

Outline, Neurobiology 635

Synapses I : Neuron-Neuron and Neuron-Muscle Interactions

I. Synapse:

- site of interaction between 2 neurons or neuron and muscle
- junction through which electrical activity is transferred

II. Electrical Synapses

- gap junctions (protein structures which can open and close)
- "couple" cells (why?)

III. Chemical Synapses - Many types; many receptor mechanisms, many neurotransmitters

A. Neurotransmitter Release - Presynaptic

1. Presynaptic Action Potential induces Ca^{++} entry (voltage sensitive Ca^{++} channels)
2. Ca^{++} activates neurotransmitter (NT) release.
3. NT diffuses across synaptic cleft -> receptor proteins in postsynaptic membrane
4. Presynaptic Dynamics influenced by 2nd messenger modulation:
synaptic, NO, hormonal

B. Neurotransmitter Response - Postsynaptic

1. "Fast" Receptors: Ligand (i.e. transmitter) gated (i.e. stimulated) ion channels.
2. "Slow" Receptors: G-protein coupled receptors (a.k.a. metabotropic receptors)
3. Receptor activation regulates ionic currents
 - direct: ligand gated ion channels
 - indirect: G-protein coupled receptors
4. Postsynaptic Dynamics influenced by:
 - co-mingling of receptor types
 - complex activities of 2nd messenger induced biochemical pathways
 - duration of stimulation (time dependence)
 - "unusual" systems
 - Glutamate receptors
 - Nitric Oxide and cGMP

C. Signal Termination

1. Neurotransmitter inactivation: degradation and/or washout.
2. Presynaptic termination: terminate APs, terminate Ca^{++} entry, Ca^{++} actively removed.
3. Postsynaptic termination: degrade 2nd messengers, phosphorylation/dephosphorylation

IV. First Synaptic Model: Motor End Plate

A. Motor End Plate - (Acetylcholine / ligand gated ion channels)

B. Miniature End Plate Potentials (MEPPs); summation to Muscle AP

- Na^+ and K^+ currents - channels pass both ions (reversal potential = 0 mv)
- end plate currents:
 1. total number of channels
 2. probability that channels are open - NT dependent
 3. conductance of each channel
 4. driving force of each ion

C. quantal release theory of NT (vesicles and set numbers of vesicles)

References, Synapse I

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Problem: Due Tuesday (next lecture).

Stimulate a virtual axon. **Draw the shape of the resulting action potential (V_m vs. time) under each of the following 4 conditions;** each condition differs in the type of voltage sensitive ion channels present. **Use graph paper** and draw on top of each other so that shapes can be compared. **Differences to expect are the voltage that the action potentials "go to" and the duration of the action potential (how long from depolarization to repolarization).** Describe the differences between the curves in a few sentences.

1. Na^+ + K^+ - "DR" (the classic Squid giant axon)
2. Na^+ + K^+ - "A"
3. Na^+ channel only
4. experimentally mutated Na^+ channel with inactivation mechanism removed

Na^+ channel: fast open, self inactivating

K^+ - "DR" channel: slow open, not inactivating (delayed rectifier)

K^+ - "A" = fast open, self inactivating ("A" or Shaker type channel)

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Synapses 2: Neurotransmitters and Receptors

I. Quantal Release (From Aidley, 1998...)

1930s, debate over whether synaptic transmission was electrical or chemical
electronics and technique of intracellular recording >>>>>

1952: Spontaneous MEPPs (Fatt & Katz, 1952) (**Fig. 10.1**):
voltage dependent in a linear fashion (Liley (1956) (**Fig. 10.2**)
size spontaneous MEPPS sensitive to curare and anticholinesterase
(curare - treefrog poison - blocks ACh sensitive ion channels (i.e. receptors))
(anticholinesterase - blocks degradation of ACh)
approx. 10,000 ACh molecules (Kuffler & Yoshikami, 1975b)

1954: Quantal Release Theory (del Castillo & Katz, 1954a,b)
Mg⁺⁺ block of neuromuscular transmission
quantity of ACh per impulse reduced (del Castillo & Eugbaek, 1954)
degree of disruption dependent on ration of Mg⁺⁺:Ca⁺⁺
Reduce # of MEPPs by Mg⁺ block
size of successive MEPPs fluctuated in a stepwise manner (**Fig. 10.3**)
******suggested ACh released in packets or quanta**
Normal EPP >> 100s of quanta
MEPP >> single quantum
Statistical Analysis of many impulses (**Fig. 10.4**)

Why Quanta? What are Quanta?

1954: Vesicles observed in nerve terminals (DeRoberts & Bennett '54; Palade & Pakey '54)
500 angstroms diameter
1956: del Castillo & Katz propose these contain neurotransmitter
1 quantum = 1 vesicle

proposals that 1 quantum = multiple vesicles refuted in 1990
Hurlbut et al., 1990:
frog muscle treated with α -latrotoxin (Black Widow Spider toxin)
 α -latrotoxin causes massive quantal release of ACh
depletion takes about 20 min
during this treatment - prepare tissue for EM analysis
plot # of vesicles remaining vs. time
plot # of quanta secreted vs. time
plot # vesicles remaining vs. number quanta secreted
observe 1:1 relationship between vesicles and quanta arguing strongly that
1 vesicle = 1 quantum

Note: MEPPs are spontaneous, occurring whether or not there is an AP
their occurrence is probabilistic: there is a large population of quanta at a nerve terminal, each having a
small probability of being released (del Castillo & Katz, 1954a). An AP GREATLY increases this
probability.

- in muscle:

- 1 quantum = 10,000 ACh molecules activating 1500 receptors creating MEPP
(iontophorese ACh onto endplate to mimic MEPP, quantify)
- summation of 50-100 quanta (and MEPPs) required for Action Potential
(many more probably released)
- Acetylcholine Esterase is in synaptic cleft,
degrades ACh en route to receptor as well as after receptor activation
- from Kandel, p 204:
 - at -90 mV (Vm) each ACh channel passes -2.7pA current (30 pS conductance)
 - constant amplitude per channel
 - mean open time (variable) of 1 msec per channel
 - 17,000 Na ions move inward
 - somewhat smaller number of K channels move outward
 - about 200,000 ACh receptors in a motor endplate synapse

"The function of ligand-gated channels at the synapse is to respond to the stimulus provided by the neurotransmitter by altering rapidly the potential of the postsynaptic membrane. This is achieved by opening a pathway across the membrane that is selectively permeable to either small cations or small anions. The channels usually have only slight preferences among the types of ions that they allow through and so are unlike voltage-gated channels, which are strongly selective for a particular ion, and unlike gap junction channels or porin, which are almost nonselective. The acetylcholine, serotonin (5HT₃) and glutamate gated ion channels, at excitatory synapses, create an environment that allows the passage of cations, whereas the glycine and γ -aminobutyric acid (GABA_A)-gated ion channels, at inhibitory synapses, create the same for anions." [Unwin N (1993) Neurotransmitter action: opening of ligand-gated ion channels. Cell 72/Neuron 10, 31-41.]

II. Neurotransmitter diversity

Acetylcholine (ACh)	Amino Acids Glycine GABA Glutamate Aspartate	Catacholamines, or Biogenic amines Dopamine Norepinephrine Epinephrine (adrenaline) Serotonin (5HT) Histamine	Peptides numerous (50-100s)
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Neurotransmitters must be synthesized and packaged into vesicles

Note: neurotransmitter receptors include (1) ligand gated ion channels and (2) G-protein coupled receptors. Receptors for peptides are mostly G-protein coupled receptors. However, both types of receptors are present for all of the other types of neurotransmitters, distributed by cell or tissue type. For ACh in mammals, neuromuscular receptors are of the ligand gated ion channels while the receptors in the heart (SA node from vagus nerve) are of the G-protein coupled type. Most neurotransmitters are used in diverse types of nerves, and for quite different systems in different animal phyla. However, their "use" is conserved within closely related animal groups.

III. Synaptic Biochemistry

Paul Greengard - isolation of synaptosomes, protein called synapsin
established field characterizing mobilization of vesicles, NT synthesis, processing, release

Whittaker et al., (1964) *Biochem. J.* 90, 293, (synaptosomes)

Ueda & Greengard (1977) *J. Biol. Chem.* **252**, 5155-5163. (synaptosomes, rediscovered?)

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IV. Storage and Release of Synaptic Vesicles

1. Complex biochemistry, many proteins (see fig. 10.18)

2. Two Populations of Vesicles in nerve terminals

1. Storage Pool

bound to microtubules by Synapsin I protein

Ca⁺⁺ activates Protein Kinase (PK)

PK phosphorylates Synapsin I

Vesicles released to migrate to Active Zone

2. Releasable Pool

Vesicles are arrayed near pre-synaptic membrane in active zone

Double Row of Vesicles (Aidley, fig. 10.7)

Double Row of Ca⁺⁺ channels 20-30 nm away (i.e. right there!)

Docking of Vesicles - Many Proteins (SNAPs and VAMPs)

Vesicle Membrane Proteins

Nerve Membrane Proteins

Joined by Cytoplasmic Proteins

Docked Vesicles are Fusion Ready

Docking Process is triggered by high levels of free Ca⁺⁺

3. Vesicle Release: Fusion (Fig. 10.18, 10.19)

Formation of Fusion Pore

formed by docking proteins

triggered by Ca⁺⁺

stable for only "brief fraction of a msec"

Synaptotagmin protein acts as Ca⁺⁺ sensory

[Ca⁺⁺] - rest: 0.1 uM // active: 100-200 uM

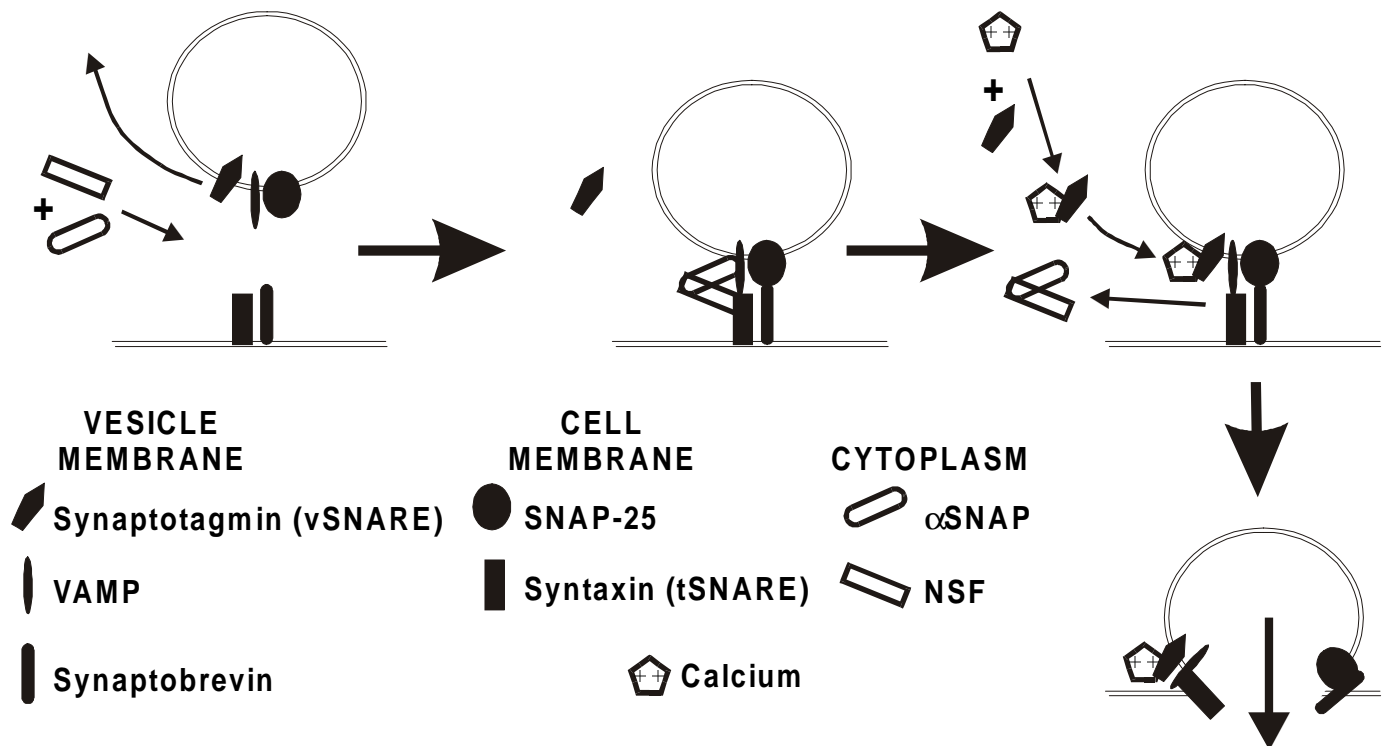
only 0.1-0.2 msec delay between Ca⁺⁺ entry and NT release

works because Ca⁺⁺ channels are so close

4. Vesicle Recycling

Fusion adds membrane to nerve terminal, must be recycled (Fig. 10.8)

Recycling process takes 45-90 sec



MODEL: VAMP/synaptobrevin and synaptotagmin (vSNARE) on the synaptic vesicle, and SNAP-25 and syntaxin (tSNAREs) on the plasma membrane, interact to form a 7S complex. Two additional soluble proteins, αSNAP and NSF, are later added to the 7S complex, accompanied by the loss of synaptotagmin. The resulting 20S complex contains syntaxin, SNAP-25, VAMP, αSNAP, and NSF. Genetic studies in several species demonstrate that mutation or deletion of synaptotagmin results in a large decrease in Ca²⁺ triggered transmitter release. Mammalian synapses that lack synaptotagmin show a selective decrease in a fast component of release, suggesting that synaptotagmin is the Ca²⁺ sensor triggering exocytosis.

III. Neurotransmitter diversity

Acetylcholine (ACh)	Amino Acids Glycine GABA Glutamate	Catacholamines, or Biogenic amines Dopamine Norepinephrine Epinephrine (adrenaline) Serotonin (5HT) Histamine	Peptides numerous (50-100s)
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Neurotransmitter receptors

Ligand-Gated Ion channels

G-protein coupled Receptors

at least for the non-peptide NTs, each NT has both types of receptor and possibly multiple sub-types

multiple types of receptors (NT sensitive and/or mechanism type) may coexist in the same synapse

thus synapses can be sites where NTs and receptor types may "compete", "cooperate" or

"synergize" with each other

and dendrites become regions where synapses may "compete", "cooperate" or "synergize"

with each other

IV. Ligand-gated Ion channels

Two types, based on homology

Type I - ACh (nicotinic), 5HT, GABA, Glycine

Type II - Glutamate

Multiple subtypes characterized by kinetic properties, pharmacological properties

V. G-protein coupled Receptors

"serpentine receptors" or 7-transmembrane domain receptors

NT+R > G-protein activation > response >>>>>>>

activation of "metabolic" or biochemical pathway (also called "metabotropic")

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Synapses 3: Receptors and Transduction Mechanisms

I. Neurotransmitter diversity

Acetylcholine (ACh)	Amino Acids: Glycine GABA Glutamate	Catacholamines, or Biogenic amines: Dopamine Norepinephrine Epinephrine (adrenaline) Serotonin (5HT) Histamine	NeuroPeptides numerous (50-100s)
NO, CO	ATP		

II. Ligand-gated Ion channels

Two types, based on homology
 Type I - ACh (nicotinic), 5HT, GABA, Glycine
 Type II - Glutamate

ACh receptor as the model ligand gated ion channel
 Five subunits: $\alpha \alpha \beta \delta \epsilon$
 Subunits are homologous
 Developmentally Specific: i.e. Fetal vs. Adult forms
 Properties to account for in molecular structure:
 Ligand binding site
 ion selective (cation vs. anion)
 gating mechanisms
 open time, current volume

III. G-protein coupled Receptors: Second Messenger Based Transduction Pathway

A. Receptor: "serpentine receptors" or 7-transmembrane domain receptors

B. G-protein

three subunits: $\alpha \beta \gamma$
 $G\alpha$ -subunit binds GDP (inactive) or GTP (active)
 receptor activation allows GTP to displace GDP, activating the $G\alpha$
 $\beta \gamma$ subunits may also be active in some cases

C. multiple different $G\alpha$ -subunits

respective $G\alpha$ -subunits are capable of activating different but specific targets (pathways)
 adenylate cyclase: ATP > cAMP
 phospholipase C: phosphatidyl inositol (PI) > inositol trisphosphate (IP₃) and diacyl glycerol (DAG).
 phosphodiesterase: cGMP > GMP

D. Second messengers can activate many targets

- cAMP: Protein Kinase A (PKA) > phosphorylates targets (activation or inactivation)
- cAMP: cyclic nucleotide gated ion channels
- cAMP: gene expression through binding to transcription factor
- IP₃: Ca⁺⁺ channels, releasing Ca⁺⁺ into the cytoplasm
- Ca⁺⁺: PKC > phosphorylates targets

E. Modulated targets can be "upstream" and/or "downstream"

IV. Nitric Oxide, cGMP, glutamate receptors

A. Glutamate Receptors: ligand gated ion channels (Type II above)

- 2 kinds, characterized by pharmacology:
 - NMDA: Na⁺ and Ca⁺⁺, slow, require both glutamate and glycine
 - Kainate: Na⁺ (fast), requires glutamate only
- both kinds co-mingle in same postsynaptic membrane

B. nitric oxide (NO)

- small, highly diffusible
- N-hydroxy-L-arginine > NO + L-cirtulline by NOS (nitric oxide synthetase)
- NO produced in POST-synaptic cell
- NOS activated by Ca⁺⁺ entry (through NMDA receptor)
- NO activates cytosolic guanylate cyclase in PRE-synaptic cell: GTP > cGMP

C. cGMP: second messenger

- cytosolic and membrane bound GCs (guanylate cyclase): different
- in NO system, cGMP regulates (reinforces) NT release in PRE-synaptic cell

D. Special role in synapses that show memory.

V. Signal Termination

A. Presynaptic: stop AP, stop Ca⁺⁺ entry

B. Postsynaptic: degrade second messengers, pump out Ca⁺⁺, remove PO₄ groups (phosphatase)

C. Synaptic Cleft: flush neurotransmitter

- permit diffusion away from synapse
- ACh: degradation by acetylcholine esterase
- other neurotransmitters: transporters in presynaptic membrane and glial cells
 - Class I: GABA, noradrenaline, dopamine, 5HT, L-glycine, L-proline
 - 12 hydrophobic domains
 - Na⁺/Cl⁻ co-transport
 - Class II: Glutamate
 - 8-10 hydrophobic domains
 - Na⁺/K⁺/OH⁻ dependent

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Synapses 4: Receptors and Transduction Mechanisms

Slow:

G-Protein Coupled Receptors

Fast:

Neurotransmitter Gated Ion Channels

I. Neurotransmitter (Ligand) Gated Ion Channels (TGIC)

5 subunits, integral membrane proteins

Excitatory: Na^+/K^+ , $V_m = E_{\text{Na}} + E_{\text{K}} = 0 \text{ mV}$ (depolarize, net positive current)

Inhibitory: Cl^- , $V_m = E_{\text{Cl}} = -60 \text{ mV}$ (hyperpolarize, no net current)

ACh Receptor

Muscarinic - + muscarine, - atropine (GPCR)

Nicotinic - + nicotine, - curare, - α -bungarotoxin

Torpedo, Muscle:

5 subunits: $\alpha_2, \beta, \gamma, \delta$

2 ACh per receptor

cloned, '83 (Numa), α more conserved than β, γ, δ between species

Na^+ and K^+

Central Nervous System

α_2, β_3

8+ α subunit types, some work as homo-channels

GABA_A, 5HT, Glycine similar

ATP receptors, also ligand gated but different - purinergic receptors

ACh + ATP in ACh vesicles

Na^+ and Cl^{++}

II. G-protein Coupled Receptors (GPCR)

A. 7-Transmembrane Domains (single proteins)

B. Receptors complexes (couples) with to G-protein

tetramer: α, β, γ subunits

α -subunit binds GDP (inactive) or GTP (active)

NT (ligand) binding to receptor causes GTP to displace GDP and complex to dissociate

C. activated α -subunit migrates around activating other proteins through contact

one α -subunit can activate more than one target

D. "Classic" Actions

1. cAMP Pathway: α -subunit > adenylate cyclase (AC) > ATP to cAMP > cAMP activates "Protein Kinase A" (PKA - A for cAMP - enzyme that phosphorylates other proteins, reversed by enzyme phosphatase).

2. IP₃ Pathway: α -subunit > Phospholipase C (PLC - C for calcium) > PLC cleaves phosphatidyl inositol into IP₃ (soluble - inositol triphosphate) and DAG (membrane - diacylglycerol). IP₃ binds to membrane receptor that is also Ca⁺⁺ channel, releasing Ca⁺⁺ into cytosol. Ca⁺⁺ activates PKC (Ca⁺⁺ dependent Protein Kinase). PKC migrates to membrane where it is further activated by DAG to phosphorylate proteins.

E. Examples.

1. Vision (vertebrate): "dark current". light > GPCR > α -subunit > Phosphodiesterase (degrades cyclic nucleotides) > degradation of cGMP. cGMP binds to and activates Na⁺ channels, establishing a Na⁺ current. Thus, light activation degrades cGMP and turns off Na⁺ currents.

2. Olfaction (vertebrate):

- "generic cAMP or IP₃, but cAMP and IP₃ bind directly to ion channels, activating them.
- "classic cAMP and IP₃ actions" follow these primary actions.

3. Multiple parallel pathways exist, with different temporal (time) properties: fast, medium, slow activation of a pathway depends on how long the second messenger is present competition between activation and inactivation of second messengers activation and inactivation of NT, synapse, etc.
cAMP >> (1) fast - direct on ion channel; (2) medium - action via PK (3) slow - gene expression

4. Influences on Gene Expression: cAMP > PK > phosphorylation of DNA binding proteins which regulate expression of specific genes (DNA binding sequence = "CRE" = "Cyclic nucleotide Response Element"; DNA binding protein = "CREB" = CRE Binding protein). Currently thought to be very important mechanisms in long term memory.

5. Combinations of different Pathways (Combinatorial Effects):

Consider synapse containing both TGIC and GPCR for same NT
Consider same for different NTs in same synapse.
If you can imagine it, it probably exists.

6. Learning and Memory: Aplysia: presynaptic modulation

Ca is regulated by phosphorylation of voltage sensitive K-channels
phosphorylation is mediated through synaptic activation of a cAMP-PKA pathway

7. Learning and Memory: Hebbian Synapses - retrograde transmission

NO - nitrous oxide.

Glutamate receptors allow Ca⁺⁺ entry

Ca⁺⁺ activates Nitrous Oxide Synthase (N-hydroxy-L-arginine + O₂ >> NO + L-citrulline)

NO is small, diffuses through membranes.

NO produced in postsynaptic side, diffuses to presynaptic side where it activates guanylate cyclase (GC) producing cGMP. cGMP increases mobilization and release of Glutamate (potentiates the synapse, strengthens the synapse, more kick per AP)