Principles of Signaling in the Nervous system

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Current flow at electrical synapses

Current flow at chemical synapses
Graded electrical transmission

Current pulse to presynaptic cell

Voltage recorded in presynaptic cell

Voltage recorded in postynaptic cell

Arrows indicate pathways of current flow
3-D MODEL OF THE GAP-JUNCTION

A. Proxynaptic cytoplasm
3.5nm

B. Postsynaptic cytoplasm
Channel formed by pores in each membrane
Normal extracellular space

B. 6 connexin subunits = 1 connexon (hemichannel)
Closed
Open
Extracellular side
Cytoplasmic side

C. Extracellular loops for homophilic interactions
Each of the 6 connexins has 4 membrane-spanning regions
What are the characteristics of a synapse?

**Electrical**

• Pre & post synaptic cell connected via ion channels

• Direct flow of current; zone of connection = gap junction (3.5 nm) (bidirectional flow of anions & cations)

• Gap junctional channels; specialized pair of cylinders = connexons in pre & postsynaptic membrane, each is a hemichannel

• Each connexon made up of x6 connexins arranged in circle = pore

• Advantage: instantaneous, rapid synchronous firing of interconnected cells. Ideal where rapid response is mandatory for function.

• Disadvantage: Size of pre-synaptic cell has to be large in order to fire smaller postsynaptic. Have a higher threshold for firing since effective resistance for each cell is decreased. ( according to Ohms Law it takes larger “ I ” to reach threshold

• Not as structurally flexible as chemical synapse
Chemical synapse

•No direct electrical continuity

•Generally slower

•But structurally much more flexible – useful when long term changes in cellular function required

•Excitation of presynaptic cell leads to release of neurotransmitter (NTx)

•There is synaptic delay (0.5 to 1 msec) for NTx to diffuse across synaptic cleft and to activate post synaptic receptors

•NTx causes some type of postsynaptic change
Gray Type 1 & 11 Synapses

Type 1
- Spine synapse
- Spine apparatus
- Prominent presynaptic dense projections
- Wide synaptic cleft
- Large active zone
- Postsynaptic density

Type 11
- Axosomatic synapse
- Flattened synaptic vesicles
- Less obvious dense projections
- Narrow synaptic cleft
- Small active zone
- Postsynaptic density
Neuromuscular synapse – Neuromuscular junction
What are the events preceding release of neurotransmitter?

• Action potential invades presynaptic terminal – membrane depolarizes

• Voltage dependent Ca\(^{++}\) channels (close to docking site) OPEN in presynaptic membrane

• Ca\(^{+}\) influx causes vesicles to dock and release NTx contents into synaptic cleft

• NTx diffuse across cleft and bind postsynaptic receptors – thereby activating receptors and leading to a postsynaptic response

• response varies with type of NTx released, receptor subtype & postsynaptic cell type

• NTx effect terminated by enzymatic degradation (ACh), or re-uptake by neuron or uptake by a glial cells
What is the quantal theory of synaptic transmission

• NTx is release in quanta or “discrete packets”

• Studies by Fatt & Katz – small (0.5mV) spontaneous potentials in frog neuromuscular junction (MEPPs)

• Katz & Miledi showed that ion flux through single cholinergic channel = 0.3μV, which is 1/2000 of amplitude of a MEPP

• Drug studies showed that MEPPs had same time course & drug sensitivities

• blocked by cholinergic antagonists & lengthened by ACh-esterase inhibitors

• shown that 2 molecules of ACh needed to activate a cholinergic channel

• Thus end plate potential is a multiple of MEPP amplitude.

• NTx is stochastic (governed by law of porbability)
Blocked by increasing [Mg++]
Synaptic vesicle cycle & regulation of NTx release by Munc-13 protein family
SYNTAXIN IN CLOSED CONFORMATION CORE COMPLEX FORMATION BLOCKED

SYNTAXIN IN OPEN CONFORMATION CORE COMPLEX FORMATION CAN PROCEED
(c) CORE COMPLEX ASSEMBLED VESICLE IS PRIMED
What are the major Proteins (Pr) involved with synaptic vesicles?

- **Cysteine string proteins;**
  Peripheral membrane Pr; Palmitoylated on +10 cysteines, cont. DNA-J homology domain. Function??

- **Cytochrome b 561;**
  Electr. transp Pr for intravesicular mono-oxygenases in vesicles. Required for DA-β-hydroxylase & peptide amidase activity

- **Neurtotransmitter (NT) transporters:**
  Transporters specific for NT accumulation in vesicle

- **Rab Proteins;**
  Rab3A, Rab3C, Rab5, Rab7. Regulates docking & fusion

- **Rabphilin-3A;**
  Binds Rab 3’s as function of GTP

- **Secretory Carrier Membr. Pr**
  Vesicle membr Pr. Function??
What are the major Proteins(Pr) involved with synaptic vesicles?

• **SV2S:** Highly glycosylated Pr (homology to bacterial & eukaryotic transporters. Function??

• **Synapsins Ia, Ib, IIa, IIb:** Fibrous phosphoPrs linking vesicles to cytoskeleton & each other

• **Synaptobrevins:** VAMP( vesicle assoc.memb Pr) – cleaved by tetanus & botulinum toxins

• **Synaptophysins:** Polytopic Pr binds to Synaptobrevins. Function??

• **Synaptogryn:** Polytopic Pr : Function??

• **Synaptogamins:**

  Binds Ca++ & Phospholipids; Interacts with neurexins, AP2 & syntaxins. ? Ca++ sensor in fast Ca++ dependent release

• **Cl- & Zn+ Transport Pr:** Mediates Cl- flux for Glut&Zn uptake

• **Vacuolar Proton pump:** Causes Electro-chem grad for NT uptake
How does docking occur?

- synaptic vesicles kept near the active zone by **synapsin & rab 3A**
- **Synapsin** binds to cytoskeleton – inhibits release of vesicle
- **Rab A3** may be necessary for localization (if mutated – random distribution occurs)
- Ca^{++} influx, **synapsin** is phosphorylated by calcium-calmodulin-dependent (CAM) – kinase
- lead to the release of vesicle from **cytoskeleton**
- In addition - when Ca^{++} enters cell **synaptotagmin** (in vesicle membrane) binds two Ca^{++} (Ca^{++} sensor)
- Effect is displacement from the **synaptobrevin/syntaxin** complex
- α-SNAP (cytosolic soluble NSF attachment proteins) combines to the complex with N-ethylmaleimide-sensitive factor (NSF) – called the docking complex – then vesicle fuses with the presynaptic membrane
Ca++ binding, unbinds synaptobrevin/syntaxin complex – allows soluble cytosolic factors to bind to complex
How are vesicles recycled?

1st theory:
• Clathrin (fibrous Pr, three limbed triskelion) – polymerizes into a lattice along the cytosolic face of the membrane, causing endocytosis
• This region expands inwards forming a clathrin coated pit that pinches off to form a new vesicle
• A chaperone protein removes clathrin coat; vesicles fuses with the endosome to recycle

2nd theory
• “kiss-&-run” hypothesis; retrieved from presynaptic membrane after releasing contents, immediately refilled with NTx
• Enter releasable pool of vesicles
• ?? Faster time scale than clathrin mechanism
What is the difference between NMJ and central synapses?

- The size of the MEPP is called the **quantal** size.
- This is much more variable in **central synapses** (100 to 400μV) and is dependent on the number of post-synaptic receptors underlying the active zone (docking & release site).
- The active zone of many **central synapses** may have only one release site (one quantum hypothesis).
- In **central synapses**, probability (p) of release varies with different sites & p may vary with time (dependent on recent history of synapse!)
- In NMJ = MEPPs, in **central synapses** mEPSPs of mIPSPs.
- In voltage clamp mode of recording called mEPSCs or mIPSCs.
**What are the main NTxs?**

<table>
<thead>
<tr>
<th>Classical</th>
<th>Amino Acids</th>
<th>Glutamante</th>
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<tbody>
<tr>
<td></td>
<td>Aspartate</td>
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<td></td>
<td>γ amino butyrate</td>
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<td></td>
<td>Glycine</td>
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<td>Monoamines</td>
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<td>Dopamine</td>
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<td>Norepinephrine</td>
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<td>Epinephrine</td>
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<td></td>
<td>Serotonin</td>
<td>indolamine</td>
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**Peptides**

- Opioids
  - Dynorphins
  - Endorphins
  - Enkephalins
- Tachykinins
  - Substance P

**Hormones**

- cholecystokinin
- somatostatin

Vesicles containing amino acid or biogenic amines are frequently co-localized with neuroactive peptides or purinergic compounds such as ATP.
SEROTONIN (5-HYDROXYTRYPTAMINE)

- Tryp → 5-HTP → 5-HT
- TrypOHase → L-AADC
- MAO
- Release-modulating autoreceptor
- Postsynaptic neuron
- Postsynaptic receptor
- Vesicular transporter
- Plasma membrane transporter (SERT)
$\gamma$-AMINO BUTYRIC ACID (GABA)
ACETYLCHOLINE
ACETYLCHOLINE
Axosomatic synapses
Axodendritic synapses
Axoaxonic synapse
A

Sensory neuron 1

Sensory neuron 2

Motor neuron

B

Temporal summation

MN

SN 1

C

Spatial summation

MN

SN 1

SN 2
Competitive effects of excitatory & inhibitory currents
Dendritic synaptic potential & AP at axon hillock
Model of the main structural and functional unit of the voltage-gated Na+ channel

Top view: 6 helices transmembrane

K+ channel: only x1 motif

Ca++ channels: 3 classes of voltage gated channels (L= Long lasting, T= Transient, N= Neuronal)
3 state model of a Sodium Channel

A
Activation gate (opened by depolarization)
Inactivation gate (closed by depolarization)

B
Activation gate (open)
Inactivation gate (open)

C
Activation gate (open)
Inactivation gate (closed)
TIME COURSE OF CHANGES IN Na⁺ & K⁺ CONDUCTANCES UNDERLYING NERVE ACTION POTENTIAL

**DEPOLARISATION**: Vm < NEG: Inward
All-or-none

**REPOLARISATION**: Vm returns towards resting Vm: I Outward: Why is there a hyperpolarising after-potential