG Ca2+ channels do not inactivate?*

The frequency, pattern and number of action potentials determines the magnitude, area and duration of the presynaptic Ca2+ signal. The location of the VG Ca2+ channels determines the initial location of the Ca2+ signal. The presynaptic Ca2+ signal then determines the amount and location of NT release. Information is encoded in the AP frequency/pattern/duration (the neural code). Since the VG Ca2+ channels are activated-opened by the APs, they decode the information that was encoded by the APs, and produce the presynaptic Ca2+ signal and NT release that reflects the AP code.

1. *The majority of neurons synthesize and release one small molecule NT and one neuropeptide NT. What are the different requirements for the release of small molecule NTs from synaptic vesicles and neuropeptide NTs from secretory vesicles.*

Many of the synaptic vesicles, which contain small molecule NTs, are localized very close to the presynaptic active zones where the voltage gated Ca2+ channels are localized and where the synaptic vesicles will fuse. The release of small molecule NTs from synaptic vesicles requires only low frequency action potentials, which is sufficient to produce small, local increases in Ca2+ near the active zone. In contrast, neuropeptide NTs, which are packaged into secretory vesicles, are localized much further away from the presynaptic active zone. Hence, to induce the release of neuropeptide NTs, high frequency action potentials are required. High frequency action potentials will activate the voltage gated Ca2+ channels repeatedly, which is required to produce a much larger increase in presynaptic Ca2+, so that enough Ca2+ will diffuse to induce fusion of the neuropeptide NT vesicles.

1. *What are the three mechanisms whereby neurotransmitter can be removed, terminated or their levels decreased at the synaptic cleft? Why is NT removal important?*

All NTs can diffuse from where they are released, so diffusion is one mechanism that leads to a decrease in NT levels. The presence of astrocytes and extracellular matrix around the synapse can affect the rate of diffusion of NS out of the synapse. Acetylcholine is the one NT that is specifically degraded at the cleft. The other NTs (amino acids and monoamines) are removed by uptake into neurons and astrocytes by NT transporters located on the plasma membrane. It’s not clear how purines are recycled. Neuropeptide NTs can also be degraded by nonselective proteases in the cleft. NT removal is important because we want neuronal responses to be fairly short lived and specific. For example, control of muscle contraction needs to be precise, and often short lived. When contraction is no longer needed, the muscle must be able to relax, which requires removal of the NT. In sensory systems, once the stimulus is gone, the transmission from the sensory neuron transmission needs to be stopped, which occurs by uptake of the NT. Then for all the integration and computation that the CNS controls, the signals and codes must be precisely controlled.