Synaptic Transmission 1

The Neuromuscular Junction.

There are 2 varieties of synaptic transmission, chemical and electrical. The electrical form uses ionic channels (called connexins) that traverse both presynaptic (input) and postsynaptic (output) membranes. Depolarising currents due to presynaptic spikes are injected directly into the postsynaptic cell through these channels, almost as though there were no synaptic gap. However, electrical synapses are rare, have very specialized roles, and will not be considered further here. Chemical synapses release neurotransmitters which act on the postsynaptic cell. For many years it was thought that chemical transmission would be too slow to explain the rapidity of synaptic action. But although like all small molecules neurotransmitters diffuse very slowly (diffusion constant about 1 $\mu m^2\ msec^{-1}$), the synaptic gap is so short (about 50 nm) that it is not a major delay. Furthermore, both the release and action of the transmitter are also extremely swift, because of the small distances involved.

[Note: when a substance diffuses its molecules undergo a random walk in space, so it gradually spreads out. Because the molecules move randomly, the highest concentration will always be at the point of origin, but the point where the concentration has fallen to half the highest concentration (i.e. the position of the advancing concentration "wave") does not advance linearly with time, but as the square root of time. This accounts for the units of the diffusion constant.]

The best studied chemical synapse, the neuromuscular junction ("nmj")of the frog, is actually a collection of several hundred synapses, each of which is composed of a specialized presynaptic half-ring (on the underside of a terminal branch of the motor axon) and a matching specialized postsynaptic plasmalemmal structure (composed of a bent slot). All these synapses work in synchrony, so the nmj behaves as one big multisynapse. Although multisynapses are also encountered in the CNS, individual independent synapses are much more common. Multisynapses are encountered wherever a single presynaptic axon spike reliably fires the postsynaptic cell.

Outline of Synaptic Transmission

1 Propagation of spike down axon ->

2Depolarisation of terminal branches by arriving spike ->

3 Brief opening of voltage dependent calcium channels ->

4 Exocytosis of contents of previously docked vesicle triggered by Ca-binding to syntagmin ->

5 Diffusion across and along synaptic cleft ->

6. Binding to nicotinic AchR on postsynaptic membrane

7. Opening of nAChR

8. Entry of Na ions through pore of nAChR and depolarization of postsynaptic cell (synaptic potential, epsp sometimes called epp at the nmj).

9. Simultaneously with (8), Ach molecules dissociate from the closing nAChRs and re-enter the cleft, where they are quickly hydrolysed by AchE

10. The synaptic potential reaches threshold and triggers a postsynaptic spike which rapidly propagates along the muscle cell, causing depolarization of the T-system and calcium release into the myoplasm, initiating actomyosin contraction.

Before examining these steps in more detail, let us consider the time course of the synaptic potential, and of the underlying synaptic conductance change. To see this, we must reduce the synaptic potential below threshold. This can be done either by reducing transmitter release, for example by lowering the external calcium concentration, or by blocking most of the nAChRs, for example suing the competitive blocker curare. This reveals that the synaptic potential, recorded at the synapse, has a rapid rise (about 1 msec) and a slower fall (a time constant close to 10 msec). The synaptic potential gets much smaller and has a much slower rise time when recorded at a point well away from the synapse; and it does not reach the tendons at all.

The flow of current which generates this potential can be determined in a voltage clamp experiment. It is a very brief inward current (if the membrane potential is held at the resting potential) which peaks in about 0.5 msec and then decays exponentially with a time constant of about 1 msec. We can understand how this current generates the synaptic potentials observed at various distances from the synapse by using passive cable theory.

What ions flow across the membrane during this synaptic current? We can regard each type of ion as flowing through a conductance in series with its associated Nernst potential. We know that the total ionic current will be zero when the sum of the Nernst potentials, weighted by their respective conductances, is zero. We can determine this "zero-current potential", also called the reversal potential (because at this potential the net synaptic current reverses direction from inward to outward) by varying the membrane and triggering synaptic currents at these various potentials. If an ion can pass through the open nAChR we expect that when we varying its external ion concentration (and hence its Nernst potential), the reversal potential will vary. In this way it has been shown that the open nAChR allows sodium and potassium to pass equally well, but no other major ions.

Although the time course of the synaptic potentials is similar in either curare or lowered calcium, there is an interesting difference. In the latter case, it is seen that the sizes of successive synaptic potentials fluctuate greatly; these fluctuations are much smaller when using curare to block the receptors. Also, in calcium small synaptic-potential-like

depolarisations are seen even when not stimulating the presynaptic axon. These spontaneous synaptic potentials are called minis (or mepsps). They have exactly the same time course as regular, nerve-evoked synaptic potentials, and they are blocked in the same way by curare. They occur randomly, at a rate of about 1 per second.
Katz theorised that the evoked synaptic potentials were due to the synchronous occurrence of many minis, triggered by nerve stimulation. This is known as the "quantal hypothesis", since it postulates that synaptic potentials are made up of many subunits (i.e. of many "quanta"). The evoked synaptic potential fluctuations would exist because the exact number of quanta would fluctuate randomly from trial to trial.
This does NOT mean that the number of quanta released ranges randomly and uniformly from zero to some upper limit. Instead what Katz suggested was that a certain number of quanta n were available for release, and that each quantum was released randomly with probability p (or not, with probability 1-p). The release of one quantum would not affect the probability of release of other quanta. The individual quanta are released randomly with some fixed probability (i.e. on the next nerve stimulus or "trial" some process restores the quanta released at the previous trial, so n is constant).
Under these conditions the actual number released from trial to trial is a random variable. For example, if 10 quanta were available to be released, the actual number released could be 3,5,4,7,1,4,7,6,6,5,4,3,9,0,4 etc. (i.e. about 4.5 quanta on average. If we examine the number released over a sufficiently large series of trials, we can estimate the probability that we see the various possible outcomes 0 released, 1 released, 2 released………10 released. In general we call the probability that x quanta are released p_x, which will be described by the binomial distribution:

$$p_x = n!\, p^x (1-p)^{n-x} / x!\, (n-x)!$$

Note that x! = factorial x = x(x-1)(x-2)……….. and also 0! = 1

The mean number of quanta released will be m = np. The actual number released fluctuates, and we can measure the degree of variability using a quantity called the variance σ^2 (which is defined as the average value of the square of the difference between the actual number released and the mean).

[Technical note: to determine the average, one sums all the observed squared differences and divides by *1 less than* the number of trials. This is because a single observation cannot provide any measure of variability)]

For the binomial distribution $\sigma^2 = np(1-p)$. If p is very high (close to 1) then almost all the time n quanta will be released and the variance will be small.

A simple illustrative application of the binomial distribution is coin-tossing. Suppose you repeatedly toss 6 coins, and record how many come up heads each time. The 7 possible outcomes are x = 0,1,2,3,4,5 or 6 heads, each with some probability p_x . The results are usually shown as a histogram of p_x versus x. In this case n = 6 and p = 0.5 (since heads and tails have equal probability). You can try to construct the histogram yourself, or go to

http://www.stat.sc.edu/~west/applets/binomialdemo.html. The sum of all the px must equal 1 (since no other outcome is possible).

To test the quantal hypothesis one first stimulates the nerve a large number of times, and estimates the probabilities p_x from the fraction of times one sees the various possible outcomes. This data can be displayed as a histogram. One then compares this with the p_x's predicted by the Binomial Distribution. To do this, one must estimate p and n. This can be done by calculating the mean number of quanta released (averaging over all trials) and the variance of the number released. The above equations for m and σ^2 can then be used to calculate p and n.

When this is done at the nerve-muscle junctions in crustacean (which incidentally use glutamate as transmitter, not ACh) a very good agreement between experiment and theory are seen, confirming the validity of the quantal hypothesis (i.e. showing that transmitter is released as independent packets in a random nanner). However, at the frog nmj, it is very difficult to estimate p and n reliably, because the former is very small and the latter very large. Under these conditions, the Binomial Distribution reduces to the Poisson distribution:

$$p_x = e^{-m} m^x / x!$$

where as before the mean number released, m = np
Katz found that the probability distribution for the number of quanta released showed very close agreement with this formula.
Another example of the application of this formula is the annual number of deaths by horse-kick in the Prussian cavalry. Each soldier has a very low probability of meeting such a fate, but there are a lot of soldiers, so most years there are deaths, and occasionally quite a few.
Another interesting limiting case of the Binomial distribution occurs when n is very large, but p is not very small – the Gaussian or bellshaped distribution. This applies to the synaptic potential in the presence of curare – large numbers of vesicles are released (large n). The size of the synaptic potential distributes fairly closely round a mean size (eg 10 mV). This mean size is given by npq where q is the size of single mini (in the presence of curare, which is typically a small fraction of a millivolt).

The formula for the Gaussian is:

$$p_x = (\exp - (x-m)^2/2\sigma^2)/ (2\pi\sigma^2)^{0.5}$$

Where I have used the notation $\exp y = e^y$. m and σ are the mean and variance of the variable x, as before. Since n is very large, x can vary over a great range, and effectively becomes a continuous, not a discrete, variable, and p_x is to be interpreted as the "probability density" of seeing a particular value of x. (the chances of seeing any particular value of x are very small in any reasonable number of trials, so all the values occurring in a particular range of values are typically binned together to create a discrete histogram). Notice that as the variance gets larger, the bell gets broader but lower – the

area under the bell, must amount to 1 (since some value of x must be observed). Gaussian or bell-curves are very often seen when measuring quantities such as height, length or weight (which are typically subject to large numbers of varying influences). In a diffusion process, where there are large numbers of randomly moving molecules, the distribution of molecules (the "concentration profile") is typically Gaussian, and the scatter in the distribution (i.e. σ) increases with time. (see http://landau1.phys.virginia.edu/classes/311/notes/dimension/diffusion.avi)

Measurement of quantal content.

If the size of the quanta (i.e. the amplitudes of the minis) were constant, it would be relatively easy to calculate the quantal content of a particular synaptic response. For small synaptic potentials (which do not approach the synaptic reversal potential) this would be given by the ratio of the sizes of the evoked synaptic potential and the mini. However, minis do not have fixed amplitudes (and in CNS neurons most of the observed minis may not be occurring at the synapses being studied). So a 2 mV epsp could be composed of 2 1-mV minis, or 4 0.5 mV minis, etc. Furthermore, instrumental noise may also contribute. This ambiguity could be resolved in 2 different ways:

1. One could count the quanta directly, if they do not occur exactly synchronously. Using extracellular recording (which improves time resolution, because the signal is proportional to the underlying synaptic current, and is not filtered by the membrane capacity) and low temperature (to prolong the window over which release occurs) greatly helps.
2. If one can characterize the mini amplitude distribution and/or recording noise, one can try to estimate the underlying statistical distribution of the quantal release. In general, this is only possible if the raw epsp amplitude distribution shows some hints of underlying regular peaks.

Interpretation of binomial parameters

Several lines of evidence suggest that the observed quantum (i.e. the mini or the subunits of the evoked synaptic potential) corresponds to the release of a single vesicle. The amount of ACh needed to mimick a mini is similar to the amount in a vesicle, as is the amount released per quantum. At synapses where large vesicles are released it is possible to measure step increases in presynaptic plasmalemmal capacity during transmitter release, reflecting exocytotic incorporation of vesicular membrane. It is also possible to freeze synapses at the moment of peak transmitter release, and then to look for vesicle fusion in the electron microscope. In such studies there is a good correlation between the number of fusion events and the number of vesicles released.