Foundations I Fall 2016
J.M. Tepper

SYNAPTIC TRANSMISSION I

I. Introduction

DuBois Reymond (1877) was the first to suggest that nerve-muscle transmission could be either chemical or electrical in nature, and favored the chemical hypothesis. Elliot (1904) observed that adrenal extract mimicked the effects of sympathetic nerve stimulation on smooth muscle contraction, and Dixon (1906) proposed that parasympathetic nerves liberated a muscarine-like substance onto their effector organs. As late as the end of the first quarter of this century, there was still a great debate as to whether central synaptic transmission was electrical or chemical in nature. Transmission from neuron to neuron or neuron to muscle was so fast that it seemed likely that an electrical phenomenon must be occurring and in the periphery, the effects of nerve stimulation or application of these proposed transmitter substances often took place with latencies and durations of hundreds of ms or seconds. However, there were certain conceptual problems. For example, how could a small fiber, only a few microns in diameter provide a large enough current source to depolarize a muscle fiber or a cell body with hundreds or thousand of times its membrane area?

A. Loewi (1921) - took a frog heart with the vagus nerve intact and placed it in a perfusing bath. He stimulated the vagus, and the heart slowed down. When he directed the perfusate over another heart, the second one also slowed down, thus establishing that stimulation of the vagus liberated a substance into the bath that slowed the heart. Subsequent bioassays revealed that the substance "vagusstoff" was ACh.

B. As late as 1949, although it was fairly well accepted that neuromuscular transmission was chemical in nature, there was still controversy regarding the nature of the CNS transmission mechanism. Not surprisingly, most of the pharmacologists believed that transmission must be chemical, whereas most of the electrophysiologists (including Sir John Eccles) favored electrical transmission. Part of the problem revolved around the assumption that ACh would also be found to be the central neurotransmitter, but levels of ACh and choline acetyltransferase were very low in primary afferents of the dorsal root, and no central cholinergic synapse had yet been identified.

C. Just when those advocating electrical transmission had just about given up, Furshpan and Potter (1957, 1959) provided conclusive evidence for its existence in a "new" synapse in the crayfish.

II. The neuromuscular junction

A. Structure

1. Myelinated motor neuron axons lose their myelin sheath and arborize into many 1.5 μm diameter processes that run for up to 100 μm (in mammals; several hundred μm in amphibians) along the surface of a single muscle fiber in a restricted region called the *motor end plate*. Each muscle fiber is innervated by only one motorneuron but a single motor neuron may innervate many muscle fibers depending on the size and function of the muscle. At hundreds of locations along the presynaptic axon, there are accumulations of 40 nm synaptic vesicles each containing approximately 10,000 molecules of ACh. Underneath these sites, there are special infoldings of the muscle fiber termed *junctional folds* into which the axon penetrates, separated from the muscle fiber by a 50 nm junctional cleft. The entire arrangement is covered by a thin layer of Schwann cells.

B. Quantal nature of synaptic transmission

Del Castillo and Katz (1954) wished to study the nature and mechanisms of neurotransmission at the neuromuscular junction, the most accessible synapse at the time. Normally, the EPSP (or more properly the EPP) at the skeletal neuromuscular junction is very large and nearly constant in amplitude, on the order of 50 or 60 mV, and when the motor neuron is stimulated, always evokes an action potential in the postsynaptic muscle fiber. This is now known to result from the near simultaneous release of several hundred ACh-containing vesicles. This is not very conducive to studying the electrophysiological and biophysical nature of synaptic transmission. However, it is possible to reduce the size of the EJP by a number of means, including blockade

of some of the postsynaptic receptors by selective antagonists such as *curare*, a competitive antagonist isolated from the bark of the cinchona tree or *a-bungarotoxin*, a nearly irreversible antagonist isolated from snake venom. Alternatively, EPP size can be reduced by bathing the preparation in low Ca^{++} and /or high Mg^{++} media to depress ACh release. [This was also necessary in order to reduce the effects of synaptic non-linearity (to be discussed later) that causes single 0.5 mV MEPPs to fail to sum linearly- in fact 200-300 MEPPs are needed to produce the full-sized EPP.] When this is done, the large EPP is abolished and stimulation of the motor neuron (or spontaneous release) produces much smaller EPPs called mEPPs. These varied in size with a minimal mean amplitude around 0.5 mV and had the same duration as the full-sized EPP. Occasionally mEPPs exhibited amplitudes that appeared to be integral multiples of this minimal size. Del Castillo and Katz were interested in explaining the variable amplitude and frequency of the MEPPs.

1. Early experiments revealed that mEPPs could not result from the effects of a single molecule of ACh since iontophoresis or bath experiments with small concentrations (many molecules) did not give a discernible response. Thus, the mEPPs must result from the action of thousands of ACh molecules.

2. A statistical treatment was necessary since they were dealing with a phenomenon (mEPP size) that varied in amplitude and frequency. They assumed that at the neuromuscular junction there was a large population of n units of undetermined nature that respond to a nerve impulse. If the probability of a single unit responding is p, and if each unit has an independent and equal p, then the mean number of units responding to each stimulus is given by:

$$m=np$$

This is sometimes referred to as the *mean quantal content*. Under these conditions, the relative occurrence of multiple events (i.e., mEPPs of sizes corresponding to integral multiples of the minimum mEPP size) is given by the *Binomial distribution*:

$$n_x=N\frac{n!}{(n-x)!x!}p^x q^{(n-x)}$$

where N is the number of trials, n_x is the number of events consisting of x units (quanta) and q is 1-p., i.e., the probability of the unit not responding. If we divide the number of events of a given magnitude by the total number of trials, we get the probability P_x that an event will consist of x units.

$$P_x=\frac{n!}{(n-x)!x!}p^x q^{(n-x)}$$

Del Castillo and Katz had a problem applying this distribution to their mEPP amplitudes, however, since it requires that one know the values of n and p, and all they knew from their experimental data was m, the product of n and p. However, in their experiments, they assumed an artificially reduced p, since they were running in low Ca^{++}/ high Mg^{++} media.

$$P_x=\frac{m^x}{x!}e^{-m}$$

This has the advantage that it can be calculated on the basis of m alone, and m can be estimated in two independent ways directly from measurement of the amplitude and/or frequency of evoked mEPPs:

1. m= mean amplitude of synaptic potential/mean amplitude of minimal synaptic potential
or

2. If one only counts the failures (x=0, mEPP amplitude = 0), the Poisson distribution degenerates to

$$n_0=Ne^{-m}$$

and thus

$$m=\ln\left(\frac{N}{n_0}\right)$$

Both measures yield very similar results using real data and predict the actual distribution of MEPP amplitudes very well. Thus, synaptic transmission at the neuromuscular junction was shown to be quantal in nature. It was later shown that there were approximately 10,00 molecules of ACh per quantum and it was inferred that the quantum was, in fact, the contents of a single synaptic vesicle.

3. Alternative Statistical Descriptions of Quantal Release

a. The analyses described above were applied to data from the neuromuscular junction and under artificial conditions of lowered transmitter release. But there are many structural differences between the neuromuscular junction and CNS synapses that may imply functional differences. Recall that the Poisson model assumes that only a small fraction of the available quanta are released with each trial, and that the probability of release for each is very small, i.e., there are many failures. If these conditions are not met, then the Poisson model does not apply and the Binomial distribution must be used. This turns out to be the case in many situations, particularly with respect to CNS synapses.

b. In 1982, Faber, Korn and associates (Korn et al., 1982) intracellularly recorded unitary-evoked IPSPs from the goldfish Mauthner cell while stimulating an inhibitory interneuron that made monosynaptic connections with the Mauthner cell with an second intracellular HRP-containing microelectrode. At the completion of the experiment, the interneuron was filled with HRP, and the number of presynaptic boutons synapsing on the Mauthner cell was examined. The binomial parameters n and p were calculated by a program that took the raw data and obtained the best fit to a binomial and Poisson distribution. *It was discovered that p was relatively constant; it was high (0.2-0.7) for some terminals (mean 0.42) and essentially 0 for others, and that n was usually exactly equal to the number of afferent boutons revealed by the HRP staining.* The IPSP amplitudes were fit much better by the binomial than by the Poisson distribution. The identity of a presynaptic terminal as the quantal unit means that the postsynaptic effect of the activation of a presynaptic bouton is relatively constant. This could happen if the activation of each bouton released a relatively large and constant number of vesicles, or conversely, if the activation of a bouton led to the release of only a single vesicle. Korn and Faber calculated the total conductance resulting from activation of a unitary PSP from the voltage change at about 3.5×10^{-8} S. Assuming, based on single channel recordings that the conductance of a single channel opened by the transmitter was 25 pS, this means that each quantal event leads to the opening of about 1400 channels. This is a very small number! Thus, at each release site, no more than one vesicle can undergo exocytosis after each presynaptic impulse.

c. Studies of synaptic depression in this preparation (a decrease in postsynaptic response following high frequency stimulation) revealed that this occurred due to a decrease in p, with n remaining unchanged. Essentially identical findings have also been reported with mammalian CNS synapses. In mammalian spinal cord and dorsal root ganglia, the binomial n was found to be consistently less than the histological n by 1 or 2, perhaps indicating that as suggested by Faber and colleagues for the Mauthner cell, but even more common in the mammalian CNS, there were a number of "silent" synapses in which p was close to zero. Ultrastructural analyses of HRP-labeled presynaptic boutons in this study revealed that many of the synapses consisted of multiple active zones (see below), but that in all cases, it still appeared that only one vesicle was released per bouton, regardless of the number of active zones (Neale et al., 1983). These and other considerations led to the suggestion that *the basic "unit" of central synaptic transmission is functionally equivalent to a presynaptic bouton, and that each bouton functions such that upon stimulation, it either releases one vesicle or not, in a binary fashion.* (Korn et al., 1982; Korn and Faber, 1987; Neale et al., 1983, Korn and Faber, 1987).

[Although the idea that presynaptic terminals operate in a binary fashion may seem heretical, in 1961 Eccles proposed that IA afferent boutons may release no more than one quantum, or at most 2 or 3 onto the spinal motor neuron on the basis of his recordings. Similar proposals were made by others studying IA EPSP fluctuations in mammalian CNS. Korn and Faber (1987) suggest that these important observations were overshadowed by the advent of the use of drugs to evoke massive, non-physiological release of transmitter, and were, for the most part, forgotten.]

III. Central Synaptic Transmission

The term synapse (*Greek*: to clasp) was originally proposed by Sir Charles Sherrington (1897) based largely on deductive reasoning obtained from his study of spinal reflexes and the neuroanatomical work of Ramon y Cajal *viz a viz* the neuron doctrine. The first good images of synapses were not obtained until the application of electron microscopy to biology in the early 1950s. Much of our basic knowledge about central synaptic

transmission was discovered by Sir John Eccles (a student of Sherrington's) with *in vivo* intracellular recordings in cats in a career stretching from the 1930's to the 1980's.

A. Structure of CNS Chemical Synapses

1. Presynaptic bouton - most range in size from about 0.5 - 2 μm in diameter in the CNS. [The mossy fiber terminals arising from the granule cells of the dentate gyrus are the largest in the brain, about 5 μm in diameter.] They often contain mitochondria.
   a. Synaptic vesicles - termed *small* (40 nm) or *large* (50 nm) in diameter, *round*, *flattened* or *pleomorphic* (many different shapes), *electron lucent* or *large dense core* .

   b. Active zone - typically circular in shape in CNS, rod shaped at neuromuscular junctions and some sensory synapses, rarely exceeds 0.2 μm in diameter, often reveal dense aggregation of vesicles - believed to be site of vesicle-membrane fusion and exocytosis. A single bouton may have more than one active zone (perforated synapses). The presynaptic membrane at the active zone has considerable structural specialization that has been described as a "crystalline lattice" by some. Projections of the plasma membrane (dense projections) and the lattice appear to form "docking sites" where synaptic vesicles are closely packed together, opposed to the plasma membrane. A protein found in the membranes of synaptic vesicles, synaptobrevin, and syntaxin, another similar one found in the membrane of the active zone, play a role in the docking of vesicles to the active zone. Evidence that these are critical for the release process comes from the effects of various bacterial botulinus toxins that destroy one or the other of these proteins, and prevents release of ACh from the neuromuscular junction.

2. Synaptic cleft - usually reflects a *widening* of the intercellular space - 20-30 nm thick, filled with osmiophillic substance.

3. Synapse classification
   a. Gray's type I (asymmetric, 30 nm cleft, small [40 nm] round vesicles) = excitatory, usually made onto the heads of dendritic spines, dendritic shafts or occasionally, somata.

   b. Gray's type II (symmetric, 20 nm cleft, small or large [50 nm] pleomorphic vesicles) = inhibitory, almost never on the heads of dendritic spines, often spine shafts, dendritic shafts, or somata.

   [The vesicle morphology is almost certainly an artifact of fixation, albeit a useful one. Nevertheless, most of the time, with aldehyde fixation, glutamate-containing vesicles appear small and round and GABA-containing vesicles appear pleomorphic or flattened.]

   The association of Gray's Type I synapses with excitatory input and Gray's Type II with inhibitory has so far been demonstrated to hold for many parts of the nervous system including hippocampus, cerebral and cerebellar cortices, and crustacean neuromuscular junctions, but is not to be considered a hard and fast rule. Note that area of synaptic contact is only a fraction of the size of the presynaptic terminal (usually something on the order of 10% of the presynaptic bouton surface or so). Thus, it is possible to miss seeing the synaptic specialization in random EM photos unless serial sections are taken. This may account for the claims of some that there are CNS presynaptic terminals that do not make synapses. One such frequently cited example is that of monoamine axon terminals in cerebral cortex or neostriatum. However, when careful serial section analysis is performed, it has been demonstrated that all such terminals make synapses. This is not the case in the peripheral nervous system, and near blood vessels where "loose in the juice" non-synaptic release of monoamines occurs.

B. Basic steps in synaptic transmission

1. A presynaptic impulse invades the terminal bouton and depolarizes it.

2. The depolarization opens voltage sensitive Ca^{++} channels leading to a transient influx of Ca^{++}.

3. $[Ca]_i$ triggers a sequence of biochemical events resulting in the fusion of a synaptic vesicle membrane with the terminal membrane (see below).

4. The vesicle contents are extruded into the synaptic cleft in a process called *exocytosis*.

4a. The vesicle membrane is incorporated into the presynaptic terminal membrane within ~ 50 msec of fusion. Excess membrane and vesicle components are recycled by endocytosis at sites outside the active zone into clatherin-coated vesicles that eventually lose the coating and are re-used.

5. $[Ca]_i$ is immediately deactivated by uptake into mitochondria and presynaptic vesicles.

6. Released transmitter diffuses across synaptic cleft (very fast, no more than a few tens of microseconds).

7. Transmitter combines with postsynaptic receptors.

8. Receptor linked with ion channels and/or intracellular second messengers is activated and alters the permeability to certain ions and/or or cause an intracellular biochemical event.

a. Neurotransmitter-sensitive ion channels are different than voltage-dependent ion channels, i.e., they are electrically inexcitable. Since they are electrically inexcitable, the synaptic potential is not regenerative like the action potential, but passive (electrotonic). [For this reason, the electrotonic properties of the membrane dictate to a large extent the integrating properties of synaptic inputs. High membrane resistance favors long time and space constants and thus increases temporal and spatial summation.]

b. Most synaptic potentials are due to an local *increase in membrane conductance* [decrease in membrane resistance] to a specific ion or ions.

1. Most depolarizing synaptic potentials are due to an increase or $G_{Na}+$ *and* G_{K+} (e.g., nicotinic ACh receptor) which produces an inward current. [Ca^{++} plays a role in some synapses as well, e.g., the NMDA channel.]

2. Hyperpolarizing synaptic potentials are due to an increase in $G_{Cl}-$ (e.g. $GABA_A$ receptor) or G_K+ (e.g., $GABA_B$, dopamine D_2, adrenergic a_2, and $5HT_1$ receptors) causing an outward current.

3. Very few ions have to move in order to achieve large changes in E_m . To produce a 130 mV change in membrane potential requires only $1.3x10^{-12}$ moles crossing a 1 cm^2 area of membrane, ~$8x10^{10}$ ions.

4. The conductance change is significantly shorter (2-5 ms) than the potential change (5-50 ms).

c. The equilibrium potential for a synaptic conductance change is called the *reversal potential* (E_{rev}). Note that this is dependent on the particular ion channel(s) opened and not the neurotransmitter per se. In other words, neurotransmitters themselves are not “excitatory” or “inhibitory” - that is something that derives from the effector coupling of the postsynaptic receptor.

d. Synaptic potentials that make the postsynaptic neuron more likely to fire an action potential are termed excitatory postsynaptic potentials (EPSP) and those that reduce the probability of the postsynaptic neuron firing are termed inhibitory postsynaptic potentials (IPSP) - note that this means that *not all depolarizing synaptic potentials are EPSPs and not all hyperpolarizing potentials are IPSPs*.

1. The distinction between EPSPs and IPSPs depends on the relation between the reversal potential of the synaptic conductance change and the spike threshold, not on whether or not the synaptic potential is depolarizing or hyperpolarizing. For example, if the spike threshold were -55 mV, and the resting membrane potential were -75 mV, then a synapse that opened up a Cl- conductance which had reversal potential of -65 mV would elicit a depolarization, but that depolarization would be an IPSP, not an EPSP. No matter how much such a conductance were increased, the membrane potential would never rise above threshold, and the neuron would be effectively voltage-clamped at -65 mV.

2. For inhibitory synapses, the primary mechanism is often a shunting of the effects of other, excitatory inputs or of intrinsic membrane currents due to the increased conductance resulting from the action of the inhibitory synapse. In other words, inhibition can (and often is) achieved without significant changes in the membrane potential of the postsynaptic neuron. More on this later.

e. The synaptic current that flows when a synapse is activated is dependent on the *driving force* on the ion[s] whose conductance is increased. Note that this is not E_{rev} but the difference between E_m and E_{rev} as we showed previously in our consideration of the Nernst equilibrium potential.

C. What are the critical steps in transmitter release?

Katz and Miledi (1965-1967) - Simultaneous pre- and postsynaptic intracellular recordings from the giant synapse of the stellate ganglion of the squid.

1. Na^+ influx is not necessary . Transmitter release is proportional to presynaptic depolarization but Na influx is not necessary - blockade of presynaptic spike by TTX eliminates the postsynaptic response to nerve stimulation, but not to direct depolarization. There is a threshold of about a 50 mV depolarization prior to which presynaptic depolarization fails to evoke release.

2. K^+ efflux is not necessary. Blockade of presynaptic K channels by TEA does not affect transmitter release evoked by direct intracellular depolarization.

3. Ca^{++} influx is necessary. In a Ca^{++} free medium, neither nerve stimulation nor intracellular depolarization elicits release. Furthermore, extracellular Ca^{++} must be present during a critical time, just prior to the presynaptic depolarization. In more recent voltage clamp experiments by Llinas (a student of Eccles), clamping to a membrane potential near the E_{Ca} does not induce transmitter release until the clamp is released (thus allowing the membrane potential to decrease to below E_{Ca} whereupon Ca^{++} can enter.

3a. It's not really the influx of Ca^{++}, i.e., the Ca^{++} spike itself that causes transmitter release since direct intracellular injection of Ca^{++} can cause release. Rather it is the $[Ca]_i$ that is critical for release. Since $[Ca]_i$ is normally kept at very low levels, under normal physiological conditions the only way to elevate $[Ca]_i$ is by increasing $g_{Ca}{}^{++}$.

1. The synaptic delay (200-500 µsec) is too long to be accounted for by transmitter diffusion (max 50 µsec). It's also too temperature sensitive for a strictly diffusional process- the delay can be extended to several seconds by cooling. Most of time is taken up by the opening of Ca^{++} channels, intracellular movement of Ca^{++} and vesicle-membrane fusion, which is a very complex process involving dozens of proteins..

2. Control of Exocytosis The precise mechanisms whereby calcium promotes vesicular release is still unclear. Ca^{++} itself may act directly to promote the fusion of the vesicle membrane with the plasma membrane, or it may act through an intermediary protein such as calmodulin, a 65 kD calcium biding protein, or a calmodulin-dependent protein kinase.

a. Synapsin I is a protein that is found in close association with synaptic vesicles. In its dephosphorylated state, synapsin is believed to bind the vesicle to cytoskeletal proteins thereby immobilizing the vesicle. When phosphorylated, synapsin loses its ability to bind to these proteins. Since synapsin is a substrate for *Ca^{++}/calmodulin-dependent protein kinase s(CAM kinase I and II)*, it has been suggested that the role of Ca^{++} in synaptic transmission is to lead to the phosphorylation of Synapsin I, thereby releasing the vesicle from cytoskeletal binding at the active zone and allowing it to fuse with the membrane for the initial stage of exocytosis.

b. Synaptophysin is a 38 kD protein present in all synaptic vesicles. It can form gap junction like ion channels between in lipid bilayers, leading to the suggestions that it is involved in the initial fusion of the vesicle membrane and the membrane of the presynaptic terminal.

c. SNARE complex is composed of 3 different proteins.

i. synaptobrevin - transmembrane vesicular protein (also know as v-SNARE)

ii. syntaxin – transmembrane presynaptic bouton plasma membrane protein (also know as t-SNARE)

iii. SNAP-25 transmembrane protein anchored to the cytoplasmic surface of the presyna[tic bouton (also a t-SNARE)

4. The presynaptic calcium conductance

a. Most Ca^{++} channels do not inactivate. Rather the calcium current decreases due to increases in $[Ca]_i$ to between 10^{-7} and 10^{-6}M.

b. Although the E_{Ca} predicted by the Nernst equation is +175 mV (based on values of $[Ca]_o/[Ca]_i = 10^{-6}$), experimentally the reversal potential for Ca currents is found to be in the range of +40 - +70 mV. This is because $[Ca]_i$ is very low, and $[K]_i$ is about 10^6 time more concentrated so that even though the permeability of the Ca channel for Ca^{++} is much greater than that for K, there is so much K inside that a significant outward current carried by K^+ antagonizes the inward Ca^{++} current when the Ca channel opens, and the reversal potential is decreased.

b. The very sharp slope of the relationship between presynaptic depolarization and postsynaptic response is due to the steep voltage sensitivity of the presynaptic Ca^{++} conductance.

D. Presynaptic modification of neurotransmitter release.

Since the single critical step in presynaptic regulation of neurotransmitter release is creating a rise in $[Ca]_i$, it stands to reason that events that modify Ca^{++} influx can modify neurotransmitter release. This is, in fact, an important principle mechanism underlying presynaptic facilitation and presynaptic inhibition, phenomena that will be discussed in greater detail next time.

1. Short term plasticity - At many synapses, a second presynaptic action potential that occurs soon after another one gives rise to a postsynaptic response that may be larger (short term facilitation) or smaller (short term depression) than the first response. This is measured by dividing the amplitude of the second response by the amplitude of the first, giving what is called a *paired pulse ratio*. The altered postsynaptic response is due to an change in the binomial parameter m, as a result of an change in p. In the case of facilitation, this is due to a residual build up of calcium in the presynaptic terminal, as the calcium buffering mechanisms are temporarily unable to keep up, leading to a progressively greater and greater probability of release.

2. Silent synapses - recall that in the experiments on CNS quantal release by Neale et al., and Korn et al., the binomial parameter p was very low for some synapses. These may be effectively treated as silent synapses, and raise the interesting speculation that one important mechanism for presynaptic modulation of neurotransmission may be by significantly raising p for these silent synapses, thereby turning them on.

3. **Presynaptic autoreceptors and heteroreceptors** - Many, perhaps most, neurons possess neurotransmitter receptors on or near the sites of transmitter release in their axon terminal fields. Receptors for the transmitter that the neuron releases are termed *autoreceptors*, whereas those for other neurotransmitters are termed *heteroreceptors*. Stimulation of these presynaptic receptors modifies the impulse-dependent (not the leak) release of transmitters by modifying the electrical characteristics of the presynaptic membrane. Sometimes the synthesis of the transmitter is also under presynaptic receptor control. In most cases, autoreceptors and heteroreceptors decrease transmitter release (e.g., catecholamine autoreceptors) but there are some cases in which facilitation of release occurs.

IV. Electrotonic Synapses

A. Structure - the Gap Junction - Pre- and postsynaptic membranes separated by 2-4 nm, or less than 10% of the distance across a chemical synaptic cleft. There are cytoplasmic bridges, or channels, called connexons, connecting the two membranes arranged in a orderly hexagonal pattern with approximately 8-10 nm between channels. The channels are composed of pairs of hexamers of a 27 k protein called connexin, forming an aqueous channel with a diameter of about 1.5 nm.

B. Function - When the gap junction is functional, pairs of connexons from each membrane are in register, and allow a low resistance pathway for current spread from one cell to the other. The coupling ratio is the ratio of presynaptic voltage change to postsynaptic voltage change, and is usually low, in the range of 0.25 or so. Low molecular weight dyes such as Lucifer yellow or biocytin can also pass through these channels, and dye coupling is often used as evidence for electrotonic coupling of cells. Gap junctions are found between cardiac muscle cells where they serve to synchronize current spread so that the entire muscle contracts nearly simultaneously. These junctions are also exist transiently between many different cells in embryos. Gap junctions are also found between many non-neuronal cells, like liver cells, as well as between CNS neurons in mature nervous systems, e.g., in retina, cerebral cortex, hippocampus, inferior olive, neostriatum and substantia nigra.

1. Electrical characteristics of electrotonic synapses

a. **Direct depolarization test** - Dual intracellular recordings will show direct depolarization or hyperpolarization in a second neuron after manipulating the membrane potential of the neuron to which it is coupled. This is the most direct test of electrotonic coupling, but is often difficult to obtain in practice because it depends on simultaneous dual intracellular recordings.

b. **Antidromic test** - If two neurons are electrotonically coupled, if one, but not the other is antidromically activated, one will see a small (the size depends on the coupling ratio) depolarization in the non-antidromic cell at constant latency that has all the characteristics of small antidromic spike. However, since it is actually elicited in a different axon, evoking a spike in the non-activated neuron will fail to cause collision extinction of the electronically-conducted antidromic spike. Thus, the antidromic test for electrotonic synapses depends on the failure of the collision test.

c. **Rectification** - Most electrotonic synapses are non-rectifying. However, Furshpan and Potter (1959) found electrotonic coupling between two nerve fibers in the abdominal nerve cord of the crayfish that allowed current flow from a lateral giant fiber to a motor fiber leaving the cord, but not in the reverse direction.

d. **Excitation or Inhibition**? - Most electrotonic synapses observed thus far seem to be excitatory, most likely since hyperpolarization is not actively propagated as depolarization can be by action potentials. However, At least one example of an electrical inhibition has been demonstrated by Furukawa and Furshpan (1963). In this case, however, the inhibition arises from an *extracellular positive field potential* that is set up by action potentials propagating into the axon cap of the goldfish Mauthner cell. The extracellular positivity induces an intracellular negativity in the axon initial segment of the Mauthner cell, thereby inhibiting action potential initiation.

e. **Modulation** - In general, electrotonic synapses are far less modifiable than chemical synapses. However, under some conditions, chemical synapses may affect the operation of nearby electrical synapses. Gap junctions are known to exist between horizontal cells in the retina of many species. One of the synaptic inputs that coupled horizontal cells receive is from interplexiform layer neurons that use dopamine as a transmitter. Application of dopamine to horizontal cells has been shown to reversibly reduce the coupling ratio between horizontal cells by increasing the junctional resistance. This has the effect of reducing receptive field size, and is likely to be a major mechanism by which receptive field sizes are reduced by increasing levels of ambient illumination. Similarly, some neurons in the neostriatum show dye coupling, and the incidence of dye-coupled cells decreases with dopamine agonist administration.

[But be careful. The existence of dye coupling is not the same as proof that an electronic junction exists. There are all sorts of artifactual conditions that can lead to dye coupling without there being a real gap junction. A physiological test for electrotonic coupling is still the only way to show it conclusively.]