Critical Steps in Transmitter Release
Critical Steps in Transmitter Release

Na⁺ influx is not necessary

K⁺ influx is not necessary
Ca++ and Transmitter Release

Hill coefficient = 3.7

Hill coefficient of 4 implies great cooperativity in the action of Ca++

Reid et al., 1998
The consensus is now that three to four bound $\text{Ca}^{2+}$ ions per $\text{Ca}^{2+}$ sensor are required and that a relatively high $[\text{Ca}^{2+}]_i$ is needed at the release site (estimates vary from a few micromolar using high affinity $\text{Ca}^{2+}$ indicators, to several hundred micromolar using low affinity reporters). This 3rd or 4th power relationship is extremely important because small changes in the $\text{Ca}^{2+}$ influx will have dramatic effects on the release probability.

The action of $\text{Ca}^{2+}$ at the release site is cooperative. More than one $\text{Ca}^{2+}$ ion must bind to the release machinery to trigger release. Second, third and fourth power relationships between $\text{Ca}^{2+}$ entry and release have been described.
The synaptic delay (200-500 μsec) is too long to be accounted for by transmitter diffusion (max 50 μsec). It’s also too temperature sensitive for a strictly diffusional process- the delay can be extended to several seconds by cooling. Most of time is taken up by the opening of Ca^{++} channels (N P/Q, possibly others), and the rise and intracellular movement of Ca^{++}. The vesicle itself responds very quickly, in under 200 μsec and possibly as fast as 30-60 μsec.
Loose Parts

“Before we start, I’d like to ask you to hold your vicious, baseless criticisms designed to further your own careers until the end of the presentation.”
Dorsal root stimulation produces slow positive potentials in cord dorsum (dorsal root potential or DRP) simultaneous with suppression of flexor reflexes (Gasser and Graham, 1933)

Muscle afferent volleys inhibit motoneuron EPSPs with no change in membrane potential or conductance of the motoneuron (Frank and Fuortes, 1957)

Remote inhibition? Depression of excitatory input?
EPSP in plantaris neuron, conditioning stimulation to biceps/semitendinosus
Recording from gastrocnemius Ia afferent in spinal cord with conditioning stimulation of biceps semitendinosus.
Presynaptic inhibition in the spinal cord is associated with a $\text{GABA}_A$-mediated depolarization of the presynaptic terminal and a reduction in the amount of transmitter released.
If Eccles hypothesis were correct, what kind of change in the membrane potentials of the monosynaptic afferent terminal knobs would be induced by the axoaxonic synapses of circuit \( X \) (Figure 7.15)? At this point we must assume without a proof a fact which has been demonstrated regarding the synaptic generation of EPSPs: the size of an EPSP is directly related to the size of the spike potential (i.e. the total shift in the membrane potential) that arrives at the presynaptic terminal. This is illustrated in Figure 7.16. If the membrane potential is at its normal resting level of about \(-70\) mV, the spike potential will be a shift of about \(110\) mV (i.e. a brief shift from \(-70\) mV to \(+40\) mV, see Chapter 6). The EPSP induced after the spike reaches the terminal knob will be of a given

![Diagram showing the relationship between presynaptic terminal potential and postsynaptic membrane response](image)

**Figure 7.16.** Hypothetical relationship of spike height in presynaptic terminal to size of EPSP induced in postsynaptic membrane.
Monoamine autoreceptors

located at or near release sites and on dendrites

dopamine - D2 receptor

NE - α₂

5-HT - 5HT₁ₐ and 5HT₁₇

on dendrites, they all hyperpolarize the neurons by activating a K⁺ channel

At nerve terminals, they all reduce stimulus-evoked transmitter release
GABA\textsubscript{B} autoreceptor

Shen and Johnson, 2001
Glowinski, 1988
Presynaptic autoreceptor-mediated inhibition of monoamine release is associated with a hyperpolarization of the presynaptic terminal.

How can this be reconciled with the spinal cord data?
Baxter and Bittner, 1981
Synaptic Transmission IV

Postsynaptic Mechanisms
Glutamate

most ubiquitous excitatory neurotransmitter in the CNS

>20 genes cloned so far

<table>
<thead>
<tr>
<th>Ionotropic (fast)</th>
<th>AMPA (4)</th>
<th>Kainate (5)</th>
<th>Non-NMDA</th>
<th>CNQX, DNQX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabotropic (slow)</td>
<td>NMDA(6)</td>
<td>NMDA</td>
<td>NMDA</td>
<td>AP-5, APV, MK-801</td>
</tr>
</tbody>
</table>

Selective Antagonists: MCPG
Fig. 2. Properties of AMPA and NMDA receptor-mediated synaptic conductances. Traces show (a) a dual-component synaptic current and the effects of (b) the AMPA antagonist CNQX, and (c) the further addition of the NMDA receptor antagonist AP5.

**Below left:** The NMDA receptor-mediated current (b) is superimposed with the AMPA receptor-mediated current (determined by subtraction). Note the slower kinetics of the NMDA receptor-mediated component.

**Below right:** The graph plots the amplitude of the NMDA receptor-mediated synaptic current against membrane potential. Whole-cell recordings were obtained from a CA1 neuron in response to low frequency stimulation of the Schaffer collateral–commissural pathway. The electrode solution comprised mainly CsF and the perfusate contained picrotoxin to block synaptic inhibition. The Mg$^{2+}$ concentration was 1 mM. [Adapted from Randall, A. D., et al. (1990) Neurosci. Lett. 114, 191–196.]
Nowak, 1984

whole cell glutamate current in mouse mesencephalic neuron
The NMDA receptor channel is 5-10 x more permeable to Ca++ than to Na+ or K+

Burnashev, 1996
NMDA receptors are allosterically modulated by glycine
The NMDA glycine receptor is different from the postsynaptic glycine receptor

Johnson and Ascher 1987
NMDA receptors are also allosterically modulated by Zn++

Chrisine and Choi, 1990
Zn++ blockade is voltage dependent

A
-60 mV

Control

100 μM Zn++

20 ms

2 pA

B
+40 mV

100 μM Mg++

Chrisine and Choi, 1990
corticosubthalamic EPSP
What is wrong with this EPSP?
Schaffer collateral-CA1 LTP is NMDAR dependent
postsynaptic increase in number of AMPAR

Collingridge and Singer, 1990
CA1 LTP

requires simultaneous presynaptic activity and postsynaptic depolarization

“Hebbian” mechanism

“… When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.”

D.O. Hebb, 1949

postsynaptic AMPA receptor stimulation depolarizes spine

depolarization unblocks NMDA receptor which lets in Ca++

calcium-calmodulin-dependent protein kinase (CaMKII)

changes in AMPAR trafficking/AMPAR number

changes in properties of AMPAR themselves
Mossy fiber-dentate mossy cell LTP

NMDAR independent

presynaptic not postsynaptic

presynaptic rise in Ca++ triggers CAMP rise and PKA activation

PKA acts on Rab3A and RIMa1 leading to increased glutamate release

Lyetskiy et al., 2005
Corticostriatal LTP

NMDAR dependent but probably presynaptic

requires D1/D5 receptor stimulation

4 second 100 Hz trains

Partridge et al., 2000
The type of corticostriatal plasticity is age dependent

Partridge et al., 2000
Choi and Lovinger, 1997

striatal LTD is also Hebbian
Is striatal LTD pre- or postsynaptic?

Corticostriatal LTD is presynaptic
Endocannabinoids mediate some forms of synaptic plasticity through CB1 receptors
Postsynaptic mGluR stimulation induces production and release of endocannabinoids that diffuse back to the presynaptic terminal and act on CB1 receptors that gate Ca2+ channels and reduce transmitter release.
FIG. 1. Synaptic modification induced by repetitively paired pre- and postsynaptic spikes in layer 2/3 of visual cortical slices from the rat

Spike-timing dependent plasticity

Dan, Y. et al. Physiol. Rev. 86: 1033-1048 2006; doi:10.1152/physrev.00030.2005
In general, AMPA receptors are permeable to K+ and Na+ while NMDA receptors are permeable to K+, Na+ and Ca2+, with the Ca2+ permeability about 5 x greater than that of Na+ and K+.

Some AMPA receptors are also Ca2+ permeable. AMPA Ca2+ permeability is conferred by the absence of the GluR2 subunit.

Vandenberghe et al., 2000
Metabotropic Receptors

mGluR1-8
Is glutamate always excitatory?

10 pulses @ 66 Hz

picrotoxin, strychnine, eticlopride, CNQX

adult VTA DA neuron - intracellular recordings

The mGluR IPSP is due to activation of SK. How?

Fiorello and Williams, 1998
mGluR IPSP depends on internal stores of Ca++

SK is activated by release of internal Ca++

Fiorello and Williams, 1998
in younger animals, the mGluR IPSP is mediated by an apamin-resistant Ca++-activated K+ conductance.

This is BK.

10-14 day old rat
SN DA neurons

Katayama et al., 2003
At least 2 pathways for mGluR-stimulated release of internal Ca+ in DA neurons

Horikawa et al., 2003
GABA IPSPs

Häusser and Yung, J. Physiol. (Lond). 479:401-422, 1994
Sir Henry Dale

Dale’s Law
(named by Sir John Eccles, 1952)

"The transmitter used by nerve-fibres of a particular kind, and concentrated at their endings in readiness for release is also to be found along the whole length of the fibres; a transmitter is characteristic, then, not only of the endings, but of the whole neurone?.....What shall we expect of such a substance? The synaptic endings in the central nervous system represent only one end of these dorsal root fibres. Are we to expect that the transmitter at their central, synaptic endings would also be functional at their peripheral endings, and possibly be there concerned with transmitting the so-called antidromic vasodilator action?"
Dale’s Law simply suggests that if a neuron releases a given transmitter at one of its release sites, then that same substance would also be released at all other release sites.
Co-localization of neuroactive substances

Differential frequency dependent release of peptides

Similar presynaptic receptor modulation

Dale’s Law is still alive (probably but see Hattori (1993), Neurosci. Res.)
Sir John Eccles

"..in the central nervous system, any particular transmitter always acts by opening the same ionic gates."

J.C. Eccles, "The Physiology of Nerve Cells", 1957

Eccle's Law
(named by me, 1990)

Eccle's Law is dead as a doornail