Lecture 1: An Introduction To Plasticity and Cellular Electrophysiology
Charge separation across a semipermeable membrane is the basis of excitability.
**Convention:** Current direction is defined by the direction of increasing positive charge.

- Na\(^{++}\) flux into a cell is an inward current.
- K\(^{+}\) flux out of a cell is an outward current.
- Cl\(^{-}\) flux into a cell is an outward current.

A depolarizing current is a net influx of positive ions or a net efflux of negative ions.

A hyperpolarizing current is a net efflux of positive ions or a net influx of negative ions.

**Outward rectification:** when a membrane allows outward current (net + charge out) to flow more easily than inward current.

**Inward rectification:** when a membrane allows inward current to flow more easily than an outward current.
Methods of Measuring Function In Excitable Membranes

**Field potentials**: measure current sources and sinks from populations of neurons across the electrode resistance.

**Microelectrode extracellular recording**: measures action potentials from a small number of neurons.

**Intracellular recording**: can measure voltage,

**Voltage clamp recording**: Can pass current to compensate for voltage change. In this way voltage is held ~ constant and current applied to compensate is a measure of the current flowing. This will give a measure of current.
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![Diagram](image)

**Voltage clamp recording:**
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Ion movement through channels is governed by their **Electrochemical Equilibrium Potential** also known as their **Reversal Potential** because at \( E_{\text{rev}} \) the net direction of the ions’ flow across the membrane switches direction.

\[
E_{\text{rev}} \text{ is given by the Nernst Equation:}
\]

\[
E_{i\text{rev}} = \left(\frac{RT}{zF}\right) \ln \left(\frac{[\text{ion}]_{\text{out}}}{[\text{ion}]_{\text{in}}}\right)
\]

\( R = \) the gas constant  
\( T = \) absolute temperature  
\( z = \) the valency of the ion  
\( F = \) the Faraday

At 20° C \( \left(\frac{RT}{zF}\right) = 25\text{mV} \)

\( E_{i\text{rev}} \) for Na+ at 20 degrees is  
58\log [140mM]/[7mM]= +75mV

\( E_{i\text{rev}} \) for Ca++ at 20 degrees is  
58\log [1.5mM]/[.0001mM]= +129 mV

Multiply by 2.3 to convert base 10 \( \log \left(\frac{RT}{zF}\right) = 58\text{mV} \). At 37°C \( \left(\frac{RT}{zF}\right) = 68\text{mV} \)

Functional synaptic plasticity is a change in the efficacy of synaptic transmission between at least 2 neurons.

Stimulate 1 input

Record

black box

This change could be due to:

1. An increase in the amount of transmitter released
2. An increase in the rate of transmitter released
3. An increase in post-synaptic receptors at each contact.
4. An increase in the number of contacts between the stimulated axon and the post-synaptic cell
How do you analyze where the change in transmission occurs? All methods depend on several assumptions. These may not be true.

All miniature synaptic currents/potentials arise from spontaneous release of 1 vesicle of neurotransmitter. Not true for all synapses.

At some synapses multiple vesicles can be released from one release site.

All vesicles contain the same amount of a particular transmitter called a quantum. The amount of transmitter loaded in vesicles can be different.

All vesicles release transmitter at the same rate.

Post-synaptic receptors are not saturated. This is not always true.
**Question:** Is \( pCa \) an accurate measure of \( pr \)?

**YES**
Using a potentiating stimulus (3, 1 sec trains of 100Hz stimuli at 20 sec intervals) $pCa$ goes up. Examination of $pCa$ in individual spines before and after potentiation show evidence of changes in $pr$ at individual release sites and in $N$, the number of active individual release sites onto that spine.

Evidence of an increase of $pr$. Only one site releasing.

Evidence of an increase of an increase in $N$.

Evidence of an increase of $pr$ and an increase in $N$.

Evidence of an increase of $pr$. Only one site releasing.

Application of adenosine to reduce $pr$. 
Short term plasticity (minutes): post-tetanic potentiation, paired-pulse facilitation, paired-pulse depression.

Long-term plasticity (hours to days): NMDA receptor dependent long-term potentiation (LTP); NMDAR independent LTP; NMDAR dependent LTD; Ca++ sensitive adenylyl cyclase dependent LTP (mossy fiber-CA3 synapse); mGluR, AMPA dependent LTD at parallel fiber to Purkinje cell synapses.

Homosynaptic plasticity: Plasticity expressed at an activated synapse as a result of the activity of the activated synapse.

Heterosynaptic plasticity: Plasticity induced by other synapses on the same synaptic relay.

Long-Long Term Potentiation: Involves growth of new contacts and is protein synthesis dependent.
Axo-axonic synapses

K⁺ channels

Pre-synaptic element (axon terminal)

N or P type (high voltage sensitive) Ca++ channels raise internal Ca++

Glutamate transporters
Voltage Gated Ca++ Channels

High threshold TYPES. L (long lasting, N (neither L or P), P (after Purkinje cell where this type was first discovered). High threshold voltage gated Ca++ channels means triggered by large depolarizations (e.g., from -90 or -80 mV to -20 or -10 mV). Generally presynaptic but P type can generate dendritic Ca++ spikes.

Identification by Pharmacology

L-type channels- Bay K 8644 agonist; Nimodopine, antagonist

N-type channels- conus toxin ω-conotoxin (antagonist)

P-type - components of funnel webb spider venom = FTX also a peptide in the venom ω-agatoxin IVA
Subunits of Voltage Gated Ca++ Channels

Pore forming $\alpha_1$ subunits

L-type Ca++ channels require phosphorylation by PKA in order to maintain functions

Fig 6.2. From HammondC. Cell & Molecular Neurobiology Academic Press 2001
Readily releaseable vesicle pool

Vesicular Uptake Transporter

Storage pool of Synaptic vesicles

Movement of vesicle from storage to readily releasable pool = dependent On phosphorylation of synapsin

T-Type Ca++ channels

At cholinergic synapses acetylcholinesterase degrades acetylcholine
Patch-Clamp Electrodes* Have Greatly Facilitated the Examination of Synaptic Currents in Neurons

Advantages:

1. In all but the cell attached patch mode there is access to the intracellular environment.

2. Recordings can be made from cells too small to be implaed with intracellular electrodes.

3. Currents can be recorded through single molecular channels.

* Neher and Sakmann won a Nobel Prize for developing this technique

FigA5.6 From Hammond C. Cellular and Molecular Neurobiology. (2001) Academic Press
T (transient/tiny)- type of low voltage Ca++ channels: Composed of one of three different α subunits, α1G, α1H, α1I.

Can be the earliest type voltage-dependent Ca++ channel expressed. Very small conductance.

T type Ca++ channels enhance excitability after membrane hyperpolarization due to activation/deactivation kinetics of the channel.

Totally inactivated near resting potential. De-inactivated during a small hyperpolarization.

Therefore readily activated when membrane depolarizes to ~-40V after a hyperpolarization (increased excitability). Fig 6.2. From HammondC. Cell & Molecular Neurobiology Academic Press 2001
Whole Cell Currents represent the temporal summation of all channel openings initiated by the same stimulus.
Long term Habituation In Aplysia Gill Withdrawal Reflex (hours)

Due to homosynaptic depression of transmitter release. This is related to a depletion of synaptic vesicles from the readily releasible pool.

* probable mechanism involves the decreased phosphorylation of synapsins which normally causes them to separate from synaptic vesicles and releases the vesicles to move to the synaptic membrane.
Before Habituation: Many vesicles in the readily releasable pool of Aplysia sensory motor synapses.

After Habituation: Readily releaseable pool is depleted.

Chen & Baily, 1995
Sensitization of the Siphon Withdrawal Reflex involves application of a noxious stimulus to another part of the body (the tail).

A Heterosynaptic Process

This activates facilitating interneurons that form 5HT mediated synapses on the presynaptic terminals of siphon sensory neurons. Two types of 5HT synapses are activated. One coupled to Gs. One coupled to Go.

What will be the effect of Gs? Increase in cyclic AMP and activation of PKA
What will be the effect of Go? Activation of phospholipase C (PLC) resulting in production of IP3, DAG and an increase in PKC activity.
PKA inhibitory subunit inactivated by cyclic AMP

1. Decreases K+ channel activity.
2. Mobilizes vesicles to the readily releasable pool.
3. Opens L-type Ca++ channels.

Phopholipase C activates PKC

1. PKC participates with PKA to mobilize vesicles to the readily releasable pool.
2. PKC participates with PKA to phosphorylate and increase L-type Ca++ channel activity.
Studies Using Siphon withdrawal Reflex of Aplysia:
Conditioned stimulus (CS) is the siphon tap
Unconditioned stimulus (US) tail shock
Response is siphon contraction (withdrawal)

Training: 3 blocks of 4 training trials.
5 min interval between trials in a block.
20 min between blocks.
Paired: CS started 500ms before US
Unpaired: interval between stimuli = 2.5 min.

Behavior: Response to CS becomes much larger when tested long after training with pairing.

Activated by hyperpolarization - produces an inward, depolarizing current that functions in rhythmically active neurons, in heart and to enhance transmitter release (Beaumont & Zucker, 2000).

Fig. 17.6 From HammondC. Cell & Molecular Neurobiology Academic Press 2001
Group III metabotropic glutamate receptors at locus coeruleus synapses mediate activity-dependent depression to high frequency trains of stimuli. High affinity glutamate uptake blockers potentiate this effect. Function of group II at this synapse remains unknown.

Metabotropic Glutamate Receptors

G-protein linked receptors

<table>
<thead>
<tr>
<th>Group</th>
<th>Coupling</th>
<th>Selective Agonist</th>
<th>G-protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>↑PLC ↑MAPK</td>
<td>DHPG</td>
<td>G_q</td>
</tr>
<tr>
<td>II</td>
<td>↑AC ↑I_{K,GIRK}</td>
<td>DCG-IV 2R, 4R-APDC</td>
<td>G_i, βγ</td>
</tr>
<tr>
<td>III</td>
<td>↑AC ↑I_{K,GIRK}</td>
<td>L-AP4</td>
<td>G_i, βγ</td>
</tr>
</tbody>
</table>

Fig 14.1 From Hammond C. *Cell & Molecular Neurobiology* Academic Press 2001
Other metabotropic receptors

**GABA\textsubscript{B} Receptors**  Pre- or post-synaptic localization
agonists, GABA, Baclofen; antagonists, phaclophen etc.
require dimerization of two 7 pass transmembrane
membrane proteins to produce a functional receptor
linked to an inhibitory G
protein (G\textsubscript{i}/G\textsubscript{o})

Presynaptic Function
declares transmitter release

Post-synaptic function
**Long-latency (20-50 msec); slow rise, slow decay (400-13000 msec)**

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Fig. 13.3 From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001
Ionotropic Glutamate Receptors

Figs. 11.2, 1, 3 From Hammond C. Cell & Molecular Neurobiology Academic Press 2001

* also acts on some mGluRs
**AMPA Receptors**

Unitary conductance of ~8pS

\[ g = \frac{I}{V_m - E_{rev}} \]

Are permeable to Na+, K+ and, if the GluR2 subunit is present and unedited also to Ca++. 

AMPARs can trigger Ca++ influx through VSCC’s

AMPARs desensitize rapidly.

Fig 11.4 From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001
NMDAReceptors

1. Both ligand and voltage-gated.
2. Channel blocked by Mg++ at ~ -40-80mV.
3. Therefore depolarization will relieve the Mg++ block.
4. Require glycine as a co-agonist.
5. Have slow kinetics.
6. Have >> greater affinity for glutamate than either AMPAR, KainateRs or mGluRs.

Kainate Currents

Subunits GluR5,6,7, KA1, KA2

Functional receptors are homomers of GluR5 & GluR6 or heteromers of KA2 & GluR5 and GluR6. Others???

Kainate currents desensitize rapidly

Larger current change for an increment in voltage when the current is inward rather than outward.

Fig 11.7. From Hammond C. *Cell & Molecular Neurobiology* Academic Press 2001
Molecules of the Post-synaptic Density Drawn to Scale

Note: Kainate receptors can also bind to PSD-95
The Post-Synaptic Density At Glutamate Synapse

En face view

Cross-sectional View

Kennedy, M (2000) Science
LECTURE 2:
Vertebrate Systems For Studying Plasticity

MCP’2003
THE HEBB RULE

When an axon of cell A is near enough to excite cell B or 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, 1948

NEURONS THAT FIRE TOGETHER WIRE TOGETHER
Mice and rats learn to associate the position of a hidden platform in a tank with landmarks in the surround.

Spatial learning is initiated in the Hippocampus.

From: Squire et al 2003, *Fundamental Neuroscience*
LTP has been studied in the dentate gyrus, the CA1 and the CA3 regions of the hippocampus.
Multi-Input LTP is Measured As A Change in an Extracellularly Recorded Field Potential

Give high frequency stimulation.

Record field potential.

Measure change as as an increase in the slope of the field potential.
Long Term Potentiation Can be Associative. This is a Heterosynaptic Interaction.

FIGURE 50.10 Features of LTP at CA3–CA1 synapses in the hippocampus. A single hippocampal pyramidal cell is shown receiving a weak and strong synaptic input. (A) Tetanic stimulation of the weak input alone does not cause LTP in that pathway (compare the EPSP before and after the tetanus). (B) Tetanic stimulus of the strong input alone causes LTP in the strong pathway, but not in the weak pathway. (C) Tetanic stimulation of both the weak and the strong pathway together causes LTP in both the weak and the strong pathway. Modified from Nicoll et al. (1998).

1. NMDAR dependent
2. Lasts hours in vitro
3. Requires post-synaptic Ca++ increase
Figure 15.10  Experiment that tested the Hebbian rule for synaptic plasticity. (A) HFS (100 Hz) given under voltage clamp (left) and HFS given with a depolarizing current injection (right). (B) The EPSPs and EPSCs before and 20 min post tetanus are indicated. LTP was elicited when HFS was given in conjunction with postsynaptic depolarization. (C) EPSP amplitude is plotted as a function of time for the different stimulus protocols. (D) Summary data are shown for two pathways (W1 and W2) at different times following HFS given separately to each pathway. On the left the mean increase in the EPSP when a voltage clamp was applied to the postsynaptic cell during HFS is indicated. On the right is the same experiment except that a depolarizing current pulse was given to the cell during the HFS to W1. (From Kelso et al. 1986.)
REQUIREMENTS FOR LTP: DEPOLARIZATION PLUS SUPERIMPOSED EPSP’S

This is often used on young synapses.

At young synapses afferents cannot follow high frequency bursts of activity.

Frequently used to convert silent synapses to active synapses.

Figure 15.11  Pairing postsynaptic depolarization with a single weak stimulus (with the pairing repeated every 20–30 sec) induces LTP at some synapses.
LTP at Glutamate Synapses can be NMDA receptor dependent or NMDA receptor independent

Or, as likely in mossy fiber to CA3 dendrite LTP, the effect can be pre-synaptic effected by a Ca++ sensitive adenylyl cyclase that activates PKA and facilitates synaptic vesicle recycling thereby increasing synaptic release.

NMDAR dependent LTP
1. Increases in AMPAR function.
   ., LLTP increases in frequency of mini AMPAR currents
3. Decrease in probability of failure when 1 input is stimulated.
NMDA Receptors Control AMPA receptor expression and internalization
Nothing in a neuron is simple. At different synapses showing NMDA dependent LTP different signaling systems may play more or less prominent roles.
Proposed Phosphorylation Model For LTP and LTD In Hippocampus and Cortex
Eye-Blink Conditioning
(motor learning)

A puff of air to the cornea causes a reflexive eye-blink.

Pairing a tone to the air puff soon entrains the eye-blink to the tone alone.
PF and CF activated together at low frequency (~ 4Hz) cause **Long Term Depression** at PF-PC synapse. This reduces output of the PC causing decreased inhibition of the conditioned response in that nucleus and increases output of the conditioned response.

If the CS were continually stimulated without the US the PF to PC synapse would potentiate leading to increased PC output, increased PC inhibition in the DCN, and extinction of the conditioned response.

1,400 synapses from 1CF / PC; (1 CF innervates ~ 10 PC)

200,000 different granule cell PF synapses/PC
LTD in Cerebellum results from stimulation of both AMPAR and mGluR1 Receptors
Long, long, term changes in synaptic strength require protein synthesis and probably reflect the development of new synapses.

Activity can trigger transcription cascades and lead to activation of immediate early genes (cfos,cjun,zif, ZENK).

Many activity sensitive genes have cyclic AMP responsive elements in their regulatory regions.

Synaptic Ca++ influx causes the phosphorylation of cyclic AMP responsive element binding protein (CREB) in the nucleus permitting it to dimerize, interact with with both the CRE element and CREB binding protein (CBP) and initiate transcription.