

CHEMICAL NEUROTRANSMISSION

1. Evidence for vesicular hypothesis

- Quantal analysis
 - NMJ, mEPPs and EPPs
 - Binomial and Poisson
 - Uses and problems of quantal analysis
- Morphological evidence
 - See vesicles in EM
 - Isolated vesicles have NT
 - freeze fracture
- Vesicle recycling
 - Timing
 - kiss and run

2. Mechanisms of neurotransmission

- Dependence on calcium, calcium microdomains
- Vesicle trafficking (CaMKinase II, synapsin)
- Docking (SNARE complex (synaptobrevin, SNAP-25, syntaxin), synaptotagmin)
- Fusion and exocytosis (calcium, fusion pore, synaptotagmin)

3. Short-term plasticity (Homosynaptic Plasticity)

- facilitation, post-tetanic potentiation, depression

OVERHEAD 1. INTRO. Today I will talk about chemical communication between cells. We have all been told that information transfer from one neuron to another takes place at synapses. The action potential travels down the axon and causes neurotransmitter to be released into the synaptic cleft, neurotransmitter diffuses across the cleft, binds to post-synaptic receptors, opens channels (all within 1 msec) and causes a voltage change in the post-synaptic cell. $I_{syn} = g_{syn}(V - V_{rev})$

How does action potential invasion of the bouton or varicosity cause neurotransmitter release? To get rapid chemical communication, the nervous system has evolved so that neurotransmitters are synthesized directly in the synaptic terminal rather than being synthesized in the soma and transported down the axon via the axon transport processes (On the other hand, peptide transmitters are made in the cell body and are transported down to the synapse. Peptide transmitters are not recycled but other neurotransmitters, like glutamate, are as discussed earlier in Chap. 3 in Dr. Colvin's section. By contrast vesicles are synthesized in the soma and transported out). This solves one problem, that of having a ready supply of transmitter, but how is this

neurotransmitter released? Does depolarization open a “neurotransmitter channel” and let neurotransmitter out according to its concentration gradient? Most people don’t think so.

By now I’m sure most of you have heard of the vesicular hypothesis--that is, neurotransmitter is packaged into synaptic vesicles and when the action potential invades the terminal, one or more vesicles or quanta of neurotransmitter are released. Electron micrographs (as in OVERHEAD 1) show round organelles which have been called synaptic vesicles. Of course, these were not seen in 1950s when vesicular hypothesis was proposed.

OVERHEAD 2. Most of what we know about how synaptic transmission works comes from study of the neuromuscular junction (NMJ), the squid giant synapse, and the chromaffin cell synapse. What we know about the action potential came from study of the squid axon because it had a huge diameter--about half a mm compared to 1 um of typical axons. Similarly we study the NMJ and the squid giant synapse because of their large size--you can put electrodes directly into the synaptic terminals.

Overhead 2 shows an EM picture and a schematic drawing of the NMJ (Fig. 8.1A in the book). **OH2** We now know that there are vesicles in the presynaptic neuron near regions we call active zones. We also see dense bars whose function is largely unknown although the identity of the proteins is becoming known through some of the nice work by Harlow as illustrated in your book in Fig. 8.8 (pegs, ribs and beams). The vesicles seem to align themselves near the dense bars.

How do we know that release occurs in quanta or vesicles? There are two main types of evidence--evidence from quantal analysis and morphological evidence

The first demonstration that transmitter release occurs in quanta was given by **Katz** studying the NMJ. He was able to put electrodes into the post-synaptic muscle cell, stimulate the presynaptic axon and record the response in the muscle cell.

OVERHEAD 3. When the presynaptic axon is stimulated, an action potential is produced which reaches the terminal, releases acetylcholine (ACh) and causes what we call an end-plate potential (EPP) in the muscle of some 10s of mV. This is usually large enough to produce an action potential which results in muscle contraction. When the presynaptic neuron is not stimulated, Katz observed small spontaneous depolarizations of about 0.5 mV. The time course of these small depolarizations which Katz called miniature end-plate potentials or **mEPPS** resembled that of the EPP. To get a depolarization of 50 mV, you would need about 100 minis.

Were these spontaneous mEPPS and EPPs related? There is evidence that they were:

- 1) Curare blocks EPPs and mEPPs. The EPP could be blocked by the poison curare (Indian arrow poison) which acts postsynaptically to reduce sensitivity to ACh. Katz found that curare also blocked the mEPPS.
- 2) Anti-AChase prolongs duration of mEPPs and EPPs. It is known that the action of ACh is terminated in part by enzymatic degradation of ACh in the synaptic cleft. (How else might NT action be terminated?--Discuss). That is, there is an acetylcholinesterase that breaks down ACh. If this ACh-ase is inhibited (anti-cholinesterase), then the duration of EPPs is prolonged. Katz found that such inhibitors also prolonged the duration of the mEPPS.
- 3) Depolarization increases mEPP freq. Katz also found that if the presynaptic terminal were slightly depolarized, then the frequency of mEPPS was increased.

These three findings suggested that mEPPs and EPPs were indeed related.

Katz sought to test the hypothesis that EPPs were made up of mEPPs.

Overhead 3 bottom. It was known at that time that a low Ca/high Mg media could reduce neurotransmitter release and Katz made use of this information. With low Ca and high Mg, he stimulated the presynaptic neuron and noted that the EPP amplitude was only a few mV. Some stimuli failed to evoke an EPP. The size was not fixed as it was in normal media.

In fact it seemed that the amplitude differences of the EPPs were multiples of a unit quanta size. Some mEPPS were 2X the smallest. Some 3X., etc. (Could this be the difference between 1 vs. 2 ACh receptors activated? Well, NO, because 1 ACh receptor channel has a conductance of 20-40 pS, 1-5 pA at rest, and causes a depolarization of about 1 μ V. A vesicle has about 5000 ACh molecules and 10s-100 vesicles are released per impulse at the NMJ. (The NMJ has about 100 active zones)

OVERHEAD 4. When the amplitudes of the EPPs observed over hundreds of trials were plotted on a histogram, it was very suggestive that the EPPs were integral multiples of mEPPs. (**Compare Fig. 8.9B**) The type of histogram you get is like the one on this overhead. It would appear that there are distinct peaks spaced at equal intervals.

However this does not prove that EPPs are multiples of mEPPs. Qualitative relationships don't prove anything in science! The appearance of the data may occur by chance! To prove that EPPs were made up of mEPPs, Katz had to do some statistics.

To determine if the step fluctuations represent mepps we must have:

- 1) mean and s.d. of mEPP amplitude (get by observing mEPPs)
- 2) A theory to predict how many synaptic potentials (out of N observations) should be at each amplitude (the pattern should follow a statistical distribution), and then do a
- 3) **statistical test** to compare the observed distribution with the predicted distribution.

OVERHEAD 5. Let's make some definitions.

Let Q = mean amplitude of mepps (quantal amplitude, response to exocytosis of 1 vesicle)

n = # releasable units or vesicles or (releasable quanta) Release sites (# active zones)

These days we say release sites

p = probability of release of a vesicle

m = mean number of quanta (vesicles) released by one impulse, $m = np$

E = mean amplitude of an EPP. The amplitude varies according to a binomial dist.

Then the predicted distribution of mEPP amplitudes should follow a binomial distribution where the probability that x quanta are released by a stimulus is

$$p_x = n_x/N = \binom{n}{x} p^x (1-p)^{n-x} \quad \text{where } \binom{n}{x} = n!/(x! (n-x)!).$$

So, if there are n "release sites" available and each release site releases a quantum with probability p, then the probability that x quanta are released by an impulse can be determined by the binomial distribution. See Box 8.4 for details of this

Important point--Fitting data to a binomial distribution and extracting n, p and Q is called a quantal analysis.

OVERHEAD 6. Problem: To apply this to data we need to know n and p, but these are not known in general (at least not independently).

So we use a **trick**: In low Ca and high Mg, the probability of any one release site releasing a quantum is small. If we also assume that the number of release sites is large, then the binomial distribution can be approximated by a Poisson distribution.

The Poisson distribution is used for events that do not occur frequently (i.e. number of Prussian soldiers who died from horse kicks). The probability that x quanta are released in N trials is (The number of synaptic potentials in N trials that are at a given amplitude is:) (Again, see Box 8.4)

$$p_x = n_x/N = m^x/x! e^{-m} \quad \text{where } e \text{ is the exponential function}$$

where $m=np$ **n =# of release sites and p is probability of release**
So **m = average # of quantum released per trial**

To use the Poisson distribution you need to know m . We can calculate m in 2 ways.
 m =mean amplitude of an EPP/mean amplitude of a MEPP
or from number of failures $n_0/N = e^{-m}$ (solve for m , given n_0 and N)

Then one can calculate the expected number of EPPs at each amplitude from the Poisson distribution and compare this to the experimental data.

OVERHEAD 7. This was done and the results are those on this **next overhead 7**. The statistical test (which would you use here--Chi-square) indicated that the release of quanta could be described by the Poisson distribution and this provided the convincing evidence that neurotransmitter release is quantal.

(The predicted amplitude distribution of EPPs based on a Poisson distribution agreed well with the observed distribution for data from the NMJ.)

OVERHEAD 8. Quantal analysis has been used to attempt to explain if synaptic modification/plasticity is (pre or postsynaptic and) due to either:
--a change in the number of release sites n (pre)
--a change in the probability of release p (pre)
--a change in quantal content. m (pre) mean quanta released (not to be confused with the number of NT in a vesicle)
--a change in quantal amplitude Q (post) receptors or neurotransmitter (less likely)

Results have been controversial, because some of the assumptions of quantal analysis do not necessarily hold as the book discusses. Applications of quantal analysis are done with noise deconvolution and coefficient of variation methods, discussed in the text, but I will not describe them here.

Some outstanding questions:

- 1) Is vesicle content constant? Vesicle diameters vary 20-50 nm, so probably not.
- 2) Is quantum an artifact of receptor saturation--not at the NMJ, mEPPs add linearly, also anti-Achase has effect) If saturated then Q is determined by number of receptors only.
- 3) How many quanta are secreted per terminal?--Seems to be only one in the CNS. Release mechanism may be "refractory" to 10 msec (if prolong the action potential then can see more vesicles released) So Poisson not valid, because its use

assumes large n. Why usually only one vesicle released per synapse. Don't know. Maybe mechanical limitations although some argue that two is not so uncommon.

- 4) Is probability of release the same at all synapses? Almost certainly **not** in which case the binomial distribution does not apply! Typical synapse has 5-10 vesicles docked (out of 50-100). p seems to depend on the number of docked vesicles.
- 5) How much intersite/intrasite variability is there? Variability between synapses on the same neuron is considerable. Variability in Q (when there is release) at the same synapse is not so much.

OVERHEAD 9. MORPHOLOGICAL EVIDENCE FOR VESICULAR HYPOTHESIS

Some 20 years after Katz concluded that transmitter release must be quantal, investigators were able to visualize synapses with the electron microscope as in the first overhead.

Here one could see round spherical shaped organelles which are thought to be synaptic vesicles. Well, are they? Isolated vesicles were found to contain high concentrations of neurotransmitter, so it seemed likely. If they are synaptic vesicles, then somehow they must move to the membrane and spill their contents into the cleft. Or maybe NT leaks out of the vesicles. It would be nice to catch a vesicle in the act--find a smoking gun so to speak, to prove this.

Catching vesicles in the act became possible in the 1970s with the freeze fracture technique. Here, the presynaptic neuron is stimulated and the tissue is frozen rapidly with liquid helium at different time intervals after the stimulation. Obviously, it is important to freeze the tissue rapidly and thoroughly if there is any hope of catching the vesicles in the act. After freezing the membrane is "fractured" in the middle of the lipid bilayer. One can then observe the membrane looking at the inner leaf from the outside (P-face) or at the outer leaf from the inside (E-face)

OVERHEAD 10. This overhead shows pictures from Heuser and Reese. In the **top** picture the tissue was frozen **3 ms** after stimulation (NMJ). You see the membranous particles which may be calcium channels. There is no evidence of exocytosis here. In the **middle** figure you see very definite evidence of exocytosis (at **5 ms**). The view is the inner half of the presynaptic membrane viewed from the synaptic cleft. The **bottom** picture is the view of the outer membrane leaf from the inside of the presynaptic membrane. Here you see **domes of vesicles as well as craters**.

OVERHEAD 11 . This overhead shows the un-stimulated synapse (control) and a stimulated synapse quickly frozen. The bottom picture shows omega figures from

vesicles that have discharged their contents. These pictures give perhaps the most convincing evidence that NT release is quantal and that each quantum corresponds to a synaptic vesicle.

OVERHEAD 12. VESICLE RECYCLING. Vesicles are transported from the cell body down the axon. This is expensive. So synapses have devised a means to recycle vesicles. Vesicle recycling via endocytosis. Without recycling, the synaptic membrane area would continue to grow.

After exocytosis, membrane starts to develop a **clathrin** coat. (This procedure may be a general feature of endocytosis found in other parts of the cell). This honeycomb lattice somehow starts to form the bud of curvature. A protein called **dynamain** seems to be important for the fissure of the endocytosed vesicle to free it from the membrane. Then **actin** seems to play a role in moving the vesicle along on its journey. It seems that these clathrin coated vesicles lose their coat once they are free in the cytoplasm.

Proton pumps on the vesicle membrane set up an electrochemical gradient allowing uptake or transport of neurotransmitter into the vesicle to a concentration of 200 mM. The whole process takes about 30-60 seconds for a vesicle to be released and readied for another round (20 sec to be internalized).

If a synapse has about 50-100 vesicles, a 5 Hz stimulus would deplete the synapse in about 20 seconds! How can this be? Another mechanism has been proposed to short-cut vesicle recycling. This mechanism is called "**kiss and run**" where the vesicle fuses, opens and discharges its contents, but instead of collapsing into the membrane, the vesicle pinches back off and refills. This avoids the usual recycling route. The vesicle then just has to refill. How prevalent this mechanism is (or even if it exists) is a matter of debate. Why synapses do not regularly become depleted of vesicles is one of the unanswered mysteries at present. Or perhaps they do!

MECHANISMS OF NEUROTRANSMISSION

Mechanisms of neurotransmission

- Dependence on calcium, calcium microdomains
- Vesicle trafficking (CaMKinase II, synapsin)
- Vesicle transport & targeting (sec6/8), tethering or attachment (rab3)
- Vesicle Docking (SNARE complex (synaptobrevin, SNAP-25, syntaxin), synaptotagmin) Prefusion
- Fusion and exocytosis (calcium, fusion pore, synaptotagmin)

Short-term Plasticity--facilitation, post-tetanic potentiation, depression

MECHANISMS OF NEUROTRANSMISSION

OVERHEAD 13: DEPENDENCE of Transmitter release ON CALCIUM

It has been known for a long time that NT release depends on calcium. Katz used this fact in the experiments described earlier. Specifically,

- 1) The extracellular calcium has to be present just before the presynaptic terminal is activated. After is not good enough. (Add calcium to media to see this)
- 2) In fact neurotransmitter release depends on external calcium concentration in a highly non-linear manner. In the NMJ neurotransmitter release depends on the **fourth power** of external calcium concentration. One can change extracellular calcium to see this relationship
- 3) In the squid giant synapse 50 x 700 μm it is possible to put electrodes into the presynaptic terminal to inject calcium into the cell. When calcium is injected, neurotransmitter release is invoked and an EPP occurs. Caged Ca is released by photo-activation in these experiments
- 4) Although Ca is necessary for neurotransmitter release, is it sufficient?--or does there need to be another ion involved like Na or K. It can be shown with TTX and TEA to block Na and K that Ca is sufficient to cause release of NT.

TIMING OF RELEASE PROCESS. Once calcium gets in, it acts quickly. It takes 200-300 μs from the action potential to calcium channel opening, and then 200 μs more until you start to see the post-synaptic response. Calcium acts quickly (200 μsec from calcium current to post-synaptic response) as measured from Ca tail currents. This is fast. It takes a vesicle about 0.2 msec to fully discharge its contents!

Calcium has to get in via calcium channels. So where are these calcium channels located? In the pictures from Heuser and Reese, those membranous particles were

suggested to be calcium channels since they lined the active zone of the synapse. (qualitative argument). A vesicle with a 35 nm diameter may be surrounded by 5-10 calcium channels within 50 nm.

However to determine if this is indeed where Ca gets into the cell, we need more than that. Calcium concentration has been imaged in the squid giant axon with the use of fluorescent dyes. Early on Aequorin and Arsenazo III were used. Now fura-2 and variations of fura are used to study calcium concentration. These substances emit light when they bind to calcium. The problem with these substances is that it is hard to get both the spatial and temporal resolution to definitively and quantitatively give the calcium concentrations in different parts of the terminal.

Nevertheless the squid giant synapse is very large and imaging studies have been done. The imaging studies indicate that elevation of [Ca] occurs first near the active zones. So calcium channels are clustered near the release sites. The imaging studies cannot give the [Ca] levels very accurately--fura saturates after a few uM.

OVERHEAD 14. Calcium Microdomains. The calcium channels are open only for a short period of time. They are voltage-dependent and the action potential is over quickly. So what does the calcium concentration change look like?

This is where quantitative models help. Given the diffusion equation, and the calcium diffusion coefficient, one can model what the calcium concentration changes must be. $[Ca] = M/8(\pi Dt)^{3/2} \exp(-r^2/4Dt)$. M=moles, D=diffusion constant for Ca, r=radius from the channel mouth, t=time. The models suggest that near the mouth of the calcium channel, [Ca] reaches 100 uM or more (within 10 nm) with much lower changes a short distance away. Given that its resting concentration is about 100 nM, you can see that this is a 1000-fold change. It looks like on **THIS OVERHEAD. (compare Fig 8.3)**

As mentioned earlier there may be 5-10 channels surrounding each vesicle that act cooperatively. At SFN one year people argued small domain vs large domain and all sort of settled on medium domain.

The models suggest that this Ca microdomain increase decays to 10 uM in 5 ms and back to rest within tens of ms. So you see that any proteins near the channel mouth are exposed this steep calcium rise for a very short period of time and it is possible that calcium-sensitive proteins may activate the release process.

The release process happens quickly after calcium entry. The delay is 200 μ s to the postsynaptic response. Therefore the calcium entry can't be doing something involving

complex biochemical reactions. We need a **fast, low**-affinity trigger with cooperative Ca binding with a $K_D > 10 \text{ uM}$ (later we will suggest that synaptotagmin may have these properties). The calcium step may initiate completion of the fusion reaction.

OVERHEAD 15 VESICLE TRAFFICKING.

Vesicles have proteins in their membranes. Some are pumps that transport neurotransmitter into the vesicle. These transporters are different from transporters that take neurotransmitter back up from the synaptic cleft after release. Other proteins act to form cross-links to actin or to other vesicles. Somehow the vesicles have to move toward the membrane if they are going to release neurotransmitter.

One candidate that may be involved in trafficking is Ca/CaMKinase II or calcium/calmodulin dependent protein kinase II. This protein composes 0.5-1% of the total protein in the nervous system and it is plentiful at the synapse. It has the right characteristics for release in that calmodulin binds 4 calcium ions which might be related to the 4th power nonlinearity of release as a function of Ca concentration mentioned earlier, but its role is more likely to be in trafficking.

CaMKII phosphorylates proteins. In particular **synapsin I** (see the short filamentous vesicle proteins in Fig 3.1 on Overhead 16). When unphosphorylated, synapsin I binds to vesicles and actin tightly. When phosphorylated it loses its grip. It seems that most vesicles located within 30 nm of the release site are devoid of synapsin I. It seems that CaMKII increases the availability of vesicles for the releasable pool for exocytosis without affecting the mechanisms of release (so may increase the probability of release). When CaMKII binds calmodulin, it is active for a second or 2. The timing seems too slow to participate directly in the release. (but inject CaMKII or dephosphorylate Synapsin1 and you get the predicted effects. We are finding that Ca affinity for Calmodulin is greatly increased if calmodulin is bound to protein, so perhaps it is not correct to make judgments based on calcium binding to calmodulin in the absence of protein).

OVERHEAD 16. The top figure illustrates the crosslink attachments synapsin makes between the vesicle and actin.

The bottom figure shown some of the known proteins found in synaptic vesicles. At the top are the synapsins (and it illustrates their ATP binding), synaptobrevins (VAMPs), synaptotagmins with calcium binding domains, and Rab3. We used to think synaptophysins were involved in fusion and exocytosis, but currently their function is unknown. See Fig. 8.4 and Tables 8.1 and 8.2.

Somehow the vesicle needs to be transported to the membrane, and become attached to the membrane near calcium channels which can trigger release. The mechanisms of attachment and docking are not well known.

The process of ATTACHMENT includes transport, targeting, and tethering.

OVERHEAD 17.

VESICLE TRANSPORT When the vesicle is free it has to get to the membrane. How does this happen? Probably by diffusion and possibly molecular motors (actin). Mechanisms are not known.

TARGETING. How does the vesicle know where to meet the membrane? Unknown. sec6/8 complex or Nsec-1 and munc-18 seem to play a role.

TETHERING. Rab3 (vesicle protein) seems to be required for regulating vesicle targeting and availability and may regulate SNARE complex formation

DOCKING (Prefusion)

Somehow the vesicle needs to become attached to the membrane near calcium channels which can then trigger the release. The mechanisms of docking are not well known. The current theory is that synaptic vesicle proteins **synaptobrevin** (and possibly **synaptotagmin** form a complex with membrane proteins **SNAP-25** and **syntaxin** to form a complex called the **SNARE complex**. This creates hemifusion or prefusion of one side. The process takes 10-20 msec and may involve many separate unknown steps

VESICLE FUSION AND EXOCYTOSIS. (Calcium triggered step)

Calcium is believed to induce a conformation change in synaptotagmin, perturbing the SNARE complex and creating the fusion pore. At least that is the current hypothesis.

OVERHEAD 18 (also see Figs. 8.6 and 8.7 in book)

The fusion pore opens and closes repeatedly before opening wide finally.

Neurotransmitter is dumped into the synaptic cleft. Concentrations in the cleft reach a few mM for a very brief period of time. At the NMJ this causes the opening of 1000s of channels and a postsynaptic voltage change of 10s of mV. In the CNS, by contrast, a small number of channels opens (maybe 30) and the voltage change is less than 1 mV and often closer to 0.1 mV. So it may take lots of synapses to cause an AP.

EPSP--synaptic channel permeable to Na, K, and Ca often. Reversal potential close to 0 mV (or 60-70 mV positive to rest).

IPSP-- channel permeable to K or Cl. With K you get a hyperpolarization because E_K is negative to the resting potential. With Cl, E_{Cl} may be close to or equal to the resting potential and there may be no voltage change (have shunting inhibition because of increase in g_{syn})

$$I = g_{syn}(V - E_x)$$

SHORT-TERM PLASTICITY

OVERHEAD 19 The last thing I want to talk about is **short-term plasticity**, also called **homosynaptic plasticity**. Homosynaptic plasticity is a property of a synapse that allows the amount of NT released to change as a result of previous activity. The simplest form of short-term plasticity observed in many synapses is paired-pulse facilitation or depression, where the size of a second EPSP due to a stimulus is either larger or smaller than the first, as shown here. In paired-pulse facilitation, more vesicles are released by the stimulus whereas in paired-pulse depression, fewer vesicles are released. This can be at one synapse or over a population of synapses.

Changes in the response to a stimulus are more readily observed during or following tetanic stimulation. I will discuss three types of short-term plasticity: facilitation, post-tetanic potentiation and depression.

First there is **facilitation**. Facilitation is an increase in neurotransmitter (vesicle) release by successive action potentials during a brief stimulus train. Recall that peak calcium concentration following an action potential decays quickly, but it still takes 10s of ms for Ca to finally get back to rest. If additional action potentials arrive before Ca has returned to rest, we say that there is residual calcium remaining in the terminal when the next action potential arrives and because neurotransmitter release depends non-linearly on Ca concentration, this residual calcium adds to the action potential evoked calcium influx to promote more vesicle fusion with the membrane. What happens then is that overall a greater number of vesicles get released because p increases--probability of release). Facilitation decays quickly. Ca must act on a site other than that causing release, but at a fast acting, high affinity site that senses the residual calcium. This is the **Residual calcium hypothesis**.

Second, there is **post-tetanic potentiation (PTP)**. PTP is an increase in neurotransmitter release that may begin during a stimulus train, but which continues for

some time after the train ends (to distinguish it from facilitation). Stimuli delivered after the tetanus may produce more neurotransmitter release even if delivered several minutes after the train. Eventually the effect decays away. The mechanism is thought to be different from LTP or long-term potentiation (which lasts a long time--hours) although the mechanisms for both are not known definitively. Residual calcium averaged over the whole terminal could play a role. The site is different from that of facilitation and might involve CamKinase II and synapsin. It has been found that the calcium chelator (exogenous buffer) EGTA reduces PTP.

Another possible explanation is that PTP is may result from an overload of the calcium extrusion and uptake mechanisms. The calcium ATP pump may become saturated and uptake into stores may not be able to quickly handle the high load. Also Na also enters the terminal during a train of action potentials. Because both Na and Ca are elevated after an input tetanus, the Na/Ca exchanger may get a bit confused--doesn't know which ion to take out--and so the extrusion of Ca is prolonged from tens of ms to minutes. Anyhow, it seems that there is an increase availability of releasable vesicles. The “readily releasable pool” increases.

Finally there is depression. Depression is a decrease in neurotransmitter (vesicle) release during an action potential train. You can imagine this happening if there is depletion of neurotransmitter from the terminal (only hypothesis not excluded so far). (recently a reduction in the rate of endocytotic vesicle recovery and transport of vesicles to the readily releasable pool was considered as another possibility). However in the NMJ a very small percentage of vesicles are released with each AP, about 1%, so it takes a lot of inputs to deplete NT. In the CNS synapses with a high probability of release but with low numbers of docked vesicles might find replenishing docked vesicles a problem (could be decrease in n, number of packets secreted, or p prob of release-not quantal size). **In some cases there may be autoinhibition, where the NT acts on presynaptic receptors to inhibit release (GABAB receptors).** GABA causes IPSPs. If there are GABA receptors on the pre-synaptic neuron as well as on the postsynaptic neuron, action on the presynaptic neuron might be involved in depression.