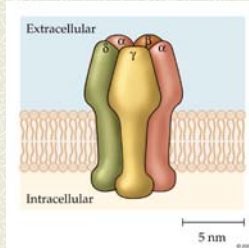
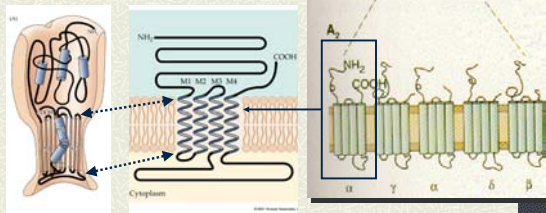


CONT...FAST SYNAPSES



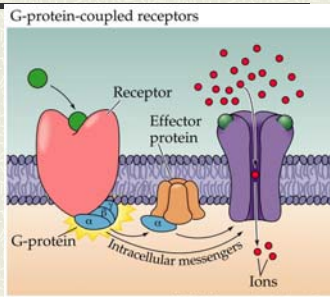
Two basic types of chemical synapse:

directly gated (ionotropic)



and indirectly gated (1) - (metabotropic)

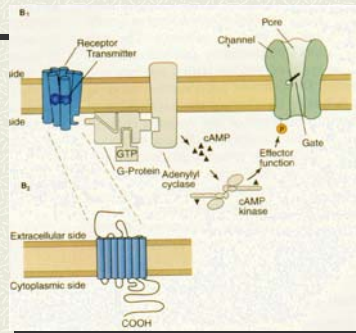
Often G-protein coupled
(G-protein α - or β/γ -subunit has direct effect on ion channel or effect via membrane bound effector protein)



(Purves et al., 2001)

And indirectly gated (2)

via intracellular
2nd messenger
system (here
cAMP)
(metabotropic)



4

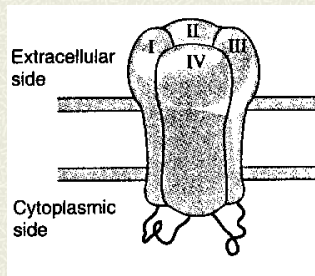
Molecular analysis has revealed *families* of ion channels

- # **Voltage-gated channels** consist of 1 polypeptide, with 4 domains, each with 6 membrane-spanning regions
- # **Ligand-gated channels** have 5 polypeptide subunits (eg. nAChR), each with 4 membrane spanning regions
- # **Gap junction channels** have 6 subunits, each with 4 membrane regions

5

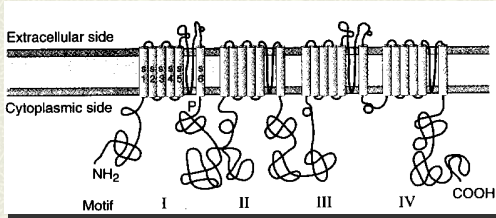
The voltage-gated Na⁺ channel

(3-D model, 4 transmembrane domains, remember single polypeptide chain)



6

... has 4 repeated domains, each with 6 membrane-spanning regions



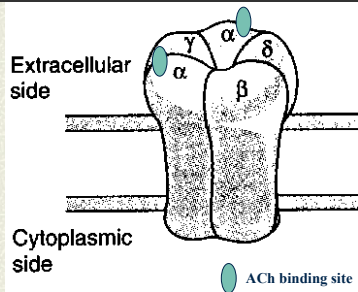
7

Cont... Voltage gated channels

Specificity for ions (Na⁺, K⁺, Ca²⁺)
Na⁺ and Ca²⁺ V-gated channels – single long polypeptide chain
Each of the domains is roughly equivalent to a subunit of a ligand-gated channel
Another similarity is the alpha-helical membrane spanning segments within each domain
Specific region (S4) believed to be responsible for voltage sensor
Region S5 – S6 region of a.a.'s that appear to form pore region which confers specificity

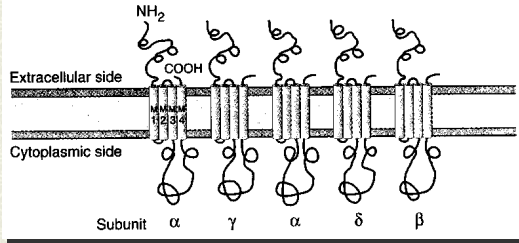
8

The ACh receptor



9

nACh receptor has 5 subunits,
each with 4 transmembrane regions



cont... nACh Receptor

- M2 region of each subunit lines pore – affects selectivity
 - M2 region flanked by cluster of acidic a.a. (glutamate and aspartate) confers cation selectivity (glu and asp –ve)
 - M2 segment flanked by cluster of basic aa's (lysine, arginine) confers anion selectivity in the pore (GABA_A, glycine)

Diversity of Neuronal AChR Subunits

- refers to those found in autonomic ganglia and brain
- α- and β- subunits similar to those from NMJ, numerous isoforms (11 different subunits – 8 α and 3 β)
- *in vitro* work (oocytes), deduced 2(α) and 3(β) subunits make up neuronal AChR

Channel Structure - Common Plan

Families: Voltage gated; ligand gated; connexon protein

1. Membrane spanning segments arranged around central hydrophilic pore which is gated
2. Structural units – subunits or domains – each that makes up channel same (connexon) or very similar
3. Ion selectivity – related to size of pore and number of subunits (roughly) – most selective (Na, Ca) only 4 “subunits” and narrowest pore; least selective is connexon (nAChR in between with 5 subunits)
4. Similarity in overall conformation – where protein is narrower/wider
5. Very minor change in conformation causes pore to open

13

More fast excitatory synapses

- ⚡ Most in the vertebrate CNS are activated by the neurotransmitter **glutamate**
- ⚡ Excitatory synapses are **cation-selective**
- ⚡ Channels are opened to Na^+ and K^+ , and sometimes to Ca^{2+}
- ⚡ Current is inward, therefore depolarizing

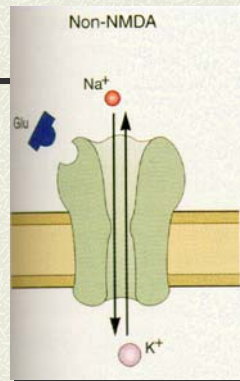
14

Glutamate receptors

Several “species” of Glu receptor/channel:

1. Non-NMDA

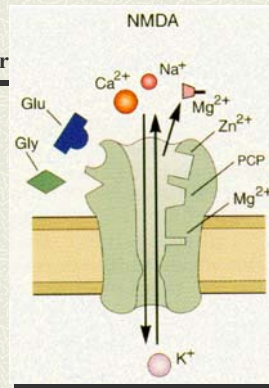
The non-NMDA receptor is like the ACh receptor, with the NT opening the channel to Na^+ and K^+ (kainate, quisqualate, & AMPA)



15

2. The NMDA glutamate receptor

- # Glutamate activates
- # At small depolarizations, Mg^{2+} blocks channel
- # At large depolarizations, channel opens to Na^+ , K^+ , and Ca^{2+}
- # Channel also binds glycine, phencyclidine and Zn^{2+}



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NMDA glutamate receptors are thought to be involved in memory (*more on this later in the term*)

- # Low levels of excitation only open non-NMDA channels (co-exist at same postsynaptic site as NMDAR channels)
- # High levels of excitation causing large depolarization also opens NMDA channels
- # NMDA channels allow Ca^{2+} to enter cell
- # This triggers intracellular messenger systems and (potentially) long-term changes to synapse

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cont...NMDA Receptors

- high conductance channel permeable to Na^+ , K^+ , and Ca^{2+}
- Calcium entry >>> activation of 2nd messenger cascades
- glycine required for operation
- gated both by glutamate and voltage (Mg^{2+} plug)
- open and close rather slowly
- glutamate excitotoxicity - various diseases/insults

Inhibit NMDA Receptor:

1. Mg^{2+} plug
2. Hallucinogenic drug phencyclidine (PCP) or MK801

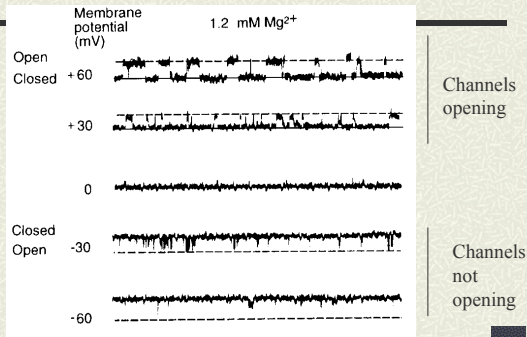
18

Special Case: NMDA Receptor Channel

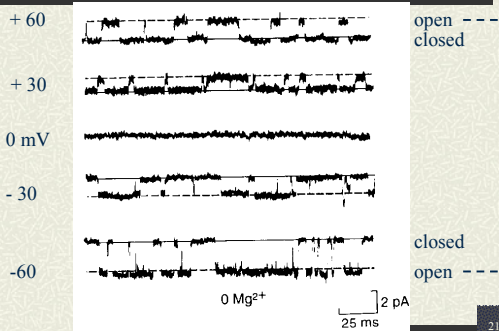
- recall, gating
 - chemical neurotransmitter (glutamate)
 - voltage (Mg^{2+} plug)
- what ions go through the channel?
- What do you think the reversal potential will be for this channel?

19

Mg^{2+} blockage of NMDA glutamate channel, outside-out patch clamp recording



With zero Mg^{2+} , the NMDA channel is voltage-independent

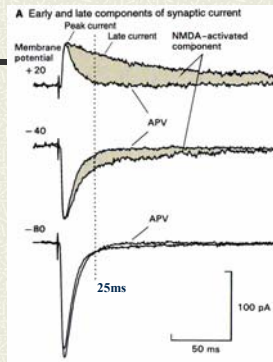


Synaptic Currents – Glu receptor channels

Component contributed by NMDA channels

APV antagonist to NMDA receptor channels

What is the significance of the late current?



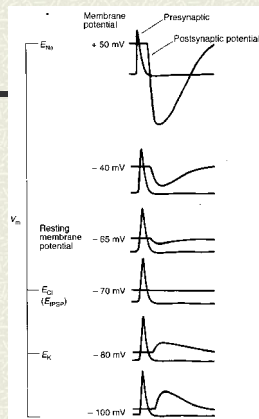
22

Fast inhibitory synapses – receptor superfamily (GABA, glycine, and 5-HT₃(fast))

- ❏ Anion channels operated (not 5-HT); 5-HT₃ subtype similar to nAChR and cation selective
- ❏ Are usually activated by γ -amino butyric acid (GABA) or glycine
- ❏ multiple subunit isoforms exist for each channel, all similar to nACh subunits
- ❏ NT opens anion-selective channels (not 5-HT)
- ❏ Ionic current is outward (carried by Cl⁻ inwards) and therefore hyperpolarizing
- ❏ Increasing G_{Cl} also short-circuits excitatory currents

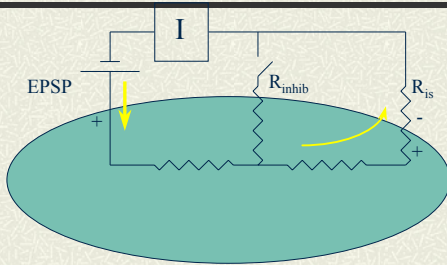
23

The reversal potential is around -70 mV, which is the same as E_{Cl}



24

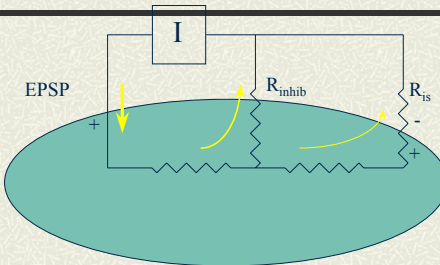
Action of synapses



R_{inhib} : inhibitory synapse (not active)
 R_{is} : membrane of initial segment (spike initiation zone)

25

cont...Action of synapses



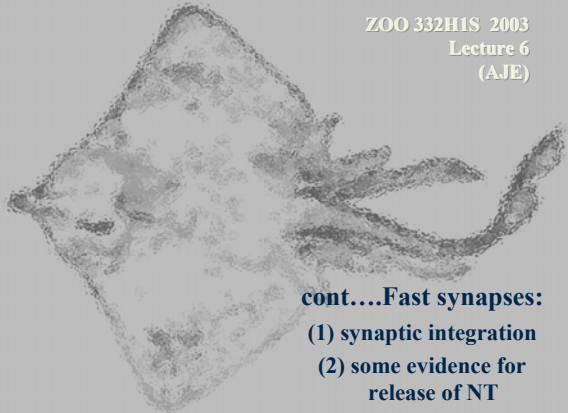
Inhibitory synapse active - shunts current, therefore less at initial segment

26

Next...Combining inputs (synaptic integration)


27

ZOO 332H1S 2003
Lecture 6
(AJE)



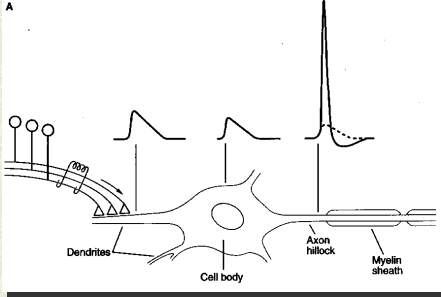
cont....Fast synapses:
(1) synaptic integration
(2) some evidence for release of NT

Synaptic integration



29

Excitatory synapses produce depolarising EPSPs that may trigger APs

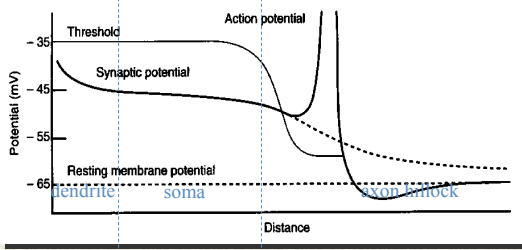


A

Dendrites
Cell body
Axon hillock
Myelin sheath

30

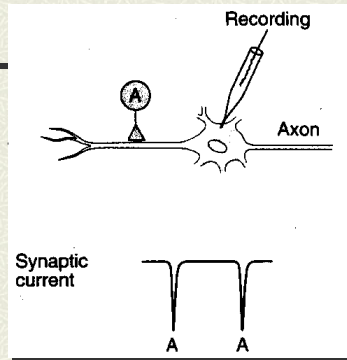
The EPSP spreads with spatial decay, initializing APs at the axon hillock



31

Temporal summation

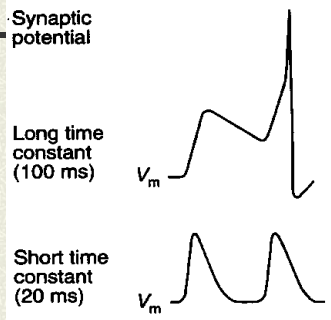
Presynaptic axon active sequentially



32

cont...Temporal Summation

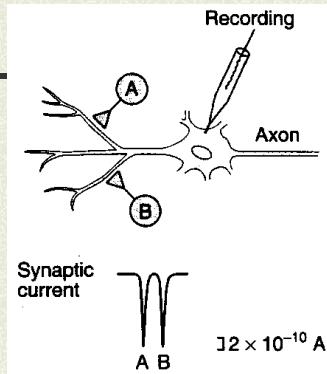
depends on time constant of membrane



33

Spatial Summation

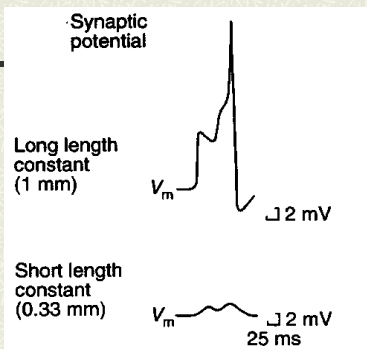
Presynaptic axons active together



34

Spatial Summation

depends on length constant of membrane

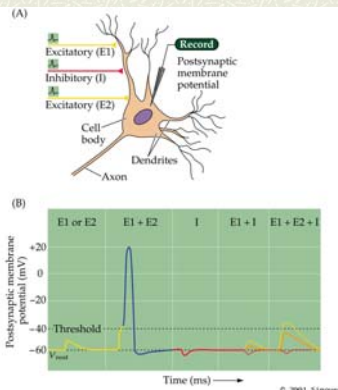


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Summary:

Summation of Postsynaptic Potentials

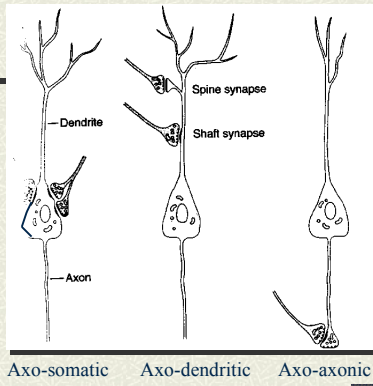
- Microelectrodes record postsynaptic activity
- E1, E2, I
- (B) shows electrical responses to synaptic activation
- E1 or E2 alone, subthreshold
- E1+E2 suprathreshold EPSP, AP
- I alone hyperpolarizing response
- yellow line shows effect of I on ability of EPSP to generate AP



(Purves et al. Fig. 7.7)

36

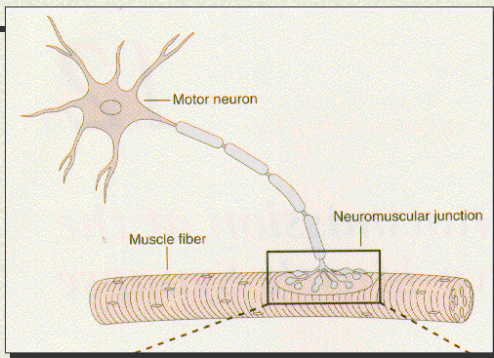
Synapses at different sites



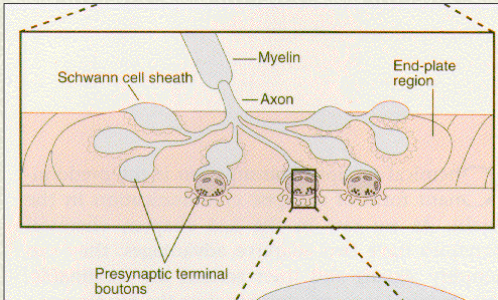
Axo-somatic Axo-dendritic Axo-axonic

Fast synapses: some evidence for NT release

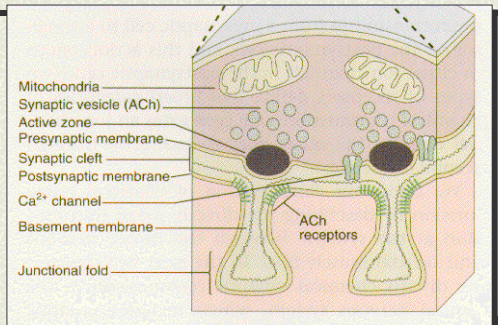
Recall...The vertebrate neuromuscular junction



An unusual, one-for-one, synapse



Large, accessible, and therefore much studied



At the NMJ

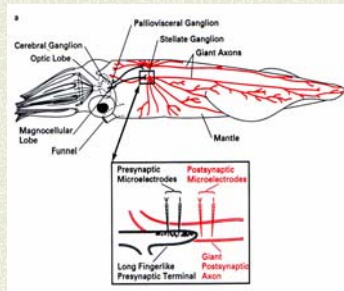
- ⚡ AP releases acetylcholine (ACh)
- ⚡ Enough ACh and enough receptor-channels (postsynaptic) to produce end-plate potential of 70 mV!
- ⚡ EPP (= EPSP) therefore triggers AP in muscle cell (also = EJP)
- ⚡ So how do we study EPP?

Neurotoxic drugs have aided research

- ✦ Curare blocks nACh receptors and reduces EPP, so can block AP
- ✦ Tetrodotoxin blocks voltage-gated Na^+ channels but doesn't affect EPP
- ✦ The snake-venom α -bungarotoxin binds to ACh receptors

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Squid Giant Synapse



L & K, Fig. 8-5

44

Synaptic efficacy

- ✦ presynaptically, depends on amount of transmitter released
- ✦ can be changed if amount of NT released by each AP changes
- ✦ So how can amount of NT be regulated?
- ✦ What is the mechanism of transmitter release?

45

Summary: Release of neurotransmitter (NT)

- Role of Na^+ and K^+ ions - "none", except to cause AP presynaptically - size of AP (amount of presynaptic depol'n)
- Experimental evidence (TTX, TEA) and electrical recordings
- Importance and evidence for involvement of Ca^{2+} (influx) - presynaptic terminal
- Quantal nature of NT release (synaptic vesicles)
- Evidence of quantal nature of release - electrical (MEPPs 0.4mV phenomenon; capacitance measurements) and morphological (freeze fracture and electron microscopy)

How is transmitter release dependent on presynaptic action potential?

Relation between presynaptic AP and PSP

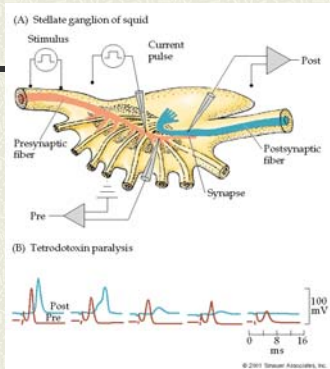


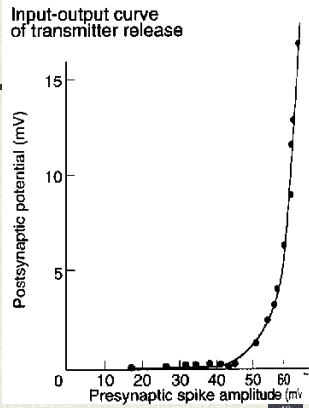
Fig. 11.1

PSP as function of presynaptic AP size

What's determining presynaptic AP amplitude?

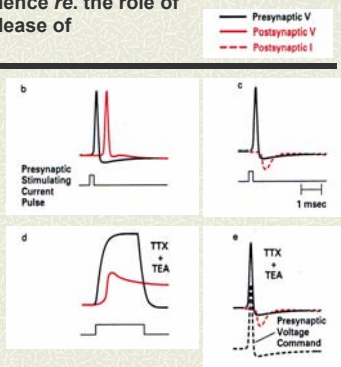
So then is Na^+ (or K^+) directly responsible for NT release?

(No)



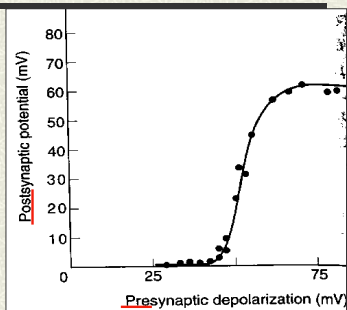
Experimental evidence re. the role of various ions in release of neurotransmitter:

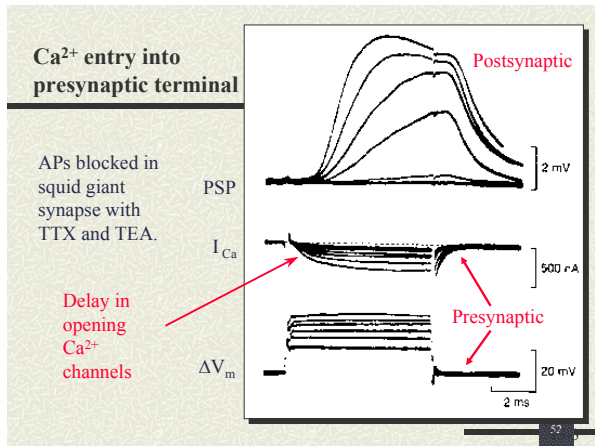
(1.) Na^+ and K^+

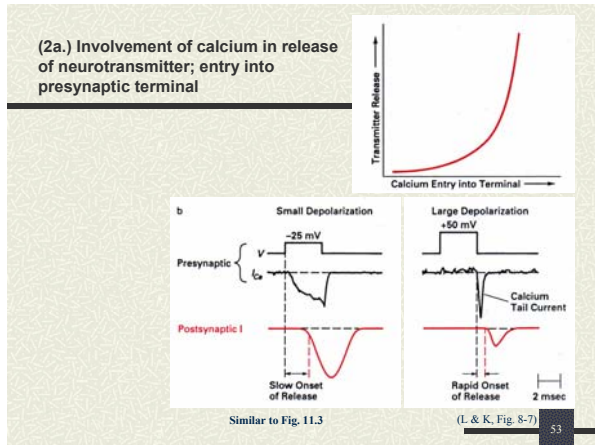


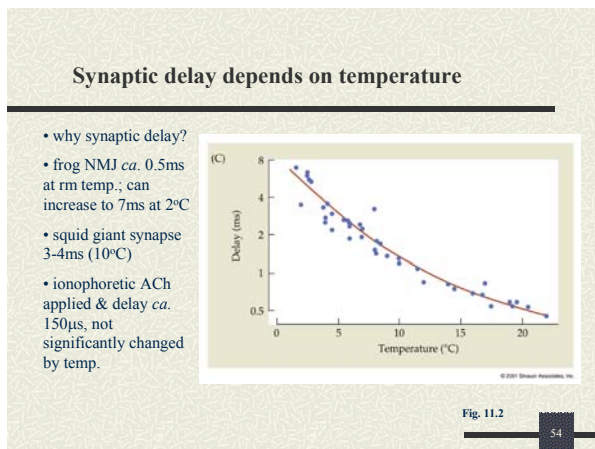
PSP vs. presynaptic ΔV

Blocking both Na^+ and K^+ channels reveals full dependence of transmitter release on presynaptic V_m

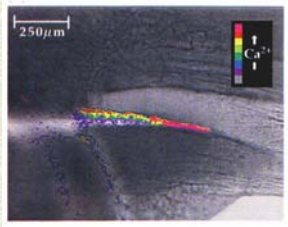








Calcium entry during presynaptic depolarization



From Smith...MP Charlton (1993). *J. Physiol. (Lond.)* 472, 573. Dr. MP Charlton's lab in the Dept. of Physiology (MSB) has contributed significantly to defining the role played by calcium in NT release.

Localizing site of Ca^{2+} entry

- (A) Recording pre- and post-synaptic axons
- (B) 4 min treatment with BAPTA

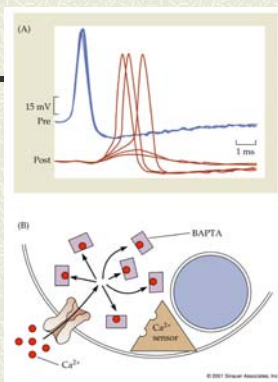


Fig. 11.5

cont...Localizing site of Ca^{2+} entry

- (A) Recording pre- and post-synaptic axons
- (B) 4 min treatment with EDTA

Site of Ca^{2+} entry must be within 100nm of Ca^{2+} trigger site

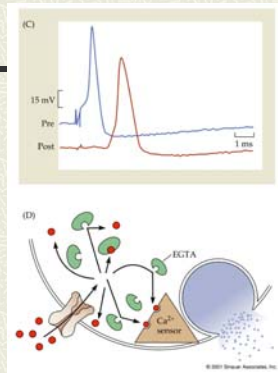
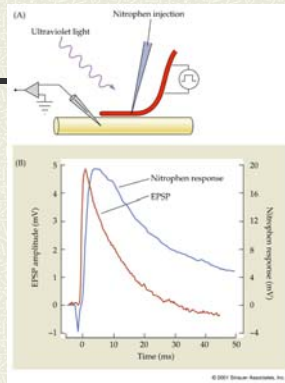


Fig. 11.5

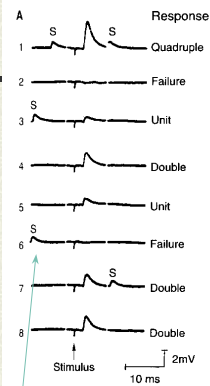
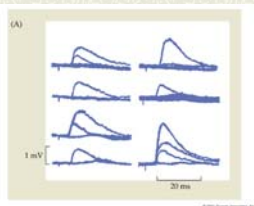
Caged Calcium



58

Quantal nature of PSP and NT release

- Muscle bathed in low $[Ca^{2+}]$
- Keeps NT output low



spontaneous m.e.p.p.

59

Size of m.e.p.p.s follows Poisson distribution

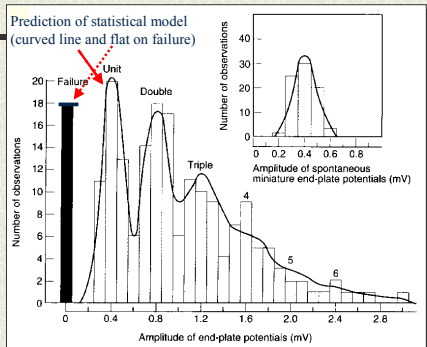


Fig. 11.9

60

How many ACh molecules in one vesicle?

- Single channel current enough to produce $0.3\mu\text{V}$ PSP
- This is about 1/2000 of 0.4 mV MEPP
- 2 ACh needed per channel opening
- Allowing for losses, estimate about 5000 molecules of ACh per vesicle
- Confirmed now by direct chemical measurements

Synaptic Ultrastructure - Diagrammatic representation of membrane from freeze-fracture EM

- support for vesicle hypothesis
- assembled multiple freeze-fracture exps
- orderly rows of vesicles
- Ca^{2+} channels and ACh receptors

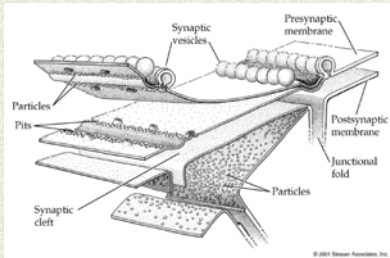
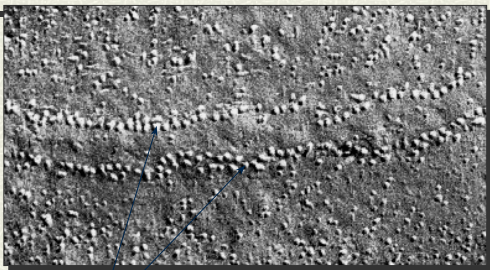


Fig. 11.14

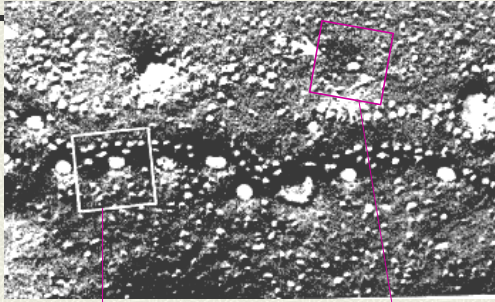
Presynaptic membrane before vesicle release



Probable Ca^{2+} channels

Fig. 11-17

Stimulus plus 5 ms

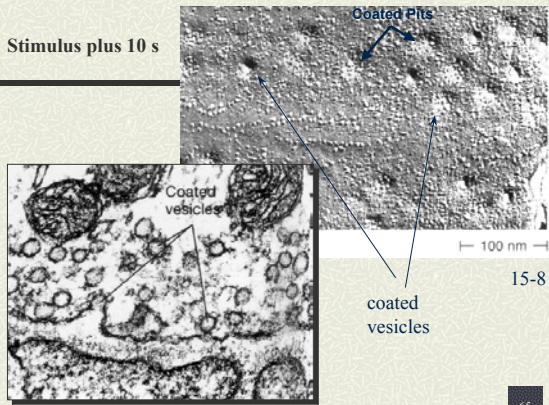


site of vesicle fusion

fused & collapsed vesicle

64

Stimulus plus 10 s



Coated pits

Coated vesicles

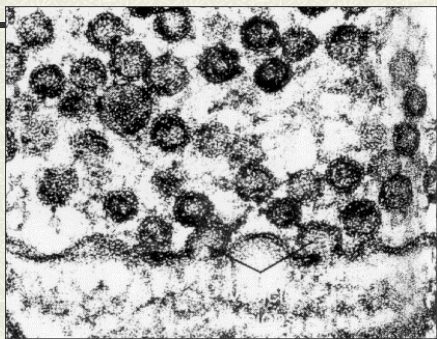
100 nm

15-8

coated vesicles

65

Fused vesicles: TEM section



omega

66

Further support for location of Ca²⁺ channels and AChR

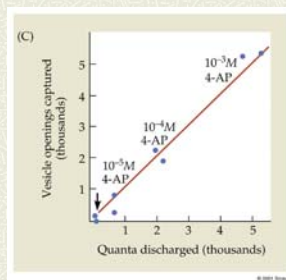
- NMJ
- fluorescent tag on α -bungarotoxin and different fluorescent tag on Ab to Ca²⁺ channel protein
- superimpose images

67

Capturing vesicles releasing NT

What effect would blocking v-gated K⁺ channels have?

Difficulty in catching vesicles in the "act" reduced by 4-AP

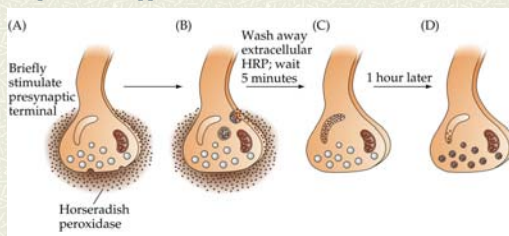


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Local recycling of synaptic vesicles in presynaptic terminals

"Synaptic vesicle cycle"

Experimental approach:



Purves *et al.* (2001) after Heuser and Reese (1973)
(Similar to Fig. 11.20)

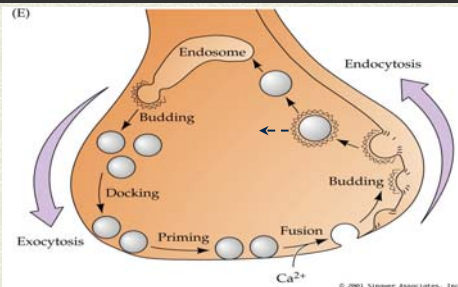
69

cont...Local recycling of synaptic vesicles

- Further support using fluorescent dyes (non-toxic)
- Advantage (living prep (neurons in culture), optical, don't require electrical recordings)
- time course of release, reuptake, 'reactivation' of vesicle
- other preparations – chromaffin cells (adrenal medulla) and mast cells (leukocyte which stores inflammatory mediators for release)

70

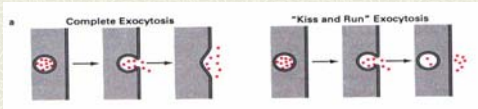
cont... Local recycling of synaptic vesicles in presynaptic terminals



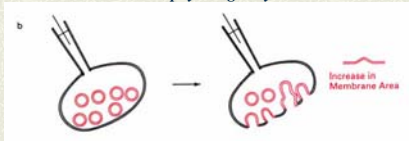
Purves *et al* (2001) after Heuser and Reese (1973) See Fig. 13.19

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Vesicle Fusion & Release of Neurotransmitter



Which is more physiologically relevant?



L&K, 1997

72

Cont...Vesicle Fusion & Release of Neurotransmitter



Complete Exocytosis

Kiss and Run

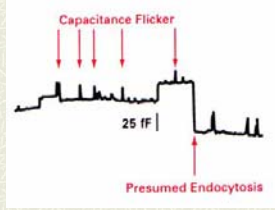
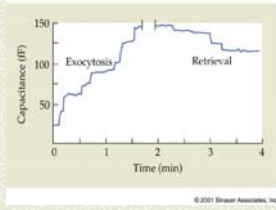
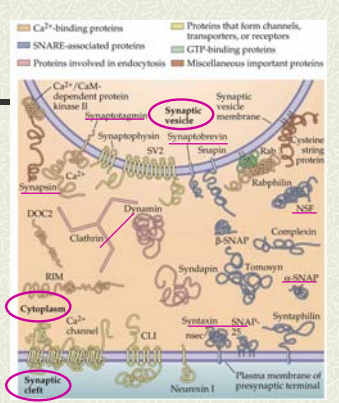


Fig. 11.24 & L&K, 1997

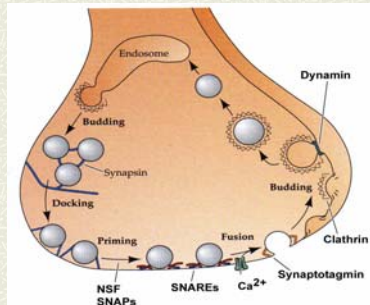
Chromaffin cells (ad med) or mast cells (here, connective tissue of peritoneum) which release contents of large vesicles by exocytosis (not neuronal presynaptic membrane)

Presynaptic proteins implicated in neurotransmitter release (after Purves et al. 2001)

Do not memorize this diagram

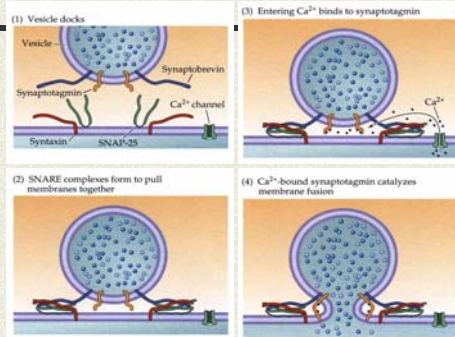


Proteins implicated in synaptic vesicle cycling



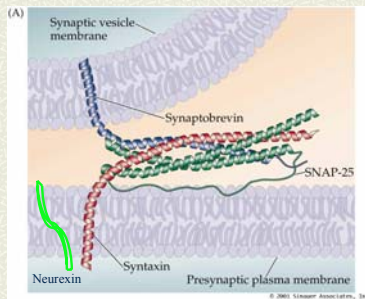
Purves et al., 2001

Steps in Fusion of Vesicles and Release of Neurotransmitter



Structure of the SNARE Complex

Vesicular SNARE, synaptobrevin (aka. VAMP), forms a helical complex with the plasma membrane SNAREs (syntaxin and SNAP-25).



Toxins That Affect Neurotransmitter Release

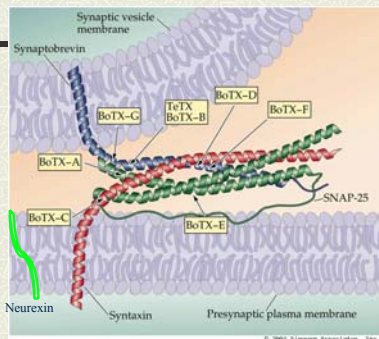
Clostridial Toxins:

botulinum toxin and tetanus toxin

- highly specific proteases that cleave SNARE proteins (synaptobrevin, syntaxin, SNAP-25)

Black Widow Spider Venom

- α -latrotoxin, massive release of NT (interaction with neurexins, which interact with synaptotagmin?), Ca²⁺-independent



Notes on Molecular Mechanism of Transmitter Secretion

- how Ca^{2+} triggers fusion and NT release not understood - although proteins and functions/interactions deduced
- **NSF** (NEM-sensitive fusion protein) and **SNAPs** (soluble NSF-attachment proteins) involved in priming synaptic vesicles for fusion
- NSF and SNAPs regulate assembly of other protein, SNAREs (SNAP receptors)
- SNARE in vesicle - synaptobrevin (also known as **VAMP**); SNAREs in plasma membrane are **syntaxin and SNAP-25**
- macromolecular complex forms between two SNAREs to bring two membranes close together
- **synaptotagmin** binds to complex and acts as Ca^{2+} sensor; Ca^{2+} acts as a regulator of NT release by binding to vesicular synaptotagmin (SNAREs do not bind Ca^{2+})
- hypothesis: binding of Ca^{2+} to synaptotagmin changes its chemical properties and allows it to insert into membranes and bind other proteins. Thus, plausible that SNAREs bring membranes close together and Ca^{2+} acts on synaptotagmin to fuse membranes.
- **clathrin and dynamin** - endocytotic budding of vesicles; **synapsin** tethers (cross-links) vesicles to cytoskeleton.

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Cont...Notes on Molecular Mechanism of Transmitter Secretion - Some Evidence

- NSF and SNAPs known to be important for fusion of vesicles with membranes of Golgi apparatus
- location of various proteins hypothesized to be involved
- in vitro interaction of proteins, ability to form macromolecular complexes
- toxins that cleave SNARE proteins block neurotransmitter release
- SNARE proteins in artificial membranes...fusion of membranes
- synaptotagmin binds Ca^{2+} at a concentration similar to those known to cause vesicular transmitter release
- alteration of properties of synaptotagmin in mice, squid, *Drosophila* affects Ca^{2+} - dependent transmitter release
- deletion of one of the genes that codes for synaptotagmin in mice is lethal

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Diseases that affect the pre- or post-synaptic terminal

- Can effect exocytosis or endocytosis of synaptic vesicles
- **eg., myasthenic (muscular weakness) syndromes** - abnormal transmission at NMJ, weakness and fatigability of skeletal muscles
- **Lambert-Eaton myasthenic syndrome (LEMS)**
 - frequent complication of certain types of cancer
 - biopsies from muscle tissue, recordings indicate LEMS impairs evoked NT release, but does not affect the size of individual quanta
 - loss of v-gated Ca^{2+} channels implicated - lower density of Ca^{2+} channel protein in presynaptic terminal
 - high titre of antibodies against Ca^{2+} channel protein
 - treatment/experiments: remove Abs, immunosuppressant drugs; injection of Abs into exp. animal

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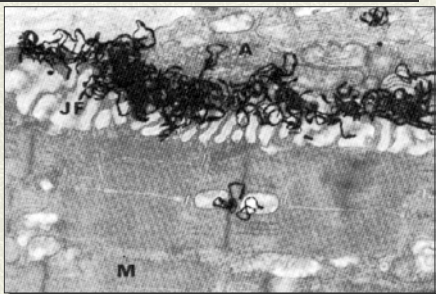
cont...Diseases that affect the pre- or post-synaptic terminal

Congenital myasthenic syndromes

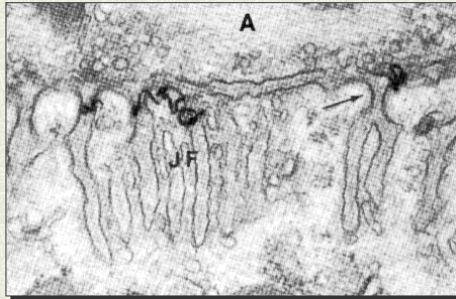
- affect acetylcholinesterase
- autoimmune attack of nACh receptors (Myasthenia Gravis)
- altered synaptic vesicle trafficking in presynaptic terminal
 - reduced number synaptic vesicles available
 - reduced size of individual quanta (smaller vesicles)
 - botulinum toxin and tetanus toxin (from *Clostridium* bacteria)(NMJ and spinal inhibitory interneurons - block release by cleaving SNARE proteins)
- more on Myasthenia Gravis later

THE END (extra slides after here)

Location of ACh receptors revealed by labelled α -bungarotoxin

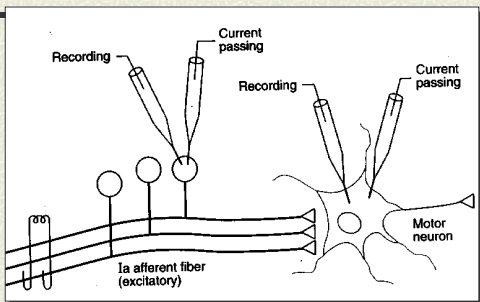


ACh receptors at peak of folds close to presynaptic membrane



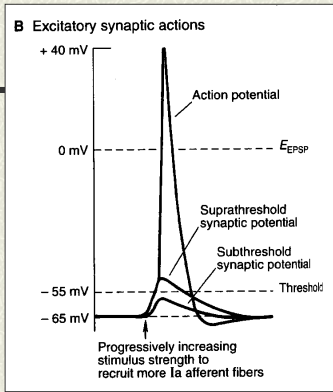
85

Intracellular recordings of the neuronal EPSP



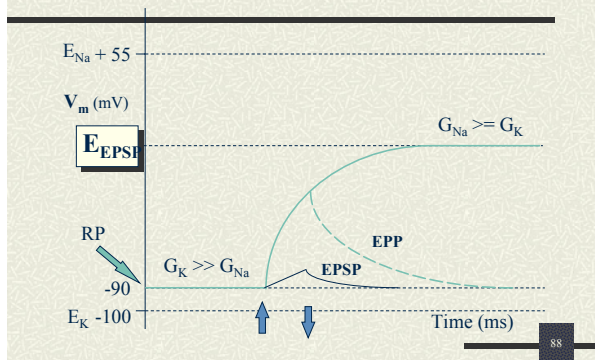
86

Increasing the number of active presynaptic axons shows summed EPSPs triggering an AP



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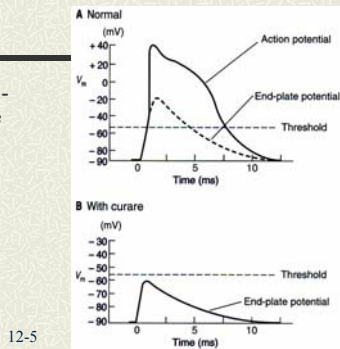
The reversal potential



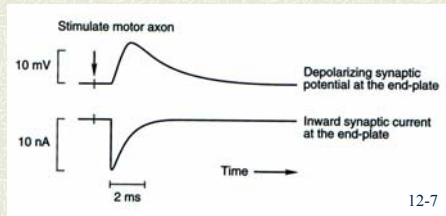
Reversal potential for EPP

- ⚡ Represent new steady state when ion channels are open during presence of ACh
- ⚡ Normally, V_m doesn't reach this value
- ⚡ If we artificially move V_m to this value before synapse is active, *EPP would be zero*
- ⚡ If move *above*, EPP becomes -ve
- ⚡ So the EPP *reverses* at this value
- ⚡ Reversal potential indicates what ions are involved

End Plate Potentials - blocking with curare

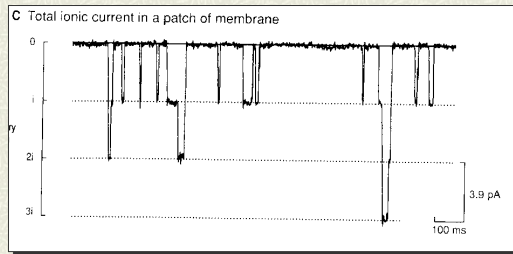


Post synaptic current causes potential change



12-7

EPP is produced by current from many channels



12-9
