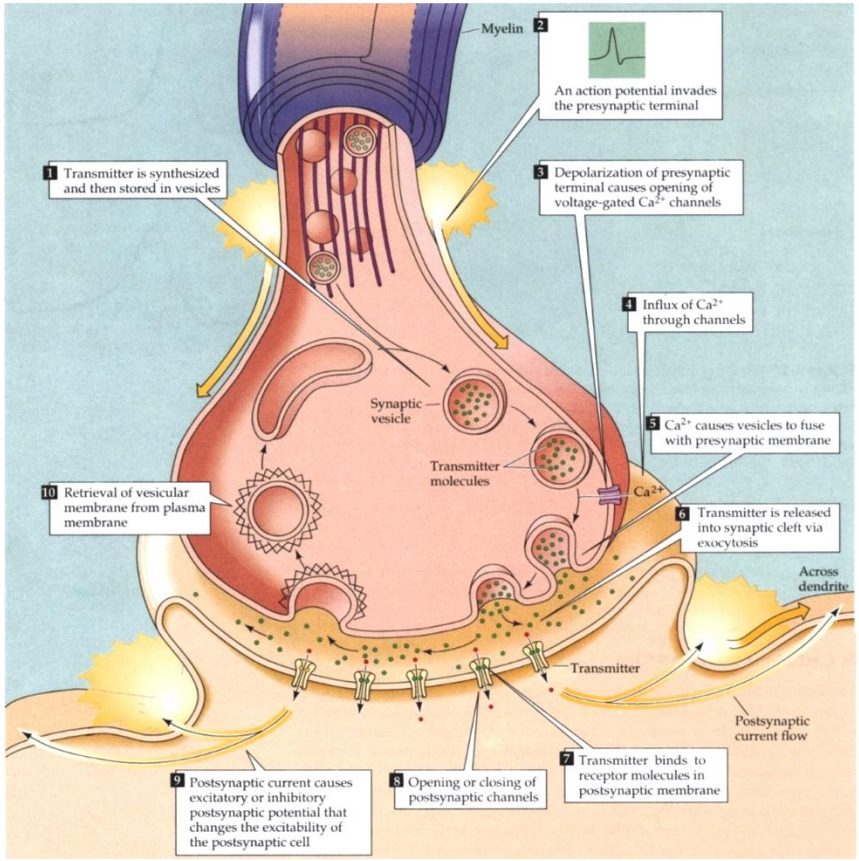
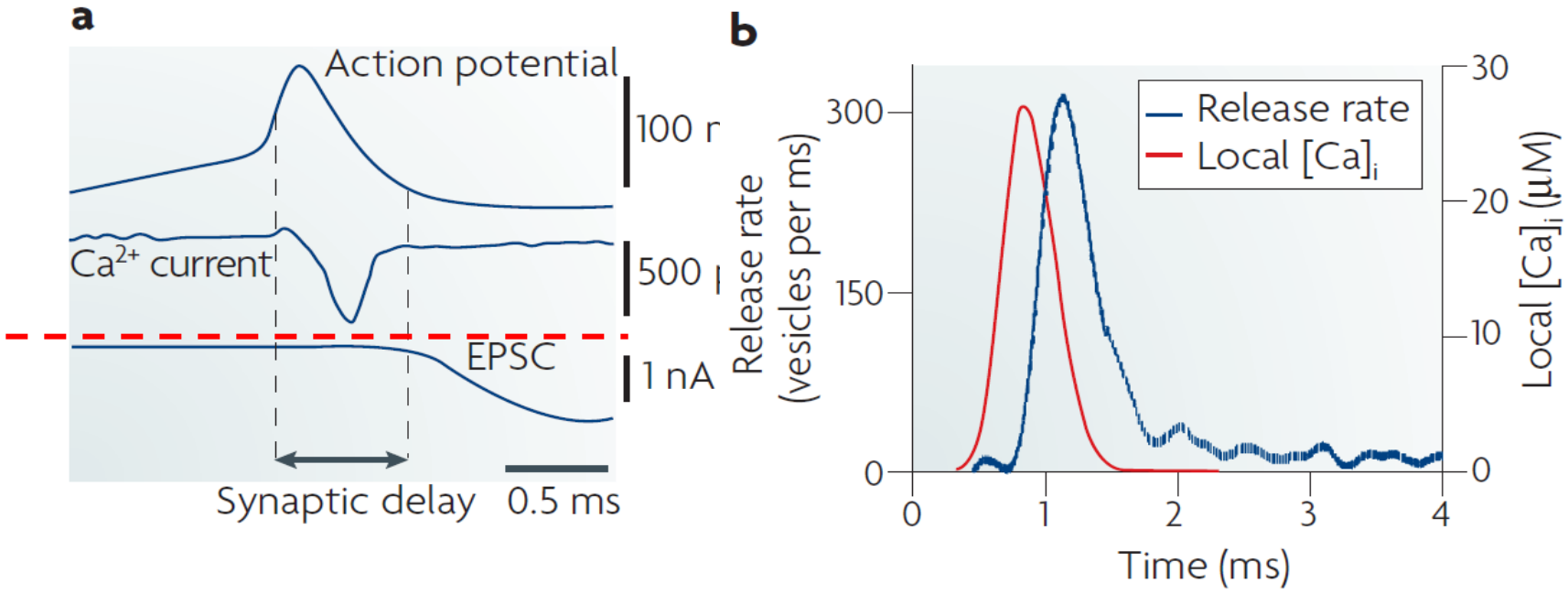
In order to secrete thousands of transmitter molecules rapidly and simultaneously, nerve terminals package them into vesicles.



Timing of steps in transmission

MF-CA3

after the presynaptic AP



At the presynaptic level the delay between Ca^{2+} flux and transmitter release in only 200 μs. Ca^{2+} in the microdomains rises to 25 μM for 300 μs.

Different types of presynaptic Ca^{2+} channels? Why??

P/Q- (Cav2.1) and N-type Ca^{2+} channels (Cav2.2) are localized to active zones, and

R-type Ca^{2+} channel (Cav2.3) may also be present (Gasparini et al., 2001; Li et al., 2007)

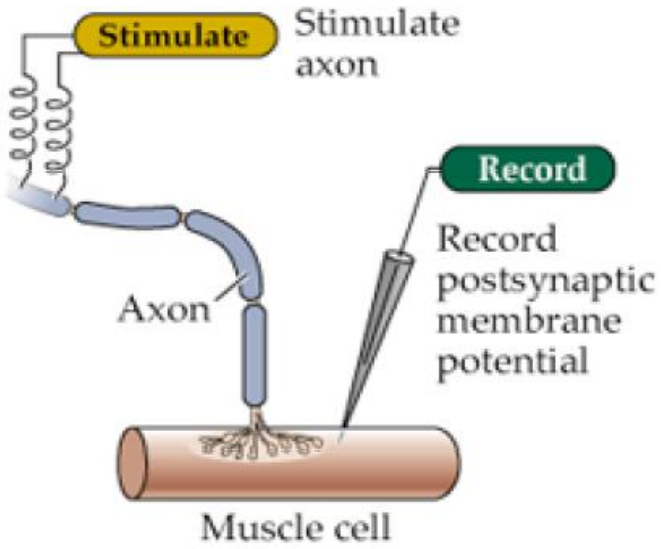
Basic features are similar (extreme Ca^{2+} selectivity, high open channel flux rate, voltage dependent gating)

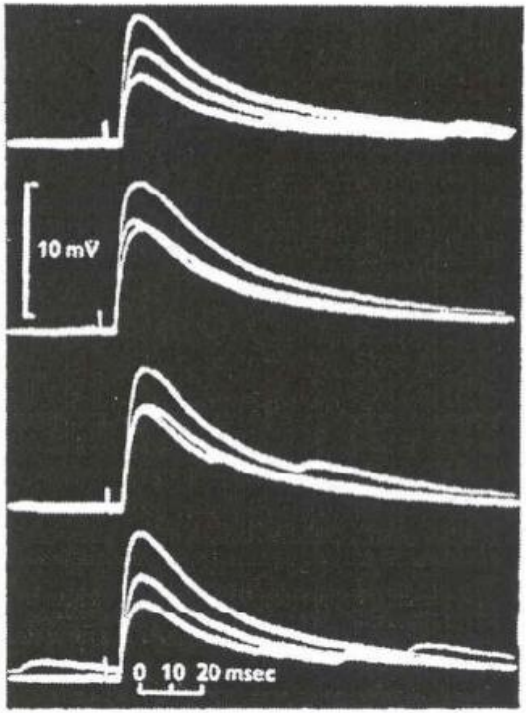
but substantial differences in channel regulation

- P/Q, N differ in their susceptibility to modulation by G protein and PK
- Several neurological disease arise from genetic modification of P/Q but not N type Ca^{2+} channels.

Quantal release

EPPs evoked in low Ca^{2+}, high Mg^{2+}





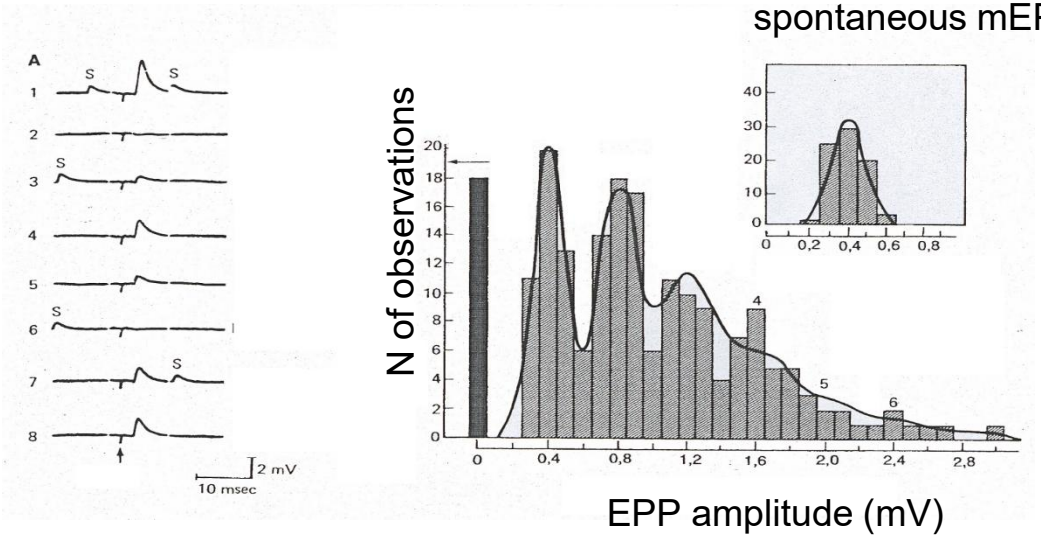
Subthreshold responses, varied amplitude of responses, occasional spontaneous potentials

Synaptic transmission is a stochastic process

Del Castillo and Katz, 1954

EEPs evoked by motoneuron stimulation in low Ca^{2+}, high Mg^{2+}

Amplitude of spontaneous mEPP

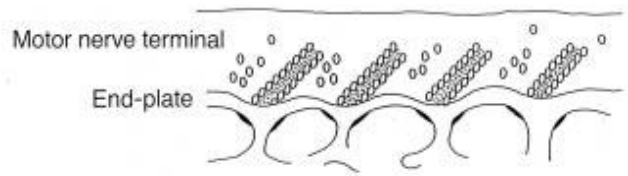


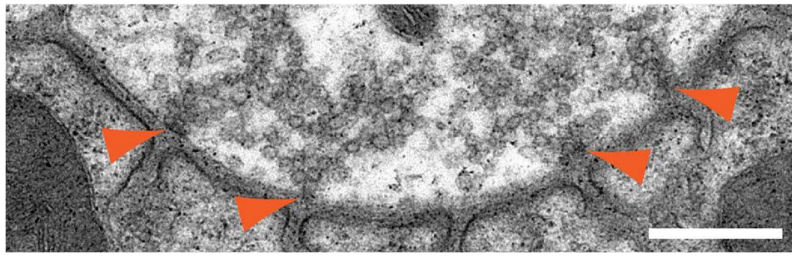
At NMJ the amplitude of EPPs induced by presynaptic stimulation fluctuated from trial to trial as integer multiples of the amplitude distribution of mEPP. Neurotransmitter release occurs in multi-molecular packets of transmitter molecules of constant size (quanta).

Failures and variability in EPP amplitude implied the probabilistic nature of transmission

Statistical model might be applied to quantitatively describe transmission

The active zone of the NMJ and its functional equivalent in the CNS





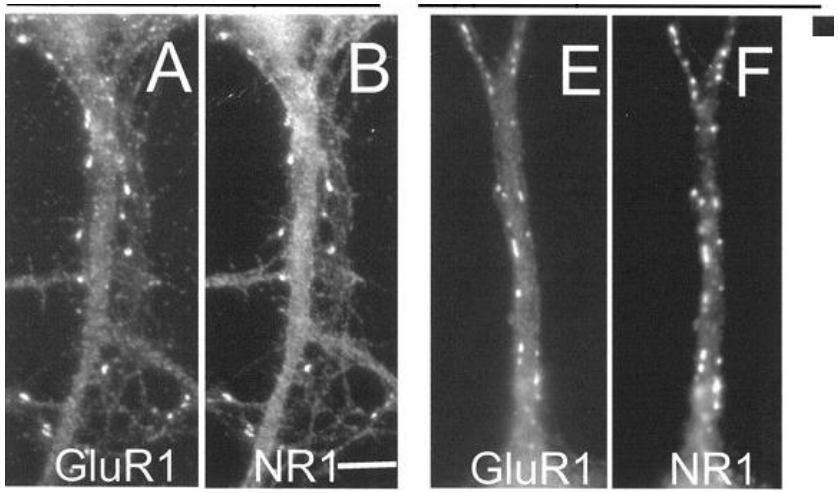
Nishimune, Ann N Y Acad Sci. 2012 December ; 1274(1): 24–32

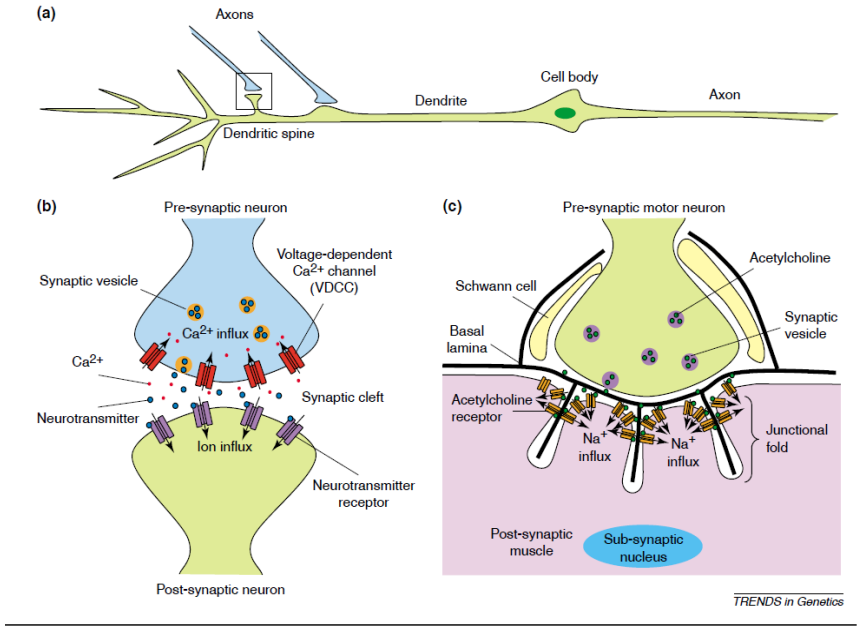
Synaptic vesicles cluster in the *disc shaped* **active zones**, also termed **release sites**. At NMJ a single nerve ending will have 1000 *elongated* active zones.

A single action potential will cause vesicles to fuse at about a third of those, to release about 300 quanta within 1.5 ms.

Each quantum gives a depolarization of 0.5 mV (mPSP).

In NMJ each postsynaptic fiber receives a single massive cholinergic input

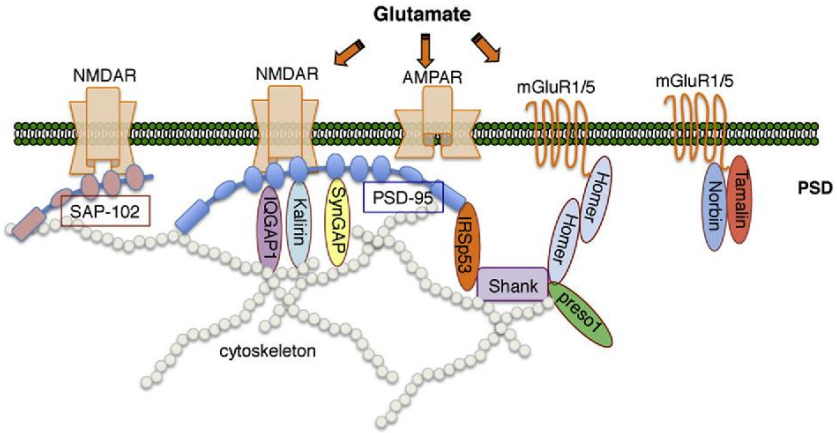


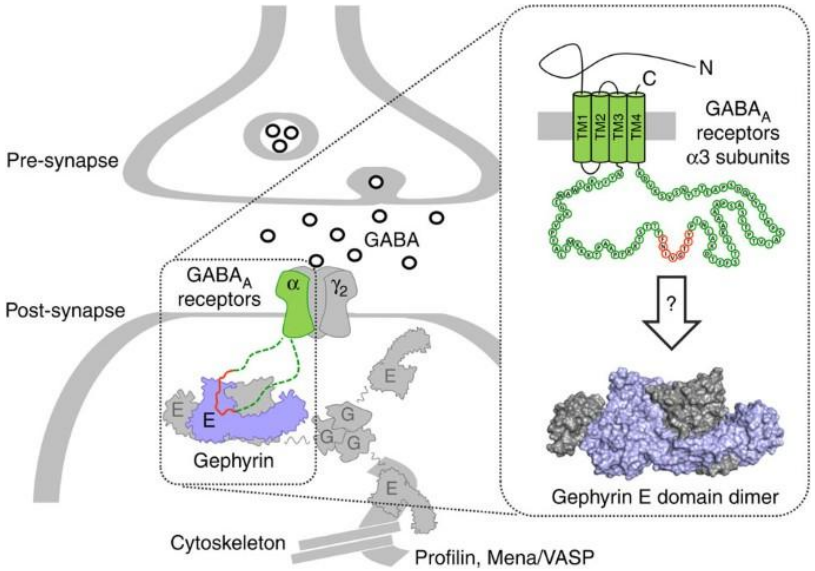


In dendritic spines and in dendritic shafts

In central synapses the postsynaptic neuron receives multiple smaller excitatory and inhibitory inputs through different synaptic connections

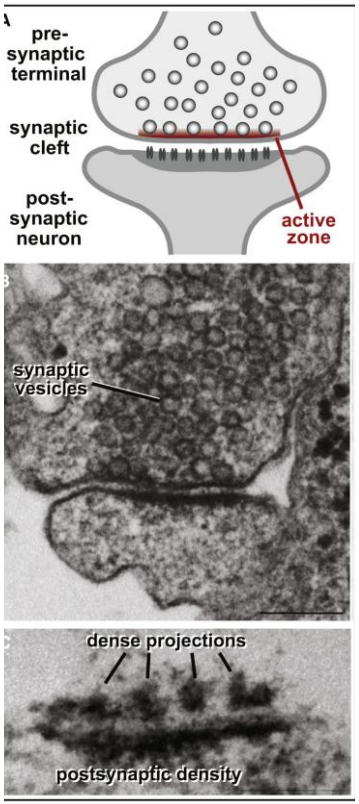
Cytoplasmic scaffold proteins, receptor associated proteins mediate clustering of receptors in the CNS but also regulate intracellular signaling



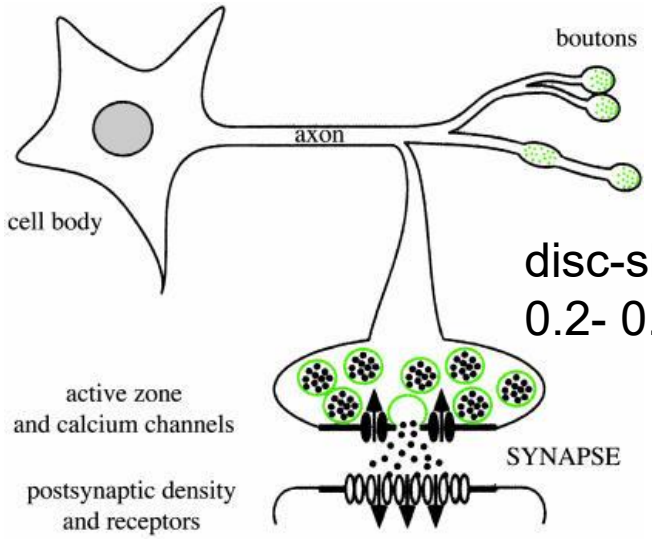


active zones recruit voltage-gated Ca2+ channels to the presynaptic membrane to allow fast synchronous excitation/release coupling. Third, active zones contribute to the precise location of pre- and postsynaptic specializations exactly opposite to each other via transsynaptic cell-adhesion molecules. Finally, active zones mediate much of the short- and long-term presynaptic plasticity observed in synapses, either directly by responding to second messengers such as Ca2+ or diacylglycerol whose production causes plasticity or indirectly by recruiting other proteins that are responsible for this plasticity.

In CNS the morphology of presynaptic specializations can vary greatly ranging from classical single active zone for each bouton (**70 %**) to boutons of various sizes harboring multiple active zones (2-4 active zones).



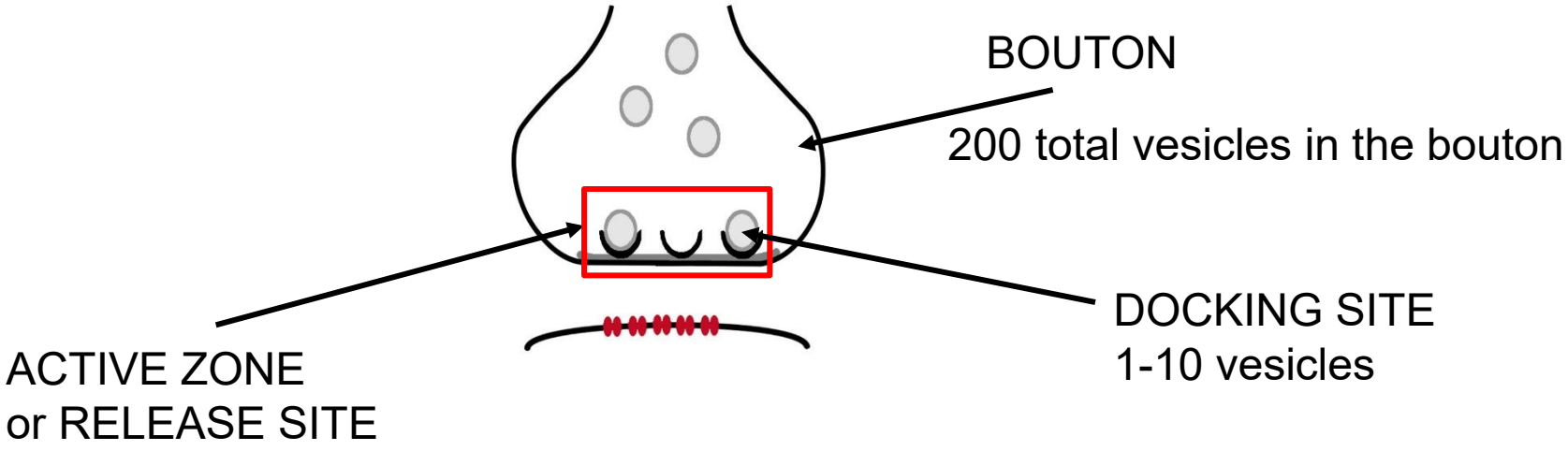
Südhof, *Neuron. 2012 75(1): 11–25.*



disc-shaped active zone
0.2- 0.5 µm

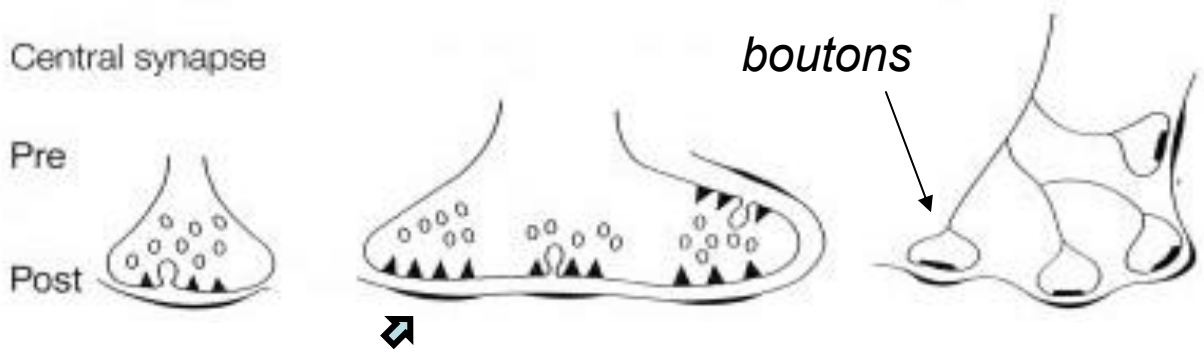
The active zone is a single large protein complex that docks and primes synaptic vesicles, recruits Ca2+ channels to the docked and primed vesicles, tethers the vesicles and Ca2+ channels to synaptic cell-adhesion molecules,
and mediates synaptic plasticity

In each active zone 1-10 docking sites can be present



A tight relationship holds between
the active zone size and the number of docked vesicles

The anatomy and physiology at **fast central synapses**



In each bouton 1-4 active zones. At any single active zone 1-10 docking sites.

A single action potential may release 0, 1, 2.. vesicle contents (**multivesicular release**). The action of each zone is additive in determining the postsynaptic response. At a typical glutamatergic synapse, each action potential releases 5-10 quanta (vesicle contents).

Fewer receptors are clustered at central synapses, each quantum activates only 30 or so ion channels within a hotspot (0.03 μm^2). Single APs generate EPSP of 1 mV

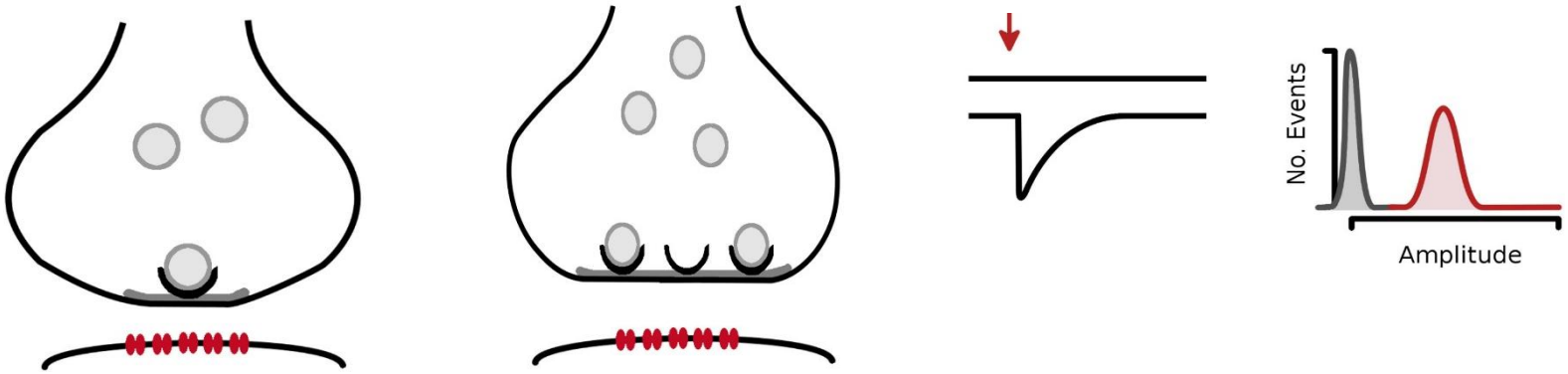
In CNS:

- Size and shape of active zones,
- the number of docked vesicles per active zone
- the area of the PSD,

vary greatly from synapse to synapse

a) Simple synapses /one active zone for bouton, one vesicle hypothesis
A single action potential might induce at single boutons the release of the content of a single vesicle, one quantum

b) Multivesicular release / more active zones for bouton, many docking sites for active zone



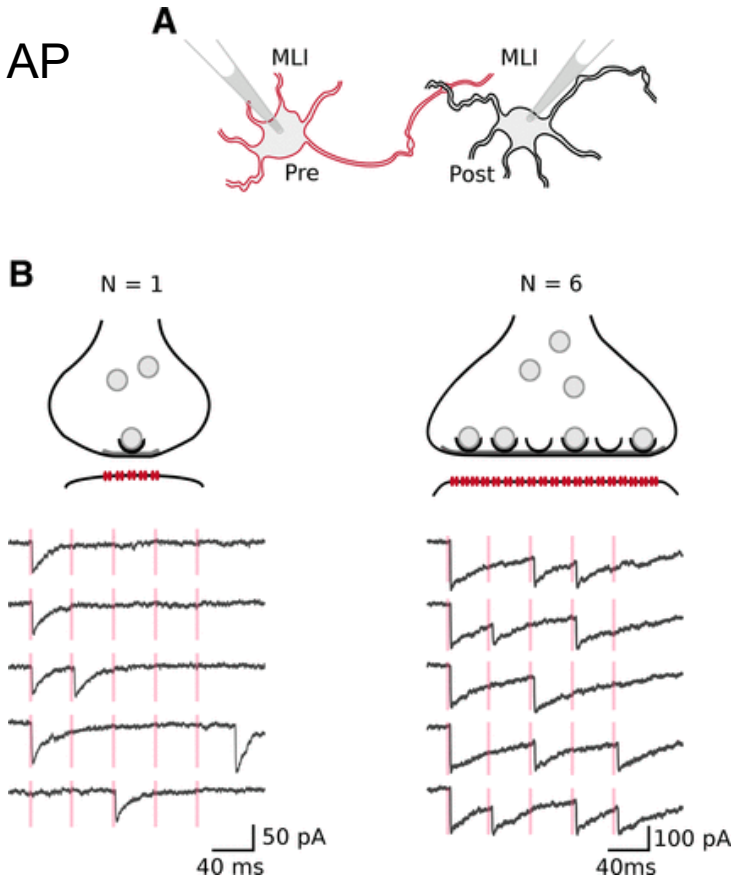
Higher release of neurotransmitter induces a higher current influx at postsynaptic level and a higher depolarizing peak, activating more receptors

But…..If the receptor are saturated, the postsynaptic peak current amplitude can be comparable for single and multiple vesicular responses

QUANTAL FLUCTUATIONS IN CENTRAL MAMMALIAN SYNAPSES: FUNCTIONAL ROLE OF VESICULAR DOCKING SITES Camila Pulido and Alain Marty, Physiol Rev 97: 1403–1430, 2017

The supply of vesicles from the reserve pool is a slow process (hundred of ms)

Burst of AP



In simple synapses with a single docking site for active zone, when bursts of AP are elicited, the limited stock of vesicles is soon exausted and can not allow sustained responses

Synapses reliably differ from each other in their properties, not only in terms of neurotransmitter type, but also in terms of basic synaptic parameters, such as

- the release probability
- post-synaptic receptor composition

Factors that may influence the release probability p

- Size, shape of depolarization
- Different functional state of Ca^{2+} channels
- Distance of vesicles from Ca^{2+} channels
- Basal $[Ca^{2+}]_i$
- Different docked, primed vesicles
- Presynaptic receptors
- Phosphorylation state of presynaptic proteins

The number of activated receptor-channel complex depends on:

- The diffusion of transmitter, which in turn depends on the geometry and structure of synaptic cleft

- The number of docking sites

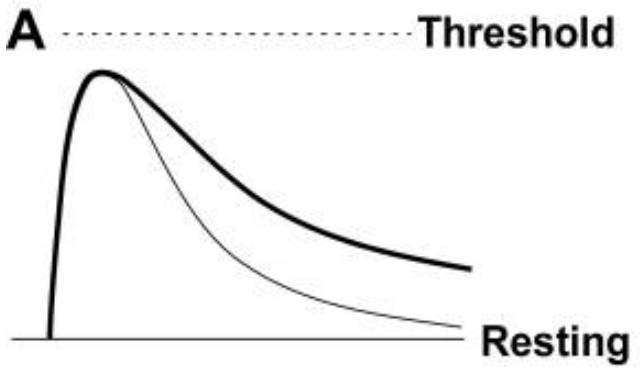
- The number of bound transmitter molecules required to activate the receptor

- The breakdown and uptake of neurotransmitter following release

- The desensitization of receptors

EPSP amplitude and time-course

- Diffusion/synaptic geometry
- Resistance and Capacitance of the postsynaptic cell
- Time-course and activation of a diverse number of postsynaptic receptors
- Reversal potential
- Enzymatic degradation
- Re-uptake



Synaptic transmission/quantal analysis

A postsynaptic response will result from the synchronous release of a number of quanta.
At any synapse **the mean number of released quanta per action potential** (**QUANTAL CONTENT, m**) may vary.
In NMJ the number of molecules in each quantum is generally fixed.

QUANTAL SIZE, q is the amplitude of the miniature event (the response to a single vescicle being released).

NeuroMuscularJunctions

Determination of quantal content, m and quantal size, q are important in establishing the site of action for treatments modulating synaptic transmission

Example of a drug reducing the transmission

A drug acting presynaptically to reduce transmitter release would be expected to reduce the mean number of quanta released by a given stimulus (quantal content, m), but not to affect the amplitude of the postsynaptic response evoked by a quantum of transmitter (quantal size, q).

A drug acting postsynaptically to antagonize the action of the neurotransmitter would reduce the quantal size q (mEPP amplitude) but does not affect the quantal content, m.

Questions related to central synapses

The quantal behavior of synaptic currents at central synapses has been the subject of much controversy

The definition of a quantum.

The postsynaptic receptor cluster in the CNS is often small and the release of a one vesicle content might open only a few channels because of the low receptor number.

What factors determine the quantal size (mEPSP or mIPSP) at a central synapse and does the postsynaptic membrane contribute to quantization?

No dogma should be considered sacred.

In the classical case of “presynaptic quantization”, the postsynaptic receptors are very far from saturation and a quantal size corresponds to the release of a single vesicle content, i.e., a quantum of neurotransmitter.

When postsynaptic receptors are saturated, the quantal size of postsynaptic response strongly depends on the number of accessible postsynaptic receptors in the vicinity of the release sites.