Lecture 7: Synaptic Transmission 1
Synaptic Transmission

General Overview
Quantal Release
Ca2+ Dependence
Vesicular Cycling
Types of Neurotransmitter
Electrical synapses

Cytoplasmatic Continuum

Chemical synapses

No Cytoplasmatic Continuum

Presynaptic neuron

Microtubule

Cytoplasm

Mitochondrion

Gap junction

Postsynaptic neuron

Presynaptic membrane

Postsynaptic membrane

Gap junction channels

Synaptic vesicle fusing

Postsynaptic neurotransmitter receptor

Synaptic cleft

Presynaptic membrane

Postsynaptic membrane

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How do we know synaptic Transmission is Chemical

Otto Loewi and Vagusstoff

One of the more colorful stories in the history of neuroscience was contributed by Otto Loewi, who, working in Austria in the 1920s, showed definitively that synaptic transmission between nerve and heart is chemically mediated. The heart is supplied with two types of innervation; one type speeds the beating of the heart, and the other slows it. The latter type of innervation is supplied by the vagus nerve. Loewi isolated a frog heart with the vagal innervation left intact, stimulated the nerve electrically, and observed the expected effect, the slowing of the heartbeat. The critical demonstration that this effect was chemically mediated came when he took the solution that bathed this heart, applied it to a second isolated frog heart, and found that the beating of this one also slowed. The idea for this experiment had actually come to Loewi in a dream. Below is his own account:

In the night of Easter Sunday, 1921, I awoke, turned on the light, and jotted down a few notes on a tiny slip of paper. Then, I fell asleep again. It occurred to me at six o’clock in the morning that during the night I had written down something most important, but I was unable to decipher the scrawl. That Sunday was the most desperate day in my whole scientific life. During the next night, however, I awoke again, at three o’clock, and I remembered what it was. This time I did not take any risk; I got up immediately, went to the laboratory, made the experiment on the frog’s heart, described above, and at five o’clock the chemical transmission of the nervous impulse was conclusively proved . . . . Careful consideration in daytime would undoubtedly have rejected the kind of experiment I performed, because it would have seemed most unlikely that if a nervous impulse released a transmitting agent, it would do so not just in sufficient quantity to influence the effector organ, in my case the heart, but indeed in such an excess that it could partly escape into the fluid which filled the heart, and could therefore be detected. Yet the whole nocturnal concept of the experiment was based on this eventuality, and the result proved to be positive, contrary to expectation.

(Loewi, 1953, pp. 33, 34)

The active compound, which Loewi called vagusstoff, turned out to be acetylcholine. As we shall see in this chapter, acetylcholine is also a transmitter at the synapse between nerve and skeletal muscle. Here, unlike at the heart, acetylcholine causes excitation and contraction of the muscle.
Otto Loewi

Nobel Prize Med. & Physiol. 1936

Henry Dale
Sequence of Events:
1. AP
2. Opening of VGCC
3. Ca influx
4. Fusion at release site
5. Transmitter release
6. Diffusion
7. Bind to receptor
8. Uptake of tr.
10. Endocytosis
11. Cycling of Vesicle
The neuromuscular junction (NMJ) of the frog

(A)

Stimulate
axon

Record
postsynaptic
membrane
potential
(the end plate potential)

Muscle cell

Stimulate
The endplate potential (EPP)

Recording from a muscle fiber

Curare: a competitive antagonist of nicotinic Ach receptors:
Reduces the amplitude of the EPP; EPP does not reach threshold, no muscle twitch
Curare: several different alcaloids
Most common curarine and tubocurarine

Strychnos Toxifera
The discovery of **miniature endplate potentials**; “**minis**”

**Sir Bernard Katz**

Nobel prize for Physiology and Medicine 1970

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2 mV

20 mV

Nerve stim.

spontaneous

evoked
Ca\(^{++}\) is necessary for transmitter release

Low Ca\(^{++}\) in the perfusion medium

Fluctuation of EPP amplitudes

Nerve stim.

evoked

Spontaneous minis
The Amplitude of the EPP fluctuates

Very low Ca$$^{++}$$

Smallest EPP not smaller than a mini.

Failures

Increments in amplitude occurs in units or “quanta”.

(one quantum is the smallest unit of transmitter release)

The increments have the size of a mini

The amplitude of evoked EPP decreases when reducing external [Ca$$^{2+}$$]. The amplitude of minis does not.
Transmitter release is “quantal”

Very low Ca\(^{++}\)

Failures of release

Transmitter release is “quantal”
Transmitter release can be described with binomial statistics

\[ P_x = \frac{n!}{(n-x)!x!} p^x q^{n-x} \]

- \( n \): number of release sites (only one vesicle (quantum) can be released for each release site)
- \( p \): probability of releasing one vesicle (assumed to be the same at each release site)
- \( q = 1-p \); i.e. the probability of not releasing a vesicle
- \( P_x \): probability of releasing \( x \) vesicles

For large \( n \) and low \( p \) (e.g. low external \([Ca^{2+}]\)) transmitter release can be described by a Poisson distribution:

\[ P_x = \frac{m^x e^{-m}}{x!} \]

\( m = p^*n \); i.e. average number of quanta released over all trials
Release is vesicular

Synaptic Vesicle exocytosis captured by quick freezing; (Hauser & Reese)

One miniature EPP correspond to the release of one vesicle. AP synchronize vesicular release.
Vesicular Cycling

| FM 1-43 structure. Three regions of FM 1-43 structure (see text).
Figure 2 | Typical FM dye experiment. (a) Synaptic vesicles near the plasma membrane. (b) FM dye is added, binds to the outer membrane, and becomes fluorescent. (c) The preparation is stimulated and a vesicle fuses with the plasma membrane exposing the luminal membrane to the FM dye. (d) The vesicle is endocytosed with FM dye inside. (e) The FM dye is washed out of the bath and a labeled vesicle is imaged. (f) The preparation is stimulated again in a dye-free medium and vesicles exocytosis is measured as dye leaves the fusing vesicle.
Figure 1 | Three vesicle pools. a | The classic three-pool model. The reserve pool makes up ~80–90% of the total pool, and the recycling pool is significantly smaller (~10–15%). The readily releasable pool (RRP) consists of a few vesicles (~1%) that seem to be docked and primed for release. b | Three kinetic components of release (indicating release of three vesicle pools) on depolarization of goldfish bipolar cells. The cell was stimulated in the presence of the styryl dye FM 1-43, and the increase in fluorescence gives a direct measure of exocytosis. Panel b modified, with permission, from REF. 12 © (1999) Blackwell Scientific Publishing.
Figure 3 | Typical images from the frog neuromuscular junction preparation. 

a | FM 1-43 fluorescence image of a nerve terminal. 
b | Electron micrograph of a cross-section through the nerve terminal. Arrows indicate active zone. 
c | Three-dimensional reconstruction of an approximately 2 μm-long nerve terminal segment. Active zones are shown in red. 
d | Postsynaptic potential recording under continuous stimulation at 30 Hz (top); a few postsynaptic responses at 0 s and 50 s after stimulation commenced are shown below (image courtesy of D. A. Richards). In the bottom trace, vertical lines are shock artefacts; synaptic responses have declined to baseline. The RRP is rapidly exhausted, followed by recycling pool depletion. Reserve pool release is maintained for at least one minute. 
e | Pool sizes and mixing rates. Blue arrows indicate endocytosis; red arrows indicate mixing between pools. Red sphere indicates total pool size relative to the other preparations. Panel c reproduced, with permission, from REF. 37 © (2004) American Association for the Advancement of Science.
Figure 4 | Typical images from the rat cultured hippocampal preparation. 

a | FM 1-43 fluorescence image of a field showing numerous labelled presynaptic boutons. 
b | Electron micrograph of a cross-section through a bouton. Arrowheads indicate the two edges of the active zone in this image; the black arrows point to two docked vesicles; a non-docked vesicle near the active zone is shown by the white arrow. 
c | Three-dimensional reconstruction of a bouton. 
d | Postsynaptic response to hyperosmotic sucrose application (bar); this treatment selectively releases the RRP, pA, picoAMP. 
e | Pool sizes and mixing rates. Blue arrows indicate endocytosis; red arrows indicate mixing between pools. Red sphere indicates total pool size relative to the other preparations. 

Two Ways to Release Neurotransmitter

Kiss and Run

Full Fusion

Rapid recycling: Short membrane residence time

Dye with slow 'off time'
Dye with fast 'off time'
Vesicle membrane
Pre-synaptic membrane
Neurotransmitter

FM1-43
FM2-10
Vesicular Cycling Kinetics
Where is Ca\textsuperscript{2+} necessary?

- Extracellular recording at NMJ without Ca\textsuperscript{2+} in the perfusion solution
- End plate potential
- stimulation artifact
- Ca\textsuperscript{2+} leak from recording pipette
- presynaptic action potential
- No Ca\textsuperscript{2+} leak from recording pipette
When is Ca\(^{2+}\) necessary?

Interval between Ca\(^{2+}\) pulse and stim
What is the relationship between Ca\(^{2+}\) and transmitter release?

Highly nonlinear relationship: EPP = k[Ca\(^{2+}\)]\(^4\)
Is an action potential essential?

Squid giant synapse

(A) Presynaptic neuron

Voltage clamp

Post V_m

Pre I

Pre V_m

Postsynaptic membrane potential

Record

B Postsynaptic Potential

Ca2+ Current

In the presence of TTX

Voltage step

Post V_m

Pre I

Pre V_m

30 mV

40 mV

52 mV

55 mV

60 mV

89 mV

98 mV

125 mV

130 mV

10 mV

150 nA

1 ms

No Ca2+ Current because ECa

ECa
The Time Course of Calcium Conductances (during an action potential)
How far away from the release site are the Ca2+ channels?

Squid giant synapse

EGTA  Slow Ca2+ buffer injected in the presynaptic terminal

Pre  Post

15mV 1ms

BAPTA  Fast Ca2+ buffer injected in the presynaptic terminal

Pre  Post

12mV 15mV 1ms

10 uM Ca

Calcium Channel  Anchor  Vesicle Fusion Mechanism

10 nm

Secretory Vesicle
Which $\text{Ca}^{2+}$ channel is responsible for Transmitter release?

*Conus Granulatus*

*conotoxins*

*Agelenopsis Aperta*

*Agatoxins*
Which $\text{Ca}^{2+}$ channel is responsible for Transmitter release?

- $\omega$ conotoxin MVII A : Blocks N-type $\text{Ca}^{2+}$ channels
- $\omega$ Agatoxin IVa: Blocks P-type $\text{Ca}^{2+}$ channels
The calcium channels that have been characterized biochemically are complex proteins composed of four or five distinct subunits that are encoded by multiple genes (Fig. 1; Catterall, 2000). The α subunit of 190 to 250 kDa is the largest subunit, and it incorporates the conduction pore, the voltage sensor and gating apparatus, and most of the known sites of channel regulation by second messengers, drugs, and toxins. Like the α subunits of sodium channels, the α1 subunit of voltage-gated calcium channels is organized in four homologous domains (I–IV), with six transmembrane segments (S1–S6) in each. The S4 segment serves as the voltage sensor. The pore loop between transmembrane segments S5 and S6 in each domain determines ion conductance and selectivity, and changes of only three amino acids in the pore loops in domains I, III, and IV will convert a sodium channel to calcium selectivity. An intracellular β subunit and a transmembrane, disulfide-linked αδ subunit complex are components of most types of calcium channels. A γ subunit has also been found in skeletal muscle calcium channels, and related subunits are expressed in heart and brain. Although these auxiliary subunits modulate the properties of the channel complex, the pharmacological and electrophysiological diversity of calcium channels arises primarily from the existence of multiple α1 subunits (Hofmann et al., 1994).
N-Type Ca²⁺ Channel

Channel name **CaV2.2**
Description voltage-gated calcium channel 1 subunit
Other names N-type, 1B; rbB-I, rbB-II (in rat)1,2, BIII (in rabbit)3
Molecular information human: 2339aa, M94172, 2237aa, M94173 (ref. 4), chr. 9q34, CACN1B
rat: 2336aa, M92905 (ref. 1)
mouse: 2329aa, NM007579, NP031605
Associated subunits 2/1, 3, 4 (ref. 5) possibly
Functional assays voltage clamp, patch clamp, calcium imaging, neurotransmitter release, 45Ca uptake into synaptosomes
Current ICa,N
Conductance 20pS (bullfrog sympathetic neurones)6; 14.3pS (rabbit BIII cDNA in skeletal muscle myotubes)3
Ion selectivity Ba²⁺ > Ca²⁺
Activation Va = +7.8mV, τa = 3ms at +10mV (human 1B /2/1-3 in HEK 293 cells, 15mM Ba²⁺ charge carrier)4,7; Va = +9.7mV, τa = 2.8ms at +20mV (rat 1B-II/1b, in Xenopus oocytes, 40mM Ba²⁺ charge carrier)2
Inactivation Vh = 61mV, τh ~200ms at +10mV (human 1B /2/1-3 in HEK 293 cells, 15mM Ba²⁺ charge carrier)4,7; Vh = 67.5mV; τh = 112ms at +20mV (rat 1B-II/1b in Xenopus oocytes, 40mM Ba²⁺)2
Activators none
Gating inhibitors none
Blockers conotoxin GVIA (1–2M, irreversible block) , -conotoxin MVIIA (SNX-111, ziconotide), -conotoxin MVIIC (ref. 8)
Channel distribution neurones (presynaptic terminals, dendrites, cell bodies)
Physiological functions peptide toxins that selectively inhibit N-type channels block a significant fraction of neurotransmission release in the mammalian peripheral and central nervous systems (ref. 10)

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P-Type Ca2+ Channel

Channel name **CaV2.1**

Description voltage-gated calcium channel 1 subunit

Other names 1A, P-type, Q-type, rbA-I (in rat)\(^1\); BI-1, BI-2 (in rabbit)\(^2\)

Molecular information human: 2510aa, AF004883, 2662aa, AF004884, chr. 19p13, CACNA1A

rat: 2212aa, M64373

mouse: 2165aa, NM007578, NP031604

(see Comments)

Associated subunits 2, possibly

Functional assays voltage clamp, patch clamp, calcium imaging, neurotransmitter release

Current ICa,P, ICa,Q

Conductance 9, 14, 19pS (P-type, cerebellar Purkinje neurones)\(^4\); 16–17pS (for 1A/2/ in *Xenopus* oocytes)\(^2\),\(^5\),\(^6\)

Ion selectivity Ba2+ > Ca2+

Activation Va = 5mV for native P-type, Va = 11mV for native Q-type (with 5mM Ba2+ charge carrier)\(^7\)

Va = 4.1mV for rat 1A-a/2/4

Va = +2.1mV for rat 1A-b/2/4 (with 5mM Ba2+ charge carrier)\(^6\)

Va = +9.5mV; a = 2.2ms at +10mV for human 1A-1/2/1b in HEK 293 cells (with 15mM Ba2+ charge carrier)\(^3\)

Inactivation Vh = 17.2mV for 1A-a/2/4, Vh = 1.6 mV for 1A-b/2/4 (with 5mM Ba2+ charge carrier); Vh = 17mV, \(\tau\)h = 690ms at +10mV human 1A-1/2/1b in HEK 293 cells (with 15mM Ba2+ charge carrier)\(^3\); h > 1s at 0mV native P-type (with 5mM Ba2+ charge carrier)\(^7\)

(see Comments)

Activators none

Gating inhibitors -agatoxin IVA (P-type Kd =1–3nM (ref. 8); Q-type Kd ~ 100–200nM (refs. 5,9)), -agatoxin IVB (ref. 6)

Blockers -conotoxin MVIIC (ref. 8)

(see Comments)

Radioligands [125I]–conotoxin MVIIC

Channel distribution neurones (presynaptic terminals, dendrites, some cell bodies), heart, pancreas, pituitary

Physiological functions neurotransmitter release in central neurones and neuromuscular junction; excitation-secretion coupling in pancreatic cells

From: www.iuphar-db.org