Synapses

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Neuronal synapses

Electrical synapses = gap junctions

Chemical synapses
Sites at which signals are propagated between cells. Usually axon to dendrite. Chemical synapses use neurotransmitter.

Final effector
Chemical synapse

Axon
Dendritic spine
Dendrite

From G Johnson, *Science*, 2005
Chemical synapse

Mitochondrion: generates ATP that is required for synaptic vesicle fusion and recycling.

Active zone

Similar to cell junction

Receptors for neurotransmitters

Axon

Dendrite

G

200 nm
Post-synapse (dendritic spine)

Axon terminal

Axon marker

Dendrite marker

post-synaptic receptors

Freeze-fracture view

super-resolution fluorescence microscopy of a pre- and a post-synaptic protein (from Dulac and Zhuang)
Green = glutamatergic (excitatory) synapses

Red = GABA-ergic (inhibitory) synapses

Majority of inhibitory synapses on initial segment of axon. Place where decision to generate action potential is made.
spine synapse (cerebellum)

Dendritic spine
Contains smooth ER for calcium storage and synthesis of lipids.

Dendrite

Axon terminal
A special synapse:
the neuromuscular junction

From Lichtmann
Synaptic vesicles store fast-acting neurotransmitters

Because axon terminus must continuously release vesicles over short period of time, vesicles are recycled through endocytosis. Neurons use small metabolites for neurotransmitters instead of peptides because peptides must be transported from cell body.

Neurotransmitters contained in synaptic vesicles:

- Gaba, glycine | inhibitory
- Glutamate
- Ach | excitatory
- Amines

+ small non peptide molecules
+ fast acting (although can also have slow actions)

Synaptic vesicles are continuously regenerated in nerve terminals by local membrane recycling
Large dense-core vesicles store neuropeptides

Are assembled in the cell body

*neuropeptides (peptide neurotransmitters) have slow modulatory actions*

If axon releases a large amount of neurotransmitter, it will also release neuropeptides that modulate the response of the post-synaptic cell. The neuropeptide prepares the post-synaptic cell to receive a barrage of neurotransmitter.
Key Steps in Synaptic Transmission
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Voltage dependent Na$^+$ channel
Voltage dependent Ca$^{2+}$ channel
Ionotropic neurotransmitter receptor (neurotransmitter-gated ion channels)

Other channels are not indicated, f.e. K$^+$ channels
Key Steps in Synaptic Transmission

The ACTION POTENTIAL travels down the axon:
Opening of voltage-gated Na⁺ channels
Key Steps in Synaptic Transmission

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Opening of voltage-gated Na\(^+\) channels

Depolarization
Key Steps in Synaptic Transmission

The ACTION POTENTIAL invades the nerve terminal: opening of voltage-gated Ca\(^{2+}\) channels

Cytosolic Ca\(^{2+}\) at release sites increases

Ca\(^{2+}\) 2.5 mM

Ca\(^{2+}\) less than 1 \(\mu\)M
Key Steps in Synaptic Transmission

Ca$^{2+}$ triggers synaptic vesicle fusion, neurotransmitter secretion

Increased calcium triggers fusions of synaptic vesicles.

Neurotransmitter content of one vesicle = quantum of neurotransmitter
Key Steps in Synaptic Transmission

Post-synaptic effects at excitatory synapses

Opening of neurotransmitter gated ion channels (ionotropic receptor)

Cations primarily $\text{Na}^+$ (excitatory synapses)

Binding of neurotransmitter opens ion channels in dendrites.
Key Steps in Synaptic Transmission

Opening of voltage-gated Na$^+$ channels: A NEW ACTION POTENTIAL STARTS

Depolarization of dendrite membrane triggers action potential.
Key Steps in Synaptic Transmission

**Post-synaptic effects at inhibitory synapses**

Opening of neurotransmitter gated ion channel
(ionotropic receptor permeable to Cl⁻)

Entry of chloride make membrane hyperpolarized
(cytoplasm has higher negative charge due to chloride ion.
Hyperpolarization makes it more difficult to initiate an
action potential. Need much more neurotransmitter to open
more ion channels.)
Cell become hyperpolarized, no action potential generated and cell becomes less excitable.
Metabotropic receptors (second messengers-linked receptors) for neurotransmitters, modulate the post-synaptic electrical response and mediate trophic effects.
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Neurotransmitters secreted via synaptic vesicles may also be involved in slow modulatory signaling.
Synaptic vesicles are well characterized organelles

from Huttner, Greengrad, De Camilli et al. 1983

Takamori et al. (Jahn lab) 2006
Synaptic vesicles undergo recycling

Black = extracellular tracer added during previous stimulation

picture by Summer Paradise
Neurotransmitter loading

Vesicle neurotransmitter transporters: load the vesicle

Plasma membrane transporters: re-uptake neurotransmitters from synaptic cleft

Required ATP to pump hydrogen ions into vesicles. Blocking proton pump prevents uptake of neurotransmitter into synaptic vesicles.

Many neuropharmacological drugs block action of pump (e.g. amphetamines), leaving neurotransmitter in synaptic cleft
Steps in vesicle recycling

Vesicle clustering:
Cytoskeletal elements cross-link vesicles to each other

from Fernandez-Busnadiego et al.
Steps in vesicle recycling

Vesicle tethering

Drosophila NMJ cross-section

Striking example of tethering structure
Steps in vesicle recycling

Vesicle tethering

mammalian central synapse
EM tomography
Steps in vesicle recycling

Vesicle docking

Docking occurs near Ca$^{2+}$ channels

Increase in calcium triggers fusion
Frog neuromuscular junction
Frog neuromuscular junction

From Heuser’s lab and McMahan’s lab
Steps in vesicle recycling

SNAREs: critical players in membrane fusion

Sollner... and J. Rothman, Nature 1993
Steps in vesicle recycling

VAMP/synaptobrevin
SNAP25
syntaxin

Vesicle fusion

Stein et al. Nature 2009
Tetanus toxin causes prolonged contraction of muscle. Blocks synaptic vesicle fusion in neurons that inhibit the activity of motor neurons.
Botulism toxin inhibits muscle contraction. Blocks fusion of synaptic vesicles at NMJ in motor neurons.
Botox

Therapeutic uses

Cosmetic uses
Clostridial toxins (tetanus, botulism) cleave SNARE proteins

Cleavage of SNAREs inhibits fusion of synaptic vesicles.
A Ca$^{2+}$ sensor: synaptotagmin

Bai and Chapman, 2004
A Ca$^{2+}$ sensor: synaptotagmin penetrates the bilayer in a Ca$^{2+}$-dependent way.
Riformation of synaptic vesicles after exocytosis
key role of clathrin-mediated endocytosis

kiss and run?
*shibire* Mutation of Drosophila due to a mutation in the dynamin gene

Koenig and Ikeda, 1989
The synaptic cleft
synapse adhesion molecules

Post-synaptic plasma membrane
neurotransmitter receptors

Ionotropic neurotransmitter receptors

Metabotropic neurotransmitter receptors (trigger second messengers signals)

E. Gouaux’s lab

glutamate (AMPA) receptor

Pre-synaptic PM

Post-synaptic PM

150 Å
A complex molecular network in dendritic spines:
+ Clustering and traffic of receptors
+ Control of their properties
+ Structural role in spine shape and size
Synapses are dynamics
dendritic spines

(GFP-ACTIN) From A. Matus
Optogenetics

Use of light to monitor and to trigger synaptic activity

Optogenetics in Neural Systems

Ofer Yizhar,¹ Lief E. Fenno,¹ Thomas J. Davidson,¹ Murtaza Mogri,¹ and Karl Deisseroth¹,²,³,⁴,*
Neuron 71, July 14, 2011 ©2011
Imaging synaptic transmission
(synaptic vesicle exocytosis)

pH sensitive variant of GFP (pHluorin) fused to a synaptic vesicle protein

pH 5.5  ΔΨ  ΔpH

Neurotransmitters

pH 7.4

Neurotransmitters
Imaging synaptic transmission
(synaptic vesicle exocytosis)

Mammalian neuromuscular junction

movie

From Bill Betz
Imaging synaptic transmission
(postsynaptic action of glutamate)

Simultaneous 2-photon Calcium Imaging and Focal Glutamate Uncaging in Living Brain Slices

Neuron, filled with the Ca-insensitive red fluorophore Alexa-594 and the Ca indicator Fluo-5F

Mike Higley (Yale CNNR)
Optogenetics
use of light and genetically encoded probes to examine and manipulate synaptic function

cations

channelrhodopsin
cation channel controlled by light from alga *Chlamydomonas reinhardti*

halorhodopsin: Cl⁻ pump (inhibitory) controlled by light *da archibatteri*
Photostimulation in living organisms

Expression of channelrhodopsin \textit{in vivo}

channelrhodopsin in inhibitory motor neurons

Channelrhodopsin expression in cortical neurons

Liewald et al. Gottshalk lab
Nature Methods 2008

Hubel et al. (Svoboda lab), Nature 2008

(five light pulses, 20 Hz, 1 ms duration)