Foundations I Fall 2016

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SYNAPTIC TRANSMISSION II

I. POSTSYNAPTIC ACTIONS

A. The Motor Endplate ACh Channel

The first and best characterized chemically gated channel is that found at the neuromuscular junction. The postjunctional receptor is a *nicotinic* cholinergic receptor (N1) that opens in response to acetylcholine or selective nicotinic agonists. This is a pentameric structure whose central region comprises a transmembrane pore. This structure resembles that of the $GABA_A$, glycine and $5HT_3$ receptor ionophores. Other acetylcholine receptors located on CNS neurons include the N2 nicotinic receptor (as well as a variety of muscarinic receptors). The EPP has a reversal potential near -5 mV, which does not correspond to the equilibrium potential of Na^+, K^+, Ca^+ or Cl^-. This receptor/channel complex is increases conductance rather non-specifically to cations, hence the reversal potential is a weighted average of the equilibrium potentials for Na^+ and K^+. It was originally proposed that ACh might simultaneously open both Na and K channels, but this idea was inconsistent with the observations that the channel current went to 0 at -5 mV, and that single channel recordings reversed at -5 mV. Analysis of the slope of a Hill plot shows that nicotinic ACh channel openings require the near simultaneous binding of two molecules of ACh, and the mean channel open time is a function of the length of time that the agonist stays bound to the receptor. This latter inference was based on the observation that channel open time varied with different agonists. The mean open time is about 1 ms with ACh, about 2 ms with suberyldicholine and about 0.5 ms with carbamylcholine.

1. Current Flow During Synaptic Activation

Synaptic current (unlike synaptic conductance) is voltage sensitive, since (from Ohm's law)

$$I_{syn}=ng(E_m-E_{syn})$$

where g is the single channel conductance (about 30 pS at the motor end plate)
and n is the number of channels open.

Thus, the synaptic current is dependent on the driving force on the ions which depends on the potential difference between the membrane potential and the reversal potential. This is just another way of saying that no net current flows at the reversal potential.

a. Reversal Potential - Although Na and K flow through the same channel at ACh synapses, it is assumed that they move independently of one another. Thus one can write separate equations for I_K and I_{Na}:

$$\Delta I_{Na}=\Delta g_{Na}(V_m-E_{Na})$$
$$\Delta I_K=\Delta g_K(V_m-E_K)$$

At the reversal potential of the EPP, by definition there is no net flow of charge, i.e.

$$I_{Na} = I_K$$

thus

$$\Delta g_{Na}(V_r-E_{Na})=-\Delta g_K(V_r-E_K)$$

(remember that the currents are opposite in sign)

If the reversal potential for a synapse is known, for example, by intracellular recording data, then the relative conductances can be calculated:

$$\frac{\Delta g_{Na}}{\Delta g_K} = \frac{-\left(V_r - E_K\right)}{\left(V_r - E_{Na}\right)}$$

Similarly, by rearranging and solving for Vr, if the conductance ratio is known, the expected reversal potential can be calculated:

$$V_r = \frac{(\Delta g_{Na}/\Delta g_K)E_{Na} + E_K}{\Delta g_{Na}/\Delta g_K + 1}$$

If we were dealing with a synapse that acted to open a conductance to only one ionic species, we could use the same logic. In this case, at the reversal potential instead of thinking of a balance between an inward Na current and an outward K current, we could think of a balance between the current induced by the synaptic conductance change and a leak current. The same equations hold, just substitute the subscripts $_{synapse}$ for $_{Na}$ and $_{leak}$ for $_{K}$

b. Experimental Reversal of Synaptic Potentials - Due to the above considerations, any synaptic potential that arises from an increase in membrane conductance should be sensitive to the membrane potential, and should change sign at the reversal potential, just as voltage-sensitive currents switch from inward to outward (or vice versa) at the reversal potential. EPSPs should reduce in amplitude with depolarizing current injections and increase with hyperpolarizing current. IPSPs should do the opposite. This is often used in intracellular recording experiments to identify depolarizing or hyperpolarizing responses to some stimulus as synaptic potentials, in contrast to extracellular field phenomena or disfacilitation or disinhibition (see below).

[In practice, it is not always possible to achieve complete reversal of synaptic potentials. If the synapse is electrotonically distant from the site of recording, it may not be possible to inject enough current to cause complete reversal (Why?). In these cases one usually extrapolates the IV curve to estimate the reversal potential. The presence of rectification can also complicate this type of analysis as well (Why?)]

c. Summation of Synaptic Potentials - The effects of multiple excitatory synaptic inputs, summed over space or time may not add linearly. Since the synaptic current is dependent on the driving force (first equation above) when a second synaptic input occurs while the membrane is still depolarized from a prior synaptic input, the driving force is reduced, and although the conductance change is identical with that of the first input, the synaptic current will be slightly less, and there will be less than linear summation. The effects of the non-linearity grow as the membrane gets farther and farther away from the resting membrane potential. The same considerations hold true for inhibitory synaptic potentials.

[This is one of the reasons that Del Castillo and Katz artificially depressed transmitter release with low Ca^{++} media for their quantal analysis studies, and is the reason that although each quantal mEPP was about 0.5 mV, it took 200-300 of them to sum to the full sized 50 mV end plate potential.]

B. Central EPSPs

The first microelectrode studies of central synaptic function were carried out in the 1950s by Sir John Eccles in the spinal cord. The synapse studied was that between the Ia sensory fiber and the a motor neuron involved in the mediation of the stretch reflex, a monosynaptic spinal cord reflex. Stimulation of a small group of Ia fibers resulted in a depolarizing potential of a few mV. More recent studies using direct intracellular stimulation of a single Ia afferent reveal that the size of the unitary EPSP is around 1 mV [a single vesicle?], far too small to evoke an action potential, although stimulation of a number of these fibers evokes an compound EPSP with maximal amplitudes of 15-20 mV.

EPSP durations and rise times vary among different CNS neurons. The rise times range from about 1 ms to as long as 20 ms, and the time constant of decay ranges from 1 to over 100 ms.

Although it was not possible to voltage clamp CNS neurons at the time, the ionic mechanisms of the Ia EPSP were inferred by Eccles and colleagues on the bases of the reversal potential of the EPSP. Similar to the neuromuscular junction, the E_{rev} was found to be near 0 mV, substantially above threshold, suggesting that conductance was simultaneously increased to Na^+ and K^+ (and perhaps also Ca^{2+}). The transmitter of the 1A fibers is glutamate.

1. Glutamate is the most ubiquitous excitatory amino acid transmitter, and is known to act through at least 4 different types of receptors: **Kainate (KA), AMPA, N-methyl-d-aspartate (NMDA) and metabotropic**. There are over 20 genes cloned at present, 7 in the NMDA family, 4 in the AMPA family, 5 in the Kainate family and 8 in the metabotropic family. Glutamate is an effective ligand at all receptors, whereas aspartate is relatively selective for the NMDA receptor. KA and AMPA receptor occupation by glutamate leads to an increase in Na^+ and K^+ conductance (and Ca^{2+} if the GluR2 subunit is *not* present – i.e. AMPA receptors lacking the GluR2 subunit **do** conduct Ca^{2+}).

a. AMPA receptors - The AMPA receptors are (semi) conventional voltage-gated channels that are permeable to Na^+ and K^+, and underlie the fast, conventional glutamate EPSP. Agonists include kainate and quisqualate. Absence of the GluR2 subunit confers (limited) permeability to Ca++ which is absent in receptors that contain the GluR2 subunit.

b. NMDA receptors - The NMDA (**N**-**m**ethyl-**d**-**A**spartate) receptor is linked to a channel with a larger conductance state that is blocked by internally by Mg^{++} when the membrane is normally polarized *(i.e., here is an example of a chemically-gated channel whose response is also voltage-dependent)*. Upon depolarization however, the NMDA receptor produces a large Ca^{++} influx along with Na^+ and K^+ (the Ca^{++} permeability is about 5x greater than that for Na^+ and K^+). This depolarization is usually achieved under physiological conditions by the simultaneous activation of AMPA receptors. Oddly enough, the NMDA-linked responses appear to be greatly facilitated or enabled by the inhibitory transmitter glycine (see below), by an allosteric mechanism whereby glycine increases the frequency of single NMDA channel openings. It is also modulated by zinc. This glycine receptor is different from the previously known glycine receptor, and is not blocked by strychnine. Glycine has no effect on the voltage-dependent conductances of AMPA receptors.

C. Central IPSPs

Central IPSPs were also first studied by Eccles in the spinal cord, at the synapse between the Renshaw cell (an inhibitory interneuron) and the a motor neuron. The time course of IPSPs is about the same as for EPSPs, although the amplitudes tend to be smaller (Why?).

1. GABA - The most ubiquitous inhibitory neurotransmitter (perhaps the most common transmitter of all) in the CNS is g-aminobutyric acid (GABA). There are 3 different GABA receptor subtypes.

a. $GABA_A$ a$GABA_A$ receptors are directly linked to Cl- channels and increase Cl- conductance when activated, thus hyperpolarizing the neuron. The IPSP is of fast onset and has a typical duration of 30-60

ms. The Renshaw cell-motor neuron synapse is a $GABA_A$ receptor synapse. $GABA_A$ receptors can be selectively blocked by *bicuculline* (competitive receptor antagonist) or *picrotoxin* (non-competitive antagonist that blocks the chloride pore).

$GABA_A$ receptors are allosterically modulated by a benzodiazepine binding site which when occupied increases the frequency of channel opening in response to GABA. There is also a barbiturate binding site which when occupied increases the binding to GABA and benzodiazepines thereby increasing the mean channel open time. There are 2 binding sites for a $GABA_A$ receptor but the binding of one molecule of GABA is sufficient to open the Cl^- channel. Binding of the 2nd molecule increases the mean channel open time. The receptor exhibits desensitization.

b. $GABA_B$ receptor - The second type of GABA receptor, the $GABA_B$ receptor produces an IPSP by increasing conductance to K^+. Unlike the $GABA_A$ receptor, this receptor is a member of the 7 transmembrane-spanning region superfamily of receptors and is G-protein linked to channel opening. Because of this, $GABA_B$ IPSPs are much slower in onset than $GABA_A$ IPSPs, and can be up to several hundred ms in duration. The $GABA_B$ receptor is a G-protein coupled receptor that opens inwardly rectifying K+ channels (GIRK). This linkage operates independent of any other 2nd messenger production. There is also evidence of direct inhibitory linkage to voltage-gated L-type calcium channels. Baclofen is a selective agonist, 2-OH-saclofen and CGP55845A selective antagonists. Location can be either pre- or postsynaptic. When is is postsynaptic, it is often found at *extrasynaptic* locations rather than subsynaptically (at the active zone) as is the case for $GABA_A$ receptors. This is one possible explanation for why it is relatively difficult to see $GABA_B$ IPSPs in the CNS unless one stimulates at high frequency. The high frequency stimulation is thought to lead to saturation of the GABA uptake transporter and the subsequent leakage of GABA to the distant extrasynaptic $GABA_B$ receptors.

c. GABA C receptor - There is also a $GABA_C$ receptor, enriched in retina but also found in the CNS that is similar to the $GABA_A$ receptor but differentiated by insensitivity to bicuculline, saclofen and benzodiazepines. It is a monomer composed of ρ (rho) subunits and is now usually called $GABA_{A\ rho}$.The receptor is slower in onset than the $GABA_A$ receptor and channel open time is longer.

2. Glycine - The next most common inhibitory neurotransmitter is glycine, found in high concentrations in the spinal cord and in brain stem. It also acts by increasing Cl conductance, but is blocked by strychnine and not by bicuculline. The Cl- ion channel is very similar to that of the $GABA_A$ receptor, and both are blocked by picrotoxin.

3. Mechanism of Inhibition - Note that even if the IPSP does not produce a significant membrane potential change, by virtue of decreasing membrane resistance, it will tend to reduce the amplitude of all electrotonic potentials, since

$$V_{EPSP} = I_{EPSP} R_m$$

Thus, the summation of EPSPs and IPSPs is not simply algebraic, and is often non-linear. An IPSP with a E_{rev} exactly equal to the resting membrane potential will still reduce the amplitude of a concurrent EPSP due to a shunting of the EPSP currents through the reduced resistance of the membrane caused by the IPSP conductance. Often the most critical synaptic action of an IPSP is to increase membrane conductance, and not necessarily to hyperpolarize.

[In practice, EPSPs and IPSPs are differentially affected by anesthetics, particularly barbiturates. These drugs tend to depress EPSP amplitudes while simultaneously increasing IPSP amplitudes. Pentobarbitone and related barbiturate anesthetics have been shown to closely mimic the effects of GABA by producing a Cl- conductance increase when administered directly to neurons, and act additively with GABA when administered together.]

D. Additional Considerations for Synaptic Potentials

1. Power test for monosynapticity

The latency of a monosynaptic response should remain invariant with increases in stimulus strength. This is because the onset latency reflects the activation of the fastest conducting fibers, which, since they will have the lowest threshold (why?) will be activated at even the lowest currents eliciting the synaptic response. A response that displays this property is said to have passed the *Power Test*, and is usually considered to be monosynaptic. The explanation for decreases in the latency of the synaptic response with increasing stimulus strength in polysynaptic pathways is that when a neuron(s) is interposed between the stimulus site and the recorded neuron, that neuron must be driven to fire a spike by the stimulus in order to propagate the chain of transmission. Increasing stimulus strength will increase the EPSP size in the interposed neurons, thus leading them to fire spikes earlier, and decreasing the latency to the observed PSP (see Park, 1987).

2. Synapse location can be inferred from the shape of the unitary EPSP

The rise time of electrotonic potentials is dependent on the membrane time constant. If two synapses differ significantly in their distance from the site of recording, there will be a greater amount of membrane between the distal synapse and the recording site versus the proximal synapse. This increased membrane area adds additional capacitance, and so the *rise time of the EPSP evoked from the distal synapse will be slower than that of the proximally evoked EPSP*. This type of analysis was used extensively by Tsukahara and colleagues (e.g., Katsamaru et al., 1986, *J. Neurosci.*) to analyze synaptic connections of the red nucleus . In this nucleus, excitatory inputs from the deep cerebellar nuclei synapse on the cell body, whereas excitatory afferents from the cerebral cortex synapse onto distal dendrites. Following lesions of the interpositus nucleus, cortical stimulation evokes EPSPs with much faster rise times, indicating that sprouting has occurred such that normally distal synapses have been relocated more proximally. [This type of analysis is only really applicable to the study of unitary PSPs or PSPs that are elicited by a single population of afferent inputs.]

E. Disinhibition and Disfacilitation - not all depolarizations and hyperpolarizations result from the action of a neurotransmitter increasing a conductance. Changes in membrane potential can also arise from a reduction in a tonic excitatory afferent tone (disfacilitation) or from a reduction in a tonic inhibitory tone (disinhibition). Because of this mechanism, these potentials respond differently to intracellular current injections than EPSPs or IPSPs, or may not be sensitive at all to membrane potential. One such example is the effect of cortical (or thalamic) stimulation on striatal neurons (Wilson et al., 1983). Afferent stimulation elicits a sequence composed of an initial, brief depolarization (20-30 ms) that may trigger action potentials, followed by a longer-lasting hyperpolarization (100-250 ms). When constant hyperpolarizing current is passed through the neuron, the initial depolarization is increased in size, and when depolarizing current is passed, it decreases in size. This demonstrates that it is a synaptic potential, and the fact that it can trigger spikes confirms it as an EPSP. However, the amplitude of the subsequent hyperpolarization shows only a small dependence on membrane potential, and it too decreases in size with depolarization and increases with hyperpolarization, just the opposite of what should happen with a synaptically induced increase in G_{Cl-} or G_{K+}. The IV curves show that the apparent reversal potential of the hyperpolarization is the same as that for the early depolarization. Finally, when one monitors the input resistance of the neuron, one finds that although conductance is increased during the EPSP, it is decreased during the hyperpolarization. Thus, the hyperpolarization is not a synaptic potential at all, but is a disfacilitation that originates from a prolonged reduction in tonic cortical excitatory input. Excitation can occur by an analogous process when a tonically active inhibitory input is removed. This is termed *disinhibition*.

F. Conductance-decreasing Synapses

1. M-Current - There exists a class of muscarinic ACh receptors that act to reduce the conductance of a K channel that is open at rest (LHRH acting on its own receptors also shuts off the M-Current). The K current that is inhibited by ACh is called the M-current. The result of cholinergic synaptic input is a depolarization accompanied by an decrease in input conductance. (What is the reversal potential for this synapse?) The increased membrane resistance serves to increase the excitability of the neuron for exactly the same reason as a conventional IPSP decreases it, i.e., a change in membrane resistance. The depolarization that results from the reduction in the M-current further contributes to increased excitability.

2. S-Current - A very similar phenomenon obtains in some Aplysia sensory interneurons in response to serotonergic synaptic input. A noxious stimulus to the head or tail of Aplysia causes a facilitation of the gill-withdrawal reflex. 5-HT closes a K^+ channel that is open at rest resulting in a prolonged presynaptic spike in an excitatory interneuron which leads to increased transmitter release onto the motor neurons by the main sensory neurons controlling the gill withdrawal reflex.

a. Slow synaptic Responses - Both of the synaptic responses mentioned above are much slower in time course than the typical "fast" EPSPs and IPSPs discussed earlier. Some, for example the late slow EPSP in frog sympathetic ganglia mediated by LHRH last for minutes. One hypothesis to account for the slow onset and long duration of these slow synaptic responses is that the linkage between the receptor occupation and ion channel opening is indirect, and is mediated by a second messenger system, e.g., cAMP. This has been shown to be the case for the S-Current in Aplysia.

G. Presynaptic inhibition - Sometimes stimulation of a pathway results in an increase or decrease in the excitability of a postsynaptic neuron that is not accompanied by any change in membrane conductance, but results from a change in the amount of transmitter released from an afferent.

1. Spinal Cord. In the late 1930s it was discovered that stimulation of the dorsal root produced a long-lasting inhibition of spinal flexor reflexes that was co-incident with a slow depolarizing potential in the dorsal cord, called the dorsal root potential. Subsequent intracellular recording studies revealed that no change in membrane potential or input conductance of the motor neuron could be detected during this inhibition, nor was there any change in motor neuron excitability to direct intracellular stimulation or antidromic activation. The original hypothesis to account for this phenomenon was one of "remote inhibition", whereby the inhibitory afferents were presumed to synapse far enough out on the dendrites of the motor neuron so that neither the inhibitory potential or the conductance change could be detected by the recording electrode at the soma, but which still summed destructively with the EPSP. [This turned out not to be the case, but it is important to note that with small CNS neurons with extensive and highly branched dendritic trees, it is often not possible to pass enough current through the recording electrode to completely reverse a synaptic potential.]

The true cause of the EPSP reduction was found to result from the action of an axo-axonic synapse onto the terminals of the Ia afferent. When presynaptic inhibition was active, stimulation of the Ia terminals in the dorsal horn caused an increase in the size of the antidromic compound action potential recorded from a group of Ia afferents outside the cord. This was interpreted as resulting from a depolarization of the Ia terminals, termed *primary afferent depolarization*. Subsequent experiments revealed that the depolarization was caused by the synaptic release of GABA onto the Ia terminals that acted to increase a Cl- conductance, thereby depolarizing the terminals.

[The results of these experiments led to confusion with respect to the mechanism of the inhibition of transmitter release by the presynaptic depolarization. A widely held view claimed that the amount of transmitter release was a direct function of the absolute presynaptic spike height (i.e., the difference between the resting potential and the peak voltage of the spike). Thus, it was claimed that a presynaptic spike in a terminal sitting at -70 mV that reached

a maximum voltage of 20 mV (for a total shift of 90 mV) would release more transmitter than a presynaptic spike in a terminal sitting at -60 mV that reached the same maximum voltage (for a total shift of 80 mV).]

2. Central Monoamine Nerve Terminals - If slices of striatum are pre-loaded with tyrosine or DOPA and then stimulated, one can measure the efflux of dopamine. If the experiment is repeated in the presence of a dopamine agonist such as dopamine itself or apomorphine, the stimulated efflux is markedly reduced. A dopamine antagonist produces increased release. This type of presynaptic inhibition is due to the action of dopamine at the dopamine terminal through a dopamine D_2 receptor which is termed an *autoreceptor*, since it is sensitive to the transmitter normally released by the neuron. Exactly the same phenomenon occurs with norepinephrine and serotonin release by stimulation of a_2 or $5HT_{1a}$ receptors, respectively. In vivo electrophysiological experiments indicate that autoreceptor activation produces a decreased excitability of the monoamine terminals, suggesting that the terminals are hyperpolarized when they release less transmitter. How can depolarization of terminals (as in the spinal cord) and hyperpolarization of terminals (as in monoamine terminals) both produce decreases in neurotransmitter release? What is the mechanism?

3. Crayfish Neuromuscular Junction - The answer can be found in an elegant demonstration by Baxter and Bittner (1981). The crayfish claw muscle is innervated by an excitor axon and an inhibitor axon. In addition, the excitor axon receives an axo-axonic synapse from a presynaptic inhibitor axon very near to the end plate. Both types of inhibitor synapse use GABA as a transmitter. The excitor axon is large enough so that intracellular recording is possible very near to the sites of transmitter release. Baxter and Bittner recorded from a number of these axons, and found that they had a mean resting membrane potential of about -70 mV, some with higher and some with lower resting potentials. In those axons with more hyperpolarized resting potentials, stimulation of the presynaptic inhibitory axon produced depolarization of the excitor axon. In those axons with less hyperpolarized resting potentials, stimulation of the presynaptic inhibitory axon produced hyperpolarization of the excitor axon terminals. This suggested that the reversal potential for GABA in this system was almost exactly equal to the resting membrane potential. But in each case, there was a reduction in the amplitude of the muscle EJP. The magnitude of the EJP reduction was independent of the sign of the presynaptic potential, and even more compelling, the time course of the EJP reduction was only a few ms, far shorter than the time course of the change in presynaptic potential. This time course was, however, almost exactly equal to that of the presynaptic conductance change. Baxter and Bittner concluded that the change in presynaptic membrane potential was epiphenomenological to the presynaptic inhibition, which only depended on the increase in terminal membrane conductance that served to shunt presynaptic action currents thereby reducing the time course and amplitude of the presynaptic spike and subsequent calcium entry.

G. Co-localization, Dale's law, and Eccles postulate - a digression

It is sometimes said that the existence of more than one neuroactive substance in a nerve terminal violates “Dale’s Law”, stated below.

1. Dale's Law (1930s)

"The transmitter used by nerve-fibres of a particular kind, and concentrated at their endings in readiness for release is also to be found along the whole length of the fibres; a transmitter is characteristic, then, not only of the endings, but of the whole neurone?.....What shall we expect of such a substance? The synaptic endings in the central nervous system represent only one end of these dorsal root fibres. Are we to expect that the transmitter at their central, synaptic endings would also be functional at their peripheral endings, and possibly be there concerned with transmitting the so-called antidromic vasodilator action?"

[It seems pretty clear that although Dale never envisioned the existence of co-transmitters (e.g., peptides), his “Law” (so named by J.C. Eccles in 1952) is not invalidated by the fact of co-localization. Dale’s Law simply

suggests that if a neuron releases a given transmitter at one of its release site, then that same substance would also be released at all other release sites. Thus far, there are no data to counter this proposal; that is, we know of no examples where a neuron releases one transmitter at one it its synaptic endings and a different one at a different ending (*at least we didn't when these note were first written*. Now, however, the evidence for colocalization of more than one classical neurotransmitter within CNS neurons, each of which can be shown to exert measurable postsynaptic effects, is growing almost weekly. Neurons that express GABA and glutamate and/or dopamine have been shown, and the postsynaptic effects are sometimes limited to subsets of the neurons that these cells innervate. Given recent findings about finely tuned mechanisms for intracellular targeting of proteins and other substances, it is not hard to envision that there might be segregation of one colocalized neurotransmitter to one set of presynaptic boutons on a neuron while a different neurotransmitter could be contained within a different set of boutons.

2. Eccles' Law (mid-1950's)

Eccles proposed another general rule for synaptic transmission that stated that

> "..in the central nervous system, any particular transmitter always acts by opening the same ionic gates."

[This law has not fared nearly as well as Dale's. Clearly, the postsynaptic effects of a given transmitter are dependent on the particular receptor/ion channel complex that is activated. Back in the 1950's all positively identified GABAergic synapses acted by opening up a conductance to chloride, but we now have identified subtypes of GABA receptors; $GABA_A$ receptors are chloride ionophores but $GABA_B$ receptors are potassium ionophores. Similar receptor subtypes exist for virtually all neurotransmitter receptors, and in many cases, the ionic mechanisms are very different among subtypes. Eccles law is thus not valid.]

3. Co-localization (1980's)

Many different examples now exist of multiple (at least potentially) neuroactive substance being localized within the same nerve terminal. Most often these are comprised of a classical neurotransmitter and a peptide, e.g., DA-CCK, NE-neuropeptide Y, GABA-enkephalin, GABA-substance P, glutamate and tachykinins, ACh and VIP, etc. Between 25 and 75% of noradrenergic neurons in peripheral ganglia contain NPY, and it is quite likely that co-localization of neuroactive substances will become the rule rather than the exception.

a. Where are the peptides stored? - In general, the peptides are not co-localized in the same synaptic vesicles as the classical transmitter. Immunoelectron microscopy reveals that classical transmitters are stored in 40-50 nm synaptic vesicles, and possibly also in larger 100 nm vesicles, whereas peptides appear to be contained only within the larger dense-core vesicles. It is possible to virtually eliminate monoamine stores with reserpine (which depletes monoamine vesicles of their contents) without affecting levels of substance P stored in the same terminals.

b. Co-localization does not necessarily imply significant co-release. - Most peptides are, in general, not present in as high a concentration in presynaptic terminals as classical transmitters.

c. Co-release does not necessarily imply synaptic function. - According to Lundberg, most co-stored peptides are probably not released at any significant concentrations under basal conditions and the concentrations detected may be too low to affect postsynaptic receptors. In some cases, postsynaptic targets lack receptors for the co-localized peptide. For example, in frog heart, somatostatin appears to be co-released with ACh from the vagus, but there is no apparent postsynaptic effect of somatostatin on heart muscle. However, there are other cases in which clear effects of the co-localized peptide have been observed (e.g., CCK from DA-CCK neurons in the CNS and TRH from 5-HT-TRH neurons in spinal cord).

[Of course, neuropeptides may influence postsynaptic neurons by means other than altering ion conductance and/or membrane potential, as do many hormones. Thus, the fact that there may be no immediately observable change in the

electrophysiological properties of a neuron as a result of neuropeptide administration is not the same thing as saying that the peptide has no effect.]

d. Can there be differential release?

Stimulation of peripheral sympathetic neurons at relatively low and steady rates evokes a substantial release of NE and some release of NPY. However, changing the pattern of stimulation to that of short bursts, while equalizing the total number of pulses per second alters the release properties such that NE release increases only slightly whereas NPY release increases significantly. Similar effects have also been proposed for CNS neurons.

On the other hand, presynaptic modulation of transmitter release appears to operate in parallel for NE and NPY. The stimulated release of both is decreased by a_2 agonists and increased for both by a_2 antagonists. This suggests that the mechanism of release of the co-stored peptide is similar or identical to that of the classical transmitter and requires Ca^{++} influx, presumably for exocytosis.

4. Co-localization (2010's)

Things are different today (Jagger, Richards, 1966). Selective activation of neurons following transduction with virally carried light sensitive opsins like channelRhodopsin 2 (ChR2) have allowed physiologists to stimulate certain neuron types with unprecedented precision. In many cases this has resulted in unexpected findings of multiple transmitters released by single populations of neurons. So far this does no appear to be an artifact and cholinergic neurons have been shown to co-release glutamate (or perhaps they are really glutamate neurons co-releasing ACh?) and dopamine neurons have been shown to co-release glutamate and GABA. The field is still struggling to make sense out of these data, but whatever the explanation, synaptic transmission in the CNS appears to be far more complex than our vision of it in the 1950s.