SYNAPSE: the specialized zone between neurons at which communication takes place. There are two major categories of synapses:

1. **Electrical** - a low resistance, high conductance channel utilizing direct connections between cells called GAP JUNCTIONS.

2. **Chemical** - NO direct contact between cells; current is dissipated in the extracellular fluid. RATHER - chemical neurotransmitters are released from synaptic vesicles in the presynaptic cell and diffuse across the synaptic cleft and bind to specialized receptors in the postsynaptic cell.

<table>
<thead>
<tr>
<th>Type of synapse</th>
<th>Distance between presynaptic and postsynaptic cell membranes (nm)</th>
<th>Synaptic continuity between presynaptic and postsynaptic cells</th>
<th>Ultrastructural components</th>
<th>Agent of transmission</th>
<th>Synaptic delay (msec)</th>
<th>Duration of transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical</td>
<td>2-5</td>
<td>Yes</td>
<td>Gap-junction channels</td>
<td>Inexcitatory</td>
<td>Usually absent</td>
<td>Usually milliseconds</td>
</tr>
<tr>
<td>Chemical</td>
<td>20-40</td>
<td>No</td>
<td>Pre-synaptic vesicles, active zones, postsynaptic receptors</td>
<td>Excitatory</td>
<td>Usually short, 1-5 sec</td>
<td>Usually several seconds</td>
</tr>
</tbody>
</table>
Chemical synapses are NOT connected structurally at the pre- and postsynaptic membranes; the synaptic cleft is actually WIDER than the adjacent extracellular space (~30-50nm). Synapses occur at specialized regions within the cells called "Active Zones" (V) which are the docking sites for the synaptic vesicles.

The general mode of transmission at all synapses is essentially the same:

3. The duration of the action potential determines how long the voltage-gated Ca channel stays open. Longer duration = greater calcium influx = more vesicles dock to membrane = more neurotransmitter released = larger post-synaptic response.

Grading of Chemical Synapses

While electrical synapses are an "all-or-none" event similar to an action potential, chemical synapses can be finely graded, based on the amount of neurotransmitter released (duration of AP) and the number of receptors available:

- as the number of synaptic vesicles released is increased, the amount of neurotransmitter available to bind to the receptors is also increased, and the probability that a receptor will be activated is also increased until all of the available receptors become saturated; this process affects the number of channels opened in the postsynaptic cell.
- therefore the PREsynaptic cell is responsible for release of the chemical messenger (neurotransmitter, size of response),
- and the POSTsynaptic cell determines the binding of the messenger to the receptor and the type of response (excitatory or inhibitory).
- The action of a neurotransmitter does NOT depend on its chemical nature or structure, but on the properties of the receptor.
- The same neurotransmitter can therefore be excitatory to one cell, but inhibitory to another, depending on the specific receptor types at each cell.
ALL chemical receptors have 2 common features:
1. They are membrane-spanning proteins.
2. They carry out an effector function in the postsynaptic cell by either directly or indirectly gating some type of response, typically via an ion channel.

Directly Gated
DIRECT: typically a single macromolecule made up of several protein subunits that forms both the receptor AND the ion channel - known as an "Ionophoric Receptor."
- The neurotransmitter binds to the receptor, which causes a conformational change and opens the ion channel.
- These types of responses are fast-acting, but typically short-lived, lasting only a few milliseconds; they typically mediate behavioral types of responses.

Indirectly Gated
INDIRECT: the receptor complex is separate from the effector complex (ion channel). Communication between the receptor and effector is accomplished by G proteins and second messengers.
- The G proteins are typically loosely bound to the postsynaptic membrane, but the second messengers are NOT, and can therefore affect distant sites within the cell such as the axon hillock, dendrites, etc.
- Indirect transmission is slow-acting, due to the numerous additional steps, but the effects can be extremely long lasting or even permanent, due to the nature of the 2nd messengers; they modulate the intrinsic excitability of the cell and may be associated with learning and memory.
INTEGRATION of synaptic input occurs at the axon hillock. In the CNS, no single synaptic event is usually sufficient to produce an AP. Therefore, you need to summate many potentials to get the necessary change in membrane potential to surpass threshold.

At the axon hillock, the membrane potential is greatly reduced, due to a higher density of voltage-gated Na⁺ channels, which results in the increased likelihood of surpassing threshold.

As the threshold decreases, the likelihood of generating an action potential increases, thereby increasing the movement of information within the CNS.

Remember that IPSPs are pulling the membrane potential AWAY from the threshold value (hyperpolarizing), while EPSPs are pushing the membrane potential TOWARDS the threshold value (depolarizing).

The axon hillock functions like an adding machine, constantly summing the EPSPs and IPSPs. Whenever the threshold is reached, an Action Potential is generated and the counter “resets.”
At all other sites within the cell, however, neuronal integration is dependent on 2 passive properties of the membrane:

1. **Time Constant** - a long time constant means a better chance for integration via "Temporal Summation."
   - typically represented as multiple inputs from a single source (cell A)

2. **Length Constant** - a long length constant means a better chance for integration via "Spatial Summation."
   - typically represented as simultaneous single input from multiple sources (cells A & B)

## Time & Length Constants

<table>
<thead>
<tr>
<th>Time Constant</th>
<th>Temporal Summation</th>
<th>Spatial Summation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long</td>
<td>Typically represented as multiple inputs from a single source (cell A)</td>
<td>Typically represented as simultaneous single input from multiple sources (cells A &amp; B)</td>
</tr>
</tbody>
</table>

## Types of Synapses

1. **Axosomatic Synapses**
   - typically inhibitory
   - via chloride channels

2. **Axodendritic Synapses**
   - typically excitatory
   - located on the shaft or specialized "spines"

3. **Axoaxonic Synapses**
   - modulatory
   - controls amount of transmitter released at the terminal

Types 1 and 2 (axodendritic and axosomatic) are known as "Mediating" synapses, because they occur before the axon hillock and can therefore aid in the production of an AP.

Type 3 (axoaxonic) synapses are strictly Modulatory, since they occur after the axon hillock and have no influence on the production of an AP, but CAN modulate its effectiveness at the presynaptic terminal.
Presynaptic Mechanisms

The propagation of the action potential to the presynaptic terminal requires primarily Na⁺ and K⁺ channels. At the presynaptic terminal, however, the presence of these channels is NOT essential to neurotransmitter release. The key ion for neurotransmitter release is Ca²⁺ through voltage-gated channels.

Increased extracellular Ca²⁺ enhances neurotransmitter release, while decreased extracellular Ca²⁺ blocks/reduces neurotransmitter release. An action potential, propagated by Na⁺ and K⁺ channels, reaches the presynaptic terminal, depolarizes the membrane, and opens voltage-gated Ca²⁺ channels, resulting in an influx of calcium.

DURATION of the AP is CRITICAL to Ca²⁺ influx; the longer the AP, the greater the influx of Ca²⁺ and the more neurotransmitter will be released.

Changes in the levels of Ca²⁺ do NOT affect the amount of neurotransmitter in a vesicle, but does affect the PROBABILITY of release of that vesicle from the presynaptic terminal.

Ca²⁺ and Vesicles

Proteins anchor vesicles to the cytoskeleton near the active zone via synapsins, which are freed by the interaction of calcium-dependent protein kinases, which make the vesicles free to bind to the active zone (guided by G proteins).

Vesicles are positioned on the membrane near the active zone and attached to the active zone; Ca²⁺ plays a vital role in the dilation of the fusion pore via proteins and protein kinases.
1. Translocation from the cytoskeleton near the active zones to the active zone itself, perhaps via an energy-dependent process involving the active zone, actin, and actin-anchoring proteins.
2. Attachment to the active zone (dense projection).
3. Contact to the docking protein at the presynaptic membrane via calcium-dependent proteins (synaptophysin, VAMP).
4. Fusion to the membrane protein (calcium dependent proteins). (see next slide)
5. Opening of the vesicle (exocytosis), which results in the extrusion of the contents into the synaptic cleft. (see next slide)
6. Collapse of the vesicle into the plasma membrane; the "vesicle" is still delimited by clathrin molecules which allows its subsequent retrieval.
7. Retrieval of the vesicle, via calcium-dependent proteins.

Calcium is required for almost every phase of neurotransmitter release and vesicle movement.

The 7 basic steps for synaptic release and retrieval are outlined below:

1. Translocation active zone to the active zone
2. Attachment to the active zone
3. Contact to the docking protein
4. Fusion to the membrane protein
5. Opening of the vesicle
6. Collapse of the vesicle
7. Retrieval of the vesicle, via calcium-dependent proteins.
Disorders Affecting the Presynaptic Terminal

Neurotransmitters

Criteria for defining a neurotransmitter:
1. It is synthesized in a neuron.
2. It is present in the presynaptic terminal and released in amounts sufficient to exert its intended action on the postsynaptic cell.
3. If applied exogenously in reasonable concentrations, it mimics exactly the action of the endogenously released substance.
4. A specific mechanism exists for its removal from the synaptic cleft.

Many neurotransmitters are synthesized “locally” at the nerve terminal, and receive the components from the soma, typically via fast axonal transport. In addition, a single synapse may release several different neurotransmitters, which may or may not interact at the postsynaptic targets — called “coexistence”.

A generalization may be made about neurotransmitters:
“A mature neuron makes use of the same combination of chemical transmitters at all of its synapses.”

Small Molecule NTs

ALL are either an amino acid or a derivative of an amino acid (and acetylcholine (ACh))

**ACh** - acetyl CoA + choline → ACh
• choline is derived ONLY from your diet
• used at ALL neuromuscular junctions, ALL preganglionic synapses, and parasympathetic postganglionic synapses, nucleus basalis (CNS).

**Biogenic Amines** - derived from amino acids
• Catecholamines - all derived from tyrosine via a common pathway:
  • tyrosine + O2 → L-DOPA → dopamine (+CO2) → norepinephrine → epinephrine
  • Dopamine is the essential neurotransmitter that “switches” for the basal ganglia (Parkinson’s).
  • Norepinephrine is used by all sympathetic postganglionic synapses, hypothalamus and the limbic system, typically via α-adrenergic receptors.
  • Epinephrine acts peripherally via β-adrenergic receptors and at the adrenal medulla.
• Indolamine - derived from tryptophan → 5-HTP → 5-HT (serotonin)
  • Serotonin is an essential neurotransmitter in the brainstem and limbic system.
• Imidazole - derived from histidine → histamine
  • Histamine is used as a “local hormone” or an “autocoid” - it acts on the cell which released it to limit the peripheral effects, found in the hypothalamus.
All amino acid transmitters are derived from universal cellular components, and therefore must be protected from degradation for other cellular processes. Therefore they are compartmentalized and isolated from the rest of the cell by placing them in synaptic vesicles. Once in a vesicle, they are NOT able to be returned to the cell; as far as the cell is concerned, they are gone.

Amino acids - such as glutamate, aspartate, glycine, GABA.

Small Molecule NTs

Principles of Neural Science, Kandel et. al., 2013
Neuroactive Peptides (NAPs)

These are secretory proteins processed by the endoplasmic reticulum and Golgi apparatus, packaged (vesicle) and then sent to the terminal via fast axonal transport mechanisms; therefore they are formed in the cell body.

There are currently over 50 different NAP's, and their actions are dependent on the target synapse; they can be excitatory, inhibitory, or act as hormones in other tissues.

Typically they are grouped as "Families" according to structure or physiological function. There are as many as 10 different families, depending on the classification used.

- Within families, many recognize similar receptors (due to similar structure), BUT the biological activities are typically not the same.
- Most are coded by genes that also translate amino acid NT's, and several NAP's are typically encoded by a single mRNA, which can therefore result in amplification and multiple copies.

Processing of NAP's takes place in the vesicle - this is a critical step in determining which peptides are released. Differences in the enzymes, cofactors, etc. within the vesicle can account for the wide variety of NAP's released. Remember that since the NAP's are created and packaged in the cell body and then transported to the synaptic terminal, there is a limited supply of neurotransmitter available at that synapse, which can limit the effectiveness of the synapse.

NAP's can coexist and be co-released along with small molecule NT's from different cells at the same synapse OR the same cell (co-transmission). The combined effect of multiple neurotransmitters can act synergistically on the target cell. Multiple neurotransmitters can also act as regulators of the presynaptic terminal of other cells at the synapse, or on its own presynaptic terminal (autocoids).

The corelease of several neurotransmitters at a synapse with the appropriate receptors permits an extraordinary diversity of actions in the postsynaptic cell.
Peptide Neurotransmitters

1. Synthesis of neurotransmitter precursor and enzymes.
2. Transport of encephaline and pre-peptide precursors. Active transport is involved.
3. Dopamine: modulates pre-peptides to produce peptide neurotransmitters.

Glial Cell uptake

AChE Transport

B. Acetylcholine

A. Monoamines

C. GABA & Glycine

D. Glutamate

Neurotransmitter Removal

Enzymatic breakdown

Return to Objectives

Postsynaptic Mechanisms

The effects of the neurotransmitter on the receptors of the postsynaptic membrane determine the response by the postsynaptic cell (excitatory or inhibitory). Recall that the response can be either direct, or indirect. The simplest form of a direct chemical synapse is the neuromuscular junction which uses a nicotinic ACh receptor.
**Total Synaptic Conductance**

The total synaptic conductance at a synapse is the sum of all of the open channels; for an entire cell an average must be used due to the large population of channels:

- \( I_{EPSP} = N \cdot P_0 \cdot \gamma \cdot (V_m - E_{EPSP}) \)

where:

- \( N \) = total number of channels in the cell
- \( P_0 \) = the probability that a channel is open
- \( \gamma \) = the conductance of an open channel
- \( V_m - E_{EPSP} \) = the driving forces acting on the ions

Of these four factors, only the total number and the probability that those channels are open is variable from cell to cell or at the NMJ, and only \( P_0 \) is typically variable at any point in time; THEREFORE the greatest factor affecting the total synaptic conductance is the probability that a channel is open.

The factor that most directly affects whether a channel is open is the concentration of available neurotransmitter at that synapse. At the NMJ this is typically about 233,333 channels!

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**NMJ vs. CNS**

Central synapses differ greatly from the NMJ in several respects:

**At the NMJ:**
1. Individual muscle fibers are innervated by a single motor neuron.
2. The synapse is excitatory ONLY.
3. The transmitter at ALL NMJ’s is ACh via nicotinic receptors.
4. The synapse is highly effective, leading to a 70mV depolarization and an AP in the sarcolemma nearly 100% of the time.

**In the CNS:**
1. Central synapses have multiple connections (thousands in the cerebellum) per cell.
2. Synapses can be either excitatory OR inhibitory.
3. There are MANY neurotransmitters used singly and in combination.
4. The effectiveness of the synapse is variable, but typically small (<2mV), and therefore dependent on the integration of MANY synapses to reach threshold.

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**CNS vs. NMJ**
**Disorders that Affect the Post-Synaptic Terminal**

<table>
<thead>
<tr>
<th>Name</th>
<th>Disease Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal NMJ</td>
<td>Normal function of the nerve terminal.</td>
</tr>
<tr>
<td>Myasthenia gravis NMJ</td>
<td>Weakened muscle contraction due to decreased acetylcholine receptor density.</td>
</tr>
</tbody>
</table>

**Synaptic Integration**

INTEGRATION of diverse inputs is THE means of communication in the CNS!

- Convergence of many excitatory inputs (EPSP's) can lead to summation.
- Inhibition (IPSP's) counteracts any excitatory input and/or decreases the output of tonically active cells, thereby “sculpturing” the firing pattern of the CNS.

**Excitatory Synapses**

Excitatory synapses generally open Na⁺ and Ca²⁺ channels. Glutamate (\(\text{glu}\)) is the major neurotransmitter, and can activate several types of receptors:

- AMPA or Kainate receptors - gate a low conductance cation channel permeable to Na⁺ and K⁺. Kainate receptors bind only glutamate, but the Kainate-Quisqualate-A receptors need zinc as a cofactor.
- NMDA receptors - gate a high conductance cation channel permeable to Na⁺, K⁺ and Ca²⁺. In addition to glutamate, these channels also bind glycine (\(\text{Gly}\)) and many cofactors such as zinc, magnesium, and PCP which regulate the opening of this channel.
- Quisqualate-B receptors - use a 2nd messenger pathway involving phospholipase C.
Inhibitory Synapses

Inhibitory synapses generally open K⁺ and Cl⁻ channels. GABA and glycine are the major neurotransmitters.

- Opening K⁺ channels hyperpolarizes the postsynaptic cell and takes that cell farther from threshold, thus making it harder for that cell to fire an action potential.
- Opening Cl⁻ channels "shunts" current away from the axon hillock by "locking" the membrane potential near the reversal potential for chloride (-60 to -65mV) and preventing the integration of subsequent EPSP’s.

Metabotropic Receptors & Second Messenger Systems

Second messenger pathways are the indirect forms of chemical transmission, and the activation of the receptor is distinct and separate from the activation of the effectors (channels). The basic outline for all 2nd messenger pathways follows the overall scheme shown here.

The neurotransmitter diffuses across the synaptic cleft and binds to the receptor. The receptor then binds and activates a transducer protein which is typically a G-protein. The activated G-protein then translocates and activates the primary effector. The activated primary effector can now assist in the production of a second messenger.

Once made, the second messenger can freely dissociate within the cell and bind to and/or activate the secondary effector (such as a channel or a protein kinase which can phosphorylate a channel), thus producing a change in the membrane potential of the postsynaptic cell.

Second messengers include cAMP, inositol triphosphate (IP₃), diacylglycerol (DAG), and Arachidonic Acid.
G-protein mediated transmission is SLOW - measured in hundreds of ms to seconds, but the effects of 2nd messengers are long-lasting, due to the creation of an intracellular messenger and the complex cascade of reactions and reaction products.

Receptors associated with G-proteins (2nd messengers) have several common features, and the process is similar for all.

G-Protein Role in Indirect Transmission

- Receptors are typically single subunit glycoproteins, with the binding site for the neurotransmitter often located within the lipid membrane portion.
- Binding of the neurotransmitter causes a conformational change which "uncovers" the binding site for the G-protein intracellularly, which then attracts an inactive G-protein (GDP) which is then replaced by an active G-protein (GTP).
- The active G-protein complex can then dissociate and the α-subunit can interact with the primary effector.
- The G-proteins typically outnumber the receptors, and therefore this is a potential site for amplification, since a single receptor can activate MANY G-proteins.

Second Messenger Systems

- The activated primary effector can now catalyze its reaction products and form its 2nd messenger. The activation of the primary effector leaves the G-protein "inactive" once again and it returns to its previous association with the other G-protein subunits. If the neurotransmitter is still bound to the receptor, the process is repeated, and this is another potential site for amplification.
- Once the neurotransmitter is broken down or dissociates from the receptor, the formation of additional 2nd messengers stops AFTER the activated G-proteins also become inactivated.

The newly created 2nd messenger is now free to interact anywhere within the cell and regulate a cellular response, depending on the nature of the 2nd messenger. Most 2nd messengers are either protein kinases or activators of protein kinases which carry out specific cellular actions.
Protein Kinases

There are many different protein kinases, and each has many functions. Most protein kinases have 2 segments: a regulatory subunit and a catalytic subunit. The regulatory subunit is the binding site or switch which activates/inactivates the kinase; the catalytic subunit is the “workhorse” part of the kinase which interacts with other proteins via a specific interaction at the phosphorylation sequence.

We will focus only on the major classifications of protein kinases: cAMP, IP$_3$/DAG, Ca$^{2+}$/Calmodulin-dependent kinase, & Arachidonic Acid.

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cAMP-dependent Kinase

cAMP activates a specific protein kinase by binding 4 molecules to the regulatory subunits, which then release two catalytic subunits into the cytoplasm, where they are free to phosphorylate proteins and activate channels.

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IP$_3$-DAG & Protein Kinase C

IP$_3$-DAG is created by phospholipase C, which cleaves inositol phosphate to produce both IP$_3$ and DAG. IP$_3$ is water soluble and diffuses into the ER and releases intracellular Ca$^{2+}$. DAG remains bound to the membrane, but activates protein kinase C, which is also dependent on Ca$^{2+}$. Protein kinase C then can phosphorylate many types of proteins.
Arachidonic Acid Pathways

Arachidonic Acid is created by phospholipase A2, which cleaves inositol phosphate to form arachidonic acid, which is then metabolized via several different pathways:

- Prostaglandins and thromboxanes - which are utilized in inflammation, injury, and control of smooth muscles in blood vessels.
- Leukotrienes - have been shown to modulate the actions of ion channels.

Termination of Kinase Activity

Phosphoprotein phosphatases terminate the actions of protein kinases. The extent and duration of phosphorylation can be controlled by inhibiting phosphatase activity via a protein labeled inhibitor-1, which is regulated by calcineurin, a Ca²⁺-activated phosphatase.

Effects of 2nd Messengers

1. Since they are not bound to the membrane, they can diffuse through the cytoplasm and affect distant areas of the cell.
2. Generally, most 2nd messengers are not voltage-dependent (but the channels they can activate may be).
3. The time course for activation of 2nd messengers is much slower than that of directly gated chemical transmission, but due to the long-lasting nature of the 2nd messengers AND the fact that these messengers are not dependent on the presence of the neurotransmitter once they are created, the effects of activation of these pathways is long-lasting - as long as the 2nd messenger and its precursors remain in the cell, they can continue to have an effect.
4. Due to the slow time course of 2nd messengers, they do NOT contribute to the formation/mediation of an action potential, but rather modulate the effectiveness of other types of synapses; they contribute to the overall excitability of a cell.
Evidence for transcellular signaling/markers

**REMEMBER:**

SLOW synaptic mechanisms **MODULATE** cellular responses;

FAST synaptic mechanisms **MEDIATE** cellular responses

Some final points about synaptic transmission through indirect receptors:

G-proteins can act directly on channels without going through a 2nd messenger cascade (this is still a form of indirect transmission because the receptor is not part of the channel).
Some final points about synaptic transmission through indirect receptors:

2nd messengers can alter and/or modulate other receptors, especially those which are part of a channel, thereby altering the effectiveness of that channel - this is the basis for many pharmaceuticals! This can result in sensitization or desensitization of a channel.

2nd messengers are typically small, and can diffuse anywhere in the cell - including the NUCLEUS - and lead to the synthesis of new proteins by altering gene expression!! This will result in extremely long term effects/changes in the cell, and can lead to the formation or loss of intracellular connections, neuronal development, the overall effectiveness of all connections, or even cell degradation and cell death. These types of changes are thought to contribute to the neuronal changes associated with long-term memory and "learning."
And finally...

Follow Rey on Instagram: reythegoldenretriever