Synaptic communication

Objectives:

after these lectures you should be able to:

- explain the differences between an electrical and chemical synapse
- describe the steps involved in synaptic communication at a chemical synapse
- design an experiment to test the dependence of chemical synapses on Ca+2 influx
- describe the quantal analysis experiments and their significance
- describe the SNARE hypothesis in general terms and design an experiment to test this hypothesis in vivo

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Reading:

Nichols (From Neuron to Brain, 4th edition)
Chapter 7 pp. 184-193, 214-235.
Chapter 9 pp. 282-300.

Shephard (Neurobiology)
Chapter 6 pp: 102 - 108, 115-131.

Hall (Introduction to Molecular Neurobiology)
Chapter 5.

Kelly, R.B. (1993)
Storage and release of neurotransmitters.
Cell 72/Neuron 10 (suppl.): 43-53.

Jessell, T.M and Kandel, E.R. (1993)
Synaptic transmission: a bidirection and self-modifiable form of cell-cell communication.
Cell 72/Neuron 10 (suppl.): 1-30.

Nonet et al. (1998)
Synaptic Transmission Deficits in *Caenorhabditis elegans* Synaptobrevin Mutants
Journal of Neuroscience 18: 70-80

Goda (1997)
SNAREs and regulated vesicle exocytosis
Proc. Natl. Acad. Sci. 94: 769 - 772.

Links:

Lecture Notes:

Sherrington (1890) studied reflex functions of spinal cord
- coined the word synapse = a functional connection between surfaces
- synapse (from the Greek: to clasp, to connect or join)
- sites of interaction between neurons and neurons and their targets

2 types of synapses

1) Electrical

- cells connect via gap junctions:
-membranes are separated by 2 nm
- gap junction channels have a large conductance
- NO synaptic delay (current spread from cell to cell is instantaneous) - important in some reflexes
- commonly found in other cell types as well i.e. glia
- can be modulated by intracellular Ca+2, pH, membrane voltage, calmodulin
- clusters of proteins that span the gap such that ions and small molecules can pass directly from one cell to another

- gap junctions:
-made up of 6 protein subunits arranged around a central pore, made up of the connexin protein
- cloned from many tissues and organisms and are extremely well conserved
- what is a way to test if your clone does indeed encode the right protein?
- injection of the cloned connexin protein into Xenopus oocytes
- results in formation of gap junctions between two oocytes

2) Chemical

- most common type of synapse
- electrical signal in the presynaptic cell is communicated to the postsynaptic cell by a chemical (the neurotransmitter),
- separation between presynaptic and postsynaptic membranes is about 20 to 30 nm
- a chemical transmitter is released and diffuses to bind to receptors on postsynaptic side
- bind leads (directly or indirectly) to changes in the postsynaptic membrane potential (usually by opening or closing transmitter sensitive ion channels)
- the response of the neurotransmitter receptor can depolarizes (excitatory postsynaptic potential; epsp) or hyperpolarizes (inhibitory postsynaptic potential; ipsp) the post-synaptic cell and changes its activity
- significant delay in signal (1 msec) but far more flexible than electrical synapse
- synaptic delay is NOT due diffusion of the neurotransmitter across the cleft (happens in about 50 msec)
- delay is in the release of neurotransmitter due to a lag in the opening of Ca+2 channels
Some types of chemical synapse include:
i) Excitatory - excite (depolarize the postsynaptic cell)
ii) Inhibitory - inhibit (hyperpolarize the postsynaptic cell)
iv) Modulatory - modulates the postsynaptic cells response to other synapses

NMJ - neuromuscular junction:

- motor nerve terminal branches run in shallow grooves on surface of muscle
- nerve terminal contains many mitochondria and vesicles
- vesicles can be seen lined up in double rows along a region of dense material attached to presynaptic membrane => active zone
- the dense material could be Ca+ channels
- used fluorescently tagged omega-conotoxin (used by sea snails to paralyze their prey) that binds specifically to Ca+ channels and saw concentrated binding in active zone
- synaptic cleft very structured (basal lamina)
- many postjunctional folds (motor endplate), grooves and fold particular to skeletal muscle synapses
- acetylcholine is the neurotransmitter for the mammalian NMJ
- glutamate is the neurotransmitter for the insect NMJ

Synapses on Nerve cells:

- usually in the form of swellings called boutons
- also contains many mitochondria and vesicles
- boutons also include electron dense active zones with vesicles clustered in rows along side
- less postsynaptic specializations
- sometimes see thickening of membrane

Basic Function of Chemical Synapse

1. Nerve impulse arrives at presynaptic terminal

2. Depolarization causes voltage-gated Ca+2 channels to open
- increases Ca+2 influx, get a transient elevation of internal Ca+2 ~100 mM

3. Vesicle exocytosis
- increase in Ca+2 induces fusion of synaptic vesicles to membrane
- vesicles contain neurotransmitters

4. Vesicle fusion to membrane releases stored neurotransmitter

5. Transmitter diffuses across cleft to postsynaptic side

6. Neurotransmitters bind to receptor either:
i) ligand-gated ion channel or
ii) receptors linked to 2nd messenger systems

7. Binding results in a conductance change
- channels open or close or
- binding results in modulation of postsynaptic side

8. Postsynaptic response
- change in membrane potential (e.g. muscle contraction in the case of a motorneuron at a neuromuscular junction)

9. Neurotransmitter is removed from the cleft by two mechanisms
i) transmitter is destroyed by an enzyme such as acetylcholine esterase
ii) transmitter is taken back up into the presynaptic cell and recycled
e.g. - acetylcholine esterase, breaks down acetylcholine in cleft, choline is recycled back into the presynaptic terminal

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Removal of neurotransmitter

i) non-peptide neurotransmitters
a) broken down by enzymes
- many nerve gases and insecticides work by blocking acetylcholine esterase
- prolongs synaptic communication
b) recycled by uptake into presynaptic terminal
- due to a specific neurotransmitter transporter
c) recycled by uptake into other cells
- glial cells will take up neurotransmitters
(GABA in crustacean NMJ, mammalian CNS)

ii) peptide transmitters
- don't appear to be broken down or recycled
- diffuse away and are broken down by nonspecific peptidases
- explains prolonged actions on synapses

One vesicle = one quantum

- the concentration of neurotransmitter within the vesicle is pretty constant
(for example ~ 3000 molecules of Ach per vesicle at the NMJ)
- neurotransmitter are released in packets or quanta = the contents of 1 vesicle
- can record the effect of the release of one quanta on the extracellular membrane
- for instance, the Ach neurotransmitter in one vesicle opens about 1500 acetylcholine receptors at the NMJ
- some vesicles contain only enough neurotransmitter to open 20 channels (depends on the neurotransmitter)
- one vesicle (or quantum) of Ach depolarizes the muscle ~1 mV
- increases in a step wise manner, i.e. 2 vesicles depolarize the postsynaptic membrane by about twice as much and 3 vesicles about three times as much etc.
- a normal depolarization at the NMJ is made up of over 200 quantal units

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Presynaptic Vesicles

2 types of secretory vesicles

1) large dense-core vesicles:

- responsible for modulatory signalling and distant signalling
- contain neuropeptide transmitters (small peptides cleaved from large precursor proteins)
- exocytosis is NOT restricted to active zones
- exocytosis is triggered by trains of action potentials
- these transmitters are produced and packaged into vesicles at the cell body and transported to nerve terminal

2) synaptic vesicles:

- responsible for fast synaptic signalling
- store and secrete non-peptide neurotransmitters,
e.g. acetylcholine, glycine, glutamate
- enough vesicles in the typical nerve terminal to transmit a few thousand impulses
- exocytosis only occurs after an increase of internal Ca+2 (due to depolarization) and at active zones (regions in the presynaptic membrane adjacent to the cleft)

Processing of different neurotransmitter vesicles

- neurotransmitters can be divided into two groups
i) low molecular weight, non-peptide
e.g. acetylcholine, glycine, glutamate
ii) peptide (over 40 identified so far and counting)
- same transmitters found widely distributed through out diverse organisms

Neurotransmitter - goes through a number of separate stages in its actions
1. Synthesis
- all transmitters except peptides are made in the nerve terminal
i) non-peptide transmitters
- responsible for fast synaptic signalling
- synthetic enzymes + precursors transported into nerve terminal
- subject to feedback inhibition (from recycled neurotransmitters)
- can be stimulated to increase activity (via Ca+2 stimulated phosphorylation)

ii) peptide transmitters
- peptide neurotransmitters are made from large precursor proteins in the cell body
- specific proteases cleave the precursor into the appropriate peptides (this can occur in the cell body, in the vesicle during transport or at the nerve terminal)
- responsible for modulatory signalling and distant signalling

2. Packaging into vesicles
- neurotransmitters packaged into vesicles
i) for small non-peptide neurotransmitters
- packaged in small "classical" vesicles
- involves a pump powered by a pH gradient between outside and inside of vesicle
- pump blocked by drugs and these block neurotransmitter release

ii) for peptide neurotransmitters
- packaged into vesicles in the cell body and transported to terminals (anterograde transport)
- found the large dense core vesicles

Vesicle Exocytosis

- many of the molecules that involved in vesicle exocytosis are now known
- related to other vesicle fusion systems such as Golgi or secretory procedures
- a group of 6 to 7 proteins work together to respond to Ca+2 influx and regulate vesicle fusion
- many of these proteins are common to other secretory pathways
- in yeast: mutations that affect the homologues of these exocytosis mediating proteins have been made and all impair exocytosis
- after exocytosis the synaptic vesicle membranes are reinternalized by endocytosis and reused (reloaded

with neurotransmitter by a transmitter transporter system)
- vesicles are also transported from the cell body to the nerve terminal
- transmitter is synthesized in the terminal and loaded into the vesicles
- enzymes and substrates necessary are present in the terminal
- i.e. acetylcholine, acetyl-CoA + choline used by choline acetyltransferase

i) non-peptide transmitters
- exocytosis only occurs after an increase of internal Ca+2 (due to depolarization)
- at active zones (regions in the presynaptic membrane adjacent to the cleft)
- many of the molecules that involved in vesicle exocytosis are now know
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Lambert-Eaton Syndrome:
- muscle weakness and reduction in transmitter release
- autoimmune disease - antibodies to own Ca+2 channels, block voltage-gated Ca+2 channels

Black Widow Spider Venom (α-latrotoxin):
- induces a massive release of transmitter by interacting with one of the complex of proteins that mediate the Ca+2 induced fusion of vesicles with the presynaptic membrane
- depletes the store of vesicles

Botulinus and Tetanus Toxins: (food poisoning)
- blocks the fusion of vesicles by interfering with another of the proteins involved in mediating fusion of vesicles
- respiratory paralysis

ii) peptide-transmitters (same as for non-peptide transmitters except:)
- exocytosis is NOT restricted to active zones
- exocytosis is triggered by trains of action potentials

SNARE hypothesis

- four components
i) V-SNARE - a vesicle membrane protein
ii) T-SNARE - a target membrane protein
iii) a cytosol protein NSF required for membrane fusion
iv) adaptors for the NSF protein called SNAPs
- vescile docking occurs between the V-SNARE and T-SNARE proteins
- the combined proteins act as a receptor for the SNAPs which then bind NSF
- this complex is called the SNARE complex

- NSF is an ATPase that hydrolyzes ATP to drive membrane fusion
- now thought that after ATP hyrolysis the complex is in a new stable form that will fuse only once Ca+2 influx occurs
- this process is known to be fast as vesicle fusion occurs 60 microseconds after Ca+2 influx

V-SNARE
synaptobrevin - found associated with vesicle membrane
- site of botulinum and tetanus toxin action will cleave this protein
- binds to syntaxin (T-SNARE)
- mutants in this protein in C.elegans have abnormal synaptic communication (J. Neursci. 18: 70-80, 1998)

synaptotagmin - associated with the vesicle membrane

- binds NSF, SNAP and SNAP25
- a Ca+2 sensor for transmitter release
- mutants in Drosophila and C. elegans: no synaptotagmin result in severe dysfunction in secretion (Cell 74: 1125-1134 (1993); Neuron 12: 909-920 (1994); Cell 73: 1291-1305 (1993))

T-SNARE
syntaxin - associated with target membrane
- binds synaptobrevin
- binds to Ca+2 channels to localize the t-snare close to the Ca+2 channels for more efficient vesicle fusion

SNAP25 - synaptosome associated protein 25K
- not be confused with the SNAPs which are different proteins

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Intracellular transport

A guide to axon transport in the squid giant axon

transport of vesicles from the nerve terminal to cell body - retrograde transport
transport of vesicles from the cell body to nerve terminal - anterograde transport

the motor is not the microtubules or MAPs
two major motor proteins, both bind to vesicles and move them along the microtubules but in different directions.

Kinesin: 350 kD protein that moves along the microtubules towards the nerve terminal (orthograde transport),

Dynein: 1200 kD protein that moves along the microtubules towards the cell body (retrograde transport) (dynein family also involved in cilia and flagella microtubule movement)

two types of axon transport
i) slow transport - 1 mm per day,
ii) fast or rapid transport - 100 to 400 mm per day

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