MEDS 370 – Introductory Neuroscience
Postsynaptic Events

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Reading: Kandel, Schwartz, and Jessell (2000) Principles of Neural Science, 4th Edition, pp. 190-196, 212-227

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Selected Milestones in the History of Electrophysiology

Electric Fish

- - Electric organ was the first indication that the body used electricity. Early Romans were familiar with electric fish and used the shock to treat various illnesses, but the nature of how the electric organ worked was a mystery. (the electric organ of the torpedo fish is also important as a very rich source of nicotinic acetylcholine receptors)

Galvani - 1790's – Father of electrophysiology (?)

- Studied frog nerve muscle junctions
- Induced twitching of frogs legs by attaching to iron bar and brass hooks
- Hypothesized that nerves were conductors of "animal electricity"

Volta - 1790's

- Refuted Volta's claims of animal electricity
- Showed that dissimilar metals in a salt solution caused the leg twitching, leading to the invention of the voltaic cell

Oersted - 1820 – Copenhagen

- First to prove the relationship between electricity and magnetism
- In a classroom presentation, passed current through a coil of wire around a magnet and deflected the magnet – the first *galvanometer*.
- Precursor for modern instruments for measuring voltage and current in biological circuits. Later perfected by wrapping coils in opposite directions to compensate for magnetic field of earth and block external interference (humbucking pickup)

Ohm's Law **V= I R**

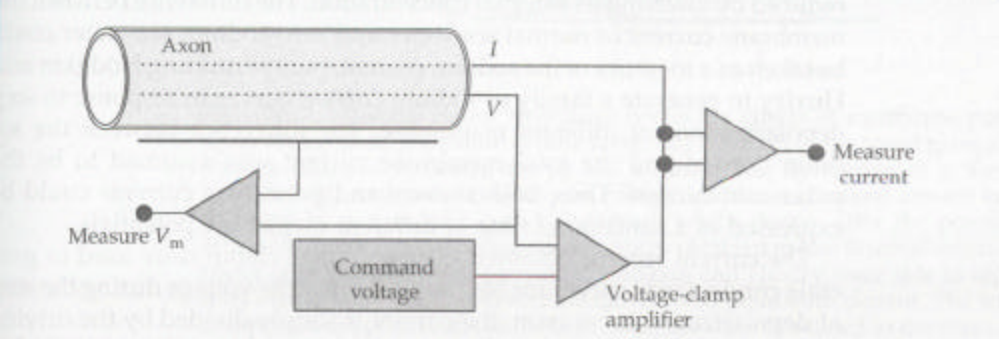
- for biological circuits, voltage difference depends on driving force of the ion, and resistance is usually stated in terms of its inverse, conductance (g)

DuBois Reymond, Von Helmholtz - ~1850

- Recorded currents from cut ends of nerves while stimulating, discovered action potential
- Measured AP propagation, synaptic delay

Hodgkin, Huxley, Katz – 1950's

- First intracellular recordings from squid giant axon. Voltage clamping the membrane led to explanation of how the action potentials are generated and propagated (Nobel Prize)
- with membrane voltage (Vm) clamped, the recorded current is directly proportional to the change in ionic conductance



Sir John Eccles – 1950's
- pioneered the use of sharp-tipped glass micropipettes to impale spinal motor neurons and conduct intracellular recordings. Can be used to record synaptic events and evoked responses.

Neher and Sakmann – 1970's – invented the Patch Clamp (Nobel Prize)
- Breakthrough in investigating single channel properties and subthreshold synaptic currents of CNS neurons
- glass micropipette with smooth, polished tip, seals onto cell membrane with extremely high resistance with slight suction

Different configurations of patch clamp recordings

Cell-attached
– Allows direct observation of the properties of individual channels, which had been predicted by the work of Hodgkin and Huxley. Provides ability to measure single channel conductance and patterns of channel activity - bursting activity and desensitization that could not be observed at the whole cell level

Whole cell
– Opens low resistance access to cell, less damage than intracellular, can record from CNS cells to small to penetrate
– Provides ability to monitor whole-cell ionic currents or membrane potential
– Diffusion into cell from pipette
  – advantage – can introduce substances selectively into cell being recorded
  – disadvantage - washout of intracellular molecules (ATP, GTP)
– Harvest cell contents for analysis of mRNA or protein expression
– Can also be used to measure cell capacitance and monitor secretion-induced changes in capacitance

Excised patch –
Inside Out – use to study effects of intracellular modulators

Outside Out – examine effects of extracellular ligands
Outside Out patches also used as sniffers of release

Two major classes of synaptic junctions in the nervous system

Electrical synapses – gap junctions – specialized channels that physically connect the cytoplasm of pre- and postsynaptic cell – fast, reliable transmission, but inflexible, and requires impedance match of pre- and postsynaptic elements. Since presynaptic terminals are usually small, there would be loss of signal strength across synapse.

Chemical synapses – cells are separated by a narrow cleft (20-40 nm) - no channel that bridges the gap, no cytoplasmic continuity. The change in potential at the presynaptic terminal leads to release of chemical transmitter, which diffuses across synaptic cleft and binds to receptors in postsynaptic membrane. These receptors are responsible for transducing the signal and communicating some change to the postsynaptic cell. This type of transmission provides for multiple points for modulation of synaptic efficacy, also allows amplification since each vesicle can dump out thousands of transmitter molecules to activate postsynaptic receptors.

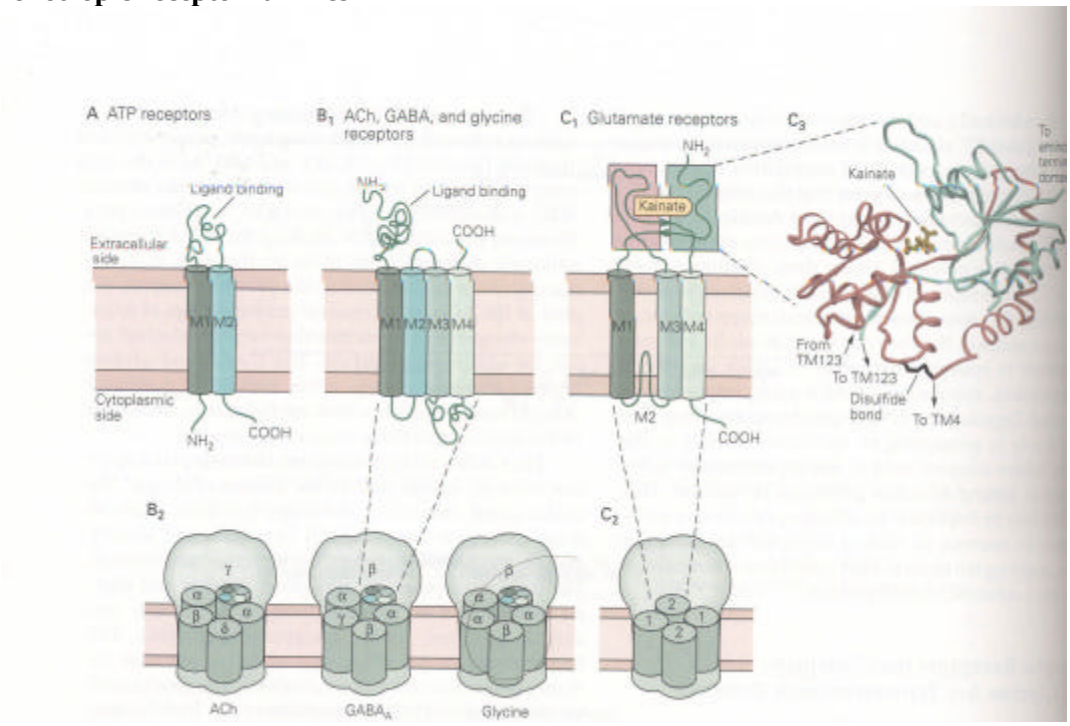
Two major types of postsynaptic receptors

Ionotropic
– Ion channel is integral part of receptor protein. Ligand binds to receptor, causing a change in protein conformation thereby opening the ion channel. Channel opening occurs rapidly and is dependent on presence of the agonist, so it is fast on, fast off. Good for rapid communication.

Metabotropic
– Receptor is coupled to G-protein, which can then alter activity of voltage-gated channels or trigger 2nd messenger pathways. Can modulate electrical activity and other intracellular processes. Slow activation in response to ligand binding, and slow offset.

Receptor tyrosine kinases – Binding of extracellular ligand (typically a growth factor) causes receptor dimerization and autophosphorylation. Subsequently phosphorylates other second messenger molecules on tyrosine, leading to activation of transcription factors and also modulation of synaptic transmission. (Also rapid channel modulation through non-phosphorylation pathways?)

Ionotropic receptor families



Most ionotropic receptors are composed of four distinct subunits. NMJ nicotinic receptor subunits are named , , ,and , with high sequence homology among the subunits. Receptor is made up of 2 , 1 , 1 , and 1 subunit (gamma switched for epsilon later in development). Each subunit has 4 membrane-spanning domains. Extracellular ligand binds to alpha subunit (both binding sites must be occupied for activation). Intracellular phosphorylation sites can modulate receptor activity.

Receptor diversity allows a relatively small number of transmitters to produce a variety of actions. *Postsynaptic signal depends on the receptor activated, not on the identity of the transmitter.*

Complexity of computation/signaling - Because of the diversity of receptors, a single neuron can release a transmitter that will have different, even opposite effects on different target cells – it can even have multiple effects on the same target cell.

Neuromuscular Junction

- Nerve muscle synapse – synapse between motor neuron and skeletal muscle fiber
- most of the principles of synaptic communication have their basis in studies of the NMJ
- **Accessibility**
- **Simplicity** - each muscle fiber innervated by one axon, no convergent inputs
- nne transmitter - ACh, one receptor - nicotinic, one type of ion channel
- each endplate potential elicits an action potential
- large safety factor

Nicotinic receptor - best understood receptor at physiological and molecular levels

- Rich source – electric organ of torpedo – tens of thousands of receptor molecules/square micron of membrane, very homogeneous

- Alpha-bungarotoxin – specific, binds irreversibly to alpha subunit – blocks ACh binding
- Enabled isolation, cloning and characterization of the individual receptor subunits

Synaptic ion current at neuromuscular junction

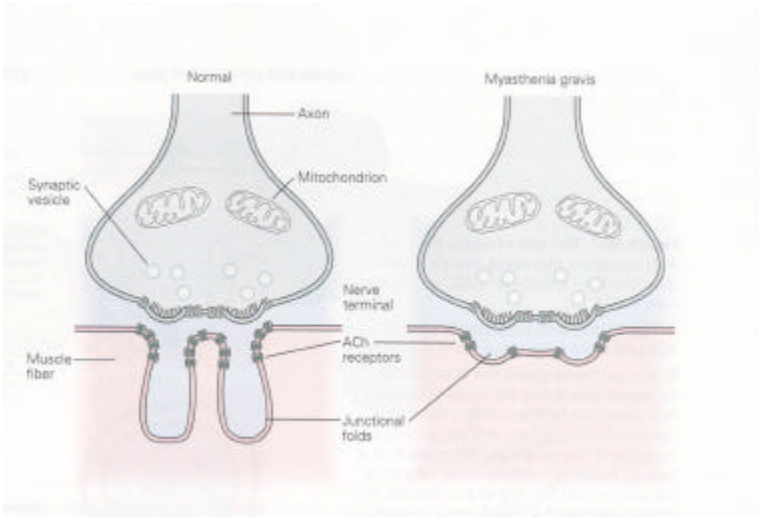
- End-plat potential (EPP) is produced by flow of both Na^+ and K^+ based on reversal potential. Small conductance to calcium as well. Anions are excluded by fixed negative charges around pore.
- Synaptic current obeys Ohm's Law: $I = g (Vm-E_{equil})$
- What happens to reversal potential with changes in extracellular K^+ or Na^+? Increasing K^+ concentration shifts reversal to right, decreased Na^+ concentration shifts reversal to left

Single channel patch clamp technique allowed first look at currents flowing through an individual channel, allows study of the molecular events of channel opening – channels seen to open and close stochastically in continuous presence of agonist

Size of single channel openings is constant a given voltage, but duration of openings and time between openings is variable, can only be described as statistical probability of opening – mean open time is fairly constant, often 2 time constants or more depending on number of open states

Myasthenia gravis

- Antibodies are produced against the nicotinic ACh receptor in the muscle
- Most common form is the autoimmune form
- These antibodies reduce the number of functional receptors, block ACh-receptor interaction, and/or cause destruction of the postsynaptic folds
- Causes muscle weakness, cranial and limb muscles, which can be reversed by cholinesterase inhibitors
- Most obvious deficit is seen with repetitive muscle stimulation – diagnose with muscle EMG (why is first AP normal, then decremented?)
- Treatment – AChE inhibitors, steroids, thymectomy



Synapses in the brain

Most fast synaptic transmission in the brain uses ionotropic receptors. As far as we know, synaptic transmission in the brain generally uses the same basic principles that are seen at the neuromuscular junction.

Added complexity in brain compared to neuromuscular junction:

- Neurons receive input from not just one neuron, but potentially hundreds or more
- Neurons receive both excitatory and inhibitory inputs.
- There are multiple transmitters and multiple receptors, that gate a variety of ion channels, both directly and indirectly
- Integration among diverse inputs is needed to produce an AP, no single input can cause sufficient depolarization

Excitatory synaptic receptors

Brain nicotinic receptor

Glutamate is major excitatory transmitter in the brain. Similar to ACH nicotinic receptor, most ionotropic glutamate receptors conduct both Na^+ and K^+, and have a reversal potential of ~0 mV.

AMPA

Kainate

NMDA receptor

Inhibitory synaptic receptors

- GABAa – blocked by bicucculine
- Glycine – blocked by strychnine
- Both open a chloride-selective channel

Morphology of central synapses- Grays type 1 - usually excitatory and type 2 - usually inhibitory

An excitatory input is one that increases the probability of firing an action potential, and an inhibitory input decreases that probability. A depolarizing input is not necessarily an excitatory input, and an inhibitory input might not change resting membrane potential at all. Since the resting membrane potential is more negative than the action potential threshold, an excitatory input is one that depolarizes the membrane above the AP threshold. The critical determinant is the relationship between the reversal potential of the synaptic current and the threshold for evoking an action potential.

NMDA receptor

– 3 distinctive features

- permeable to calcium, as well as Na^+ and K^+ – Ca^{++} not important electrically (it carries a minor component of the total synaptic current), but can activate downstream protein kinases, etc.
- NMDA channel is voltage-dependent in addition to ligand-dependent – voltage gating is a different mechanism than voltage-gated channels – Mg blocks pore, and pops out when membrane is depolarized
- Activation is also dependent on the presence of cofactor - glycine

What component of EPSP will show NMDA involvement?

What type of synaptic activity will activate NMDA conductance?
Important role for NMDA-mediated calcium influx in activity-dependent synaptic plasticity

Modulation of ionotropic receptors

Extracellular locus

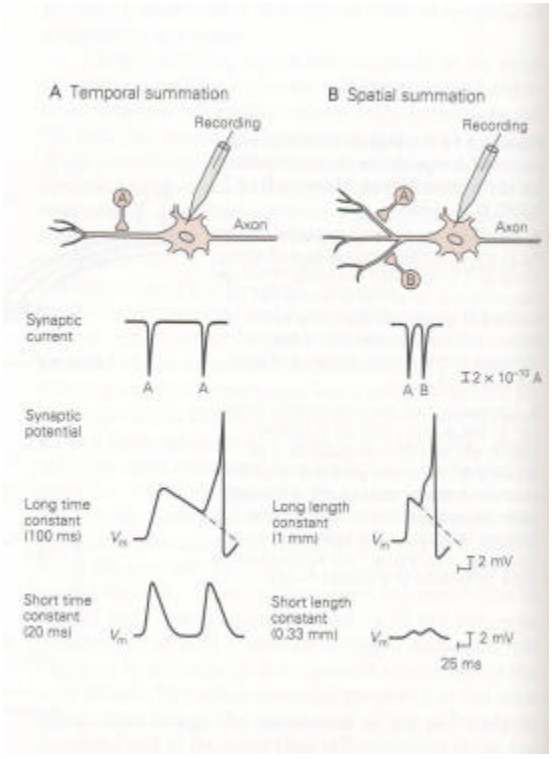
- GABA – benzodiazepines, barbiturates potentiate GABA response (is there an endogenous ligand?)
- NMDA – activity modulated by extracellular glycine

Intracellular locus

- often mediated by protein kinases
  - subunits have phosphorylation sites – phosphorylation can affect open probability and desensitization rate of channel
  - Two examples
- SP increases desensitization of ACh – not direct effect, apply SP outside a cell-attach ed patch and still see effect, can be blocked with PKC inhibitor inside cell
- NMDA-mediated calcium influx modulates number and/or conductance of AMPA receptors

Spatial and temporal summation

- These forms of summation are very important in the brain because, unlike the NMJ, central postsynaptic potentials are too small to reach threshold, summation is absolutely necessary to evoke a postsynaptic action potential.



Membrane time constant

- determines temporal summation
- time constant is a product of membrane input resistance and input capacitance

Length constant

- determines spatial summation
- length constant directly proportional to diameter of the process

Dendritic Propagation

- Important because many neurons have extensive dendritic trees with synapses far from action potential trigger zone. How can these distal synapses compete with more proximal ones to affect action potential initiation?
- Propagation along dendrite originally thought to be purely passive, but there are also voltage-gated conductances in dendrite. These help to maintain shape of EPSP, and may also play a role in amplification of synaptic potentials.
- Magee and Cook paper - Nature Neuroscience (2000) 9:895 - suggests that inputs along dendrite are scaled to allow an equalization among inputs at different dendritic locations.