

CHAP. 16 Postsynaptic potentials and synaptic integration

Ionotropic Receptors—Fast EPSPs and IPSPs

Example of ionotropic receptors in the stretch reflex

Acetylcholine, Glutamate, Glycine and GABA

Pre-synaptic inhibition

Shunting inhibition

Properties of PSPs—Reversal potential, mean open time (how we measure these)

Receptor-channel kinetics

Dual-component EPSPs—AMPA and NMDA, special properties if NMDA channels

Dual-component IPSPs—GABA_A and GABA_B

Metabotropic receptors—Slow PSPs

Key differences from ionotropic receptors

Examples

List of candidates for ionotropic and metabotropic receptors

Today I will talk more specifically about what happens after neurotransmitter binds to a post-synaptic receptor. Some time ago I discussed how neurotransmitter release occurs. Since then you have learned about the various kinds of receptors and signal transduction.

Today we will step back a bit and learn a bit more about ionotropic receptors, metabotropic receptors and the differences between the two as far as signaling is concerned. You have heard about these topics already so I won't dwell long on information you already know.

OH 1 (Fig 16.1 16.2) An example illustrating some different kinds of ionotropic receptors is the **stretch reflex**. We already discussed synaptic transmission at the neuromuscular junction and how the motoneuron releases neurotransmitter (in this case acetylcholine) and how the neurotransmitter binds to receptors to cause an EPP of 10s of mVs.

In contrast, the sensory afferent neuron that detects muscle stretch contacts the motoneuron in the spinal cord and each synapse of contact causes a voltage change of about 0.1 mV. Here the neurotransmitter is glutamate which acts on 3 different kinds of receptors--NMDA, non-NMDA, and metabotropic receptors. The NMDA receptor channel is permeable to Na, K and Ca, the non-NMDA receptor channel is permeable to Na and K (although some non-NMDA receptors also have a small Ca permeability).

Now 0.1 mV is not enough to cause an action potential. So how do we get an action potential in the motoneuron? All of the afferents in a muscle contact all of the motoneurons in a motor nucleus (about 70 afferents in cat medial gastrocnemius muscle—cat leg, cf. R.E. Burke), and each afferent makes an average of 10 contacts on a given motoneuron) so the motoneuron can fire an action potential. The action potential in the motoneurons then causes a contraction of the muscle.

The afferent fiber also contacts inhibitory interneurons which inhibit the MN enervating opposing muscles. Here the neurotransmitter is glycine which opens chloride channels. GABA is also a neurotransmitter in some cases, particularly in synapses on the soma. Recently it was found that GABA and glycine were co-released at a particular synapse. Again the response for a single synapse is small (< 1 mV, more like 0.2 mV).

Note that the reversal potential for Cl is close to the resting potential. If the reversal potential equals the resting potential, what does this say about the synaptic current? There isn't any. Is the input useless then? No. Although there is no current, the conductance is increased. An increase in conductance has what change on resistance? Resistance is reduced, and so the cell is less excitable. This is called shunting inhibition.

Not shown here is pre-synaptic inhibition. GABA receptors on the afferent bouton can be activated to inhibit the action potential from causing release. The interneuron makes an axo-axonic synapse on the afferent terminal.

OH 2 We study the properties of PSPs in cells via patch clamp techniques as introduced to you earlier. Do you recall which patch clamp configuration is used to study neurotransmitter channels?

We use the outside-out patch. With the outside facing out we can apply neurotransmitter to the outside and measure currents. As Dr. Colvin showed you, the outside out patch can be used to determine the reversal potential and single channel conductance.

The acetylcholine receptor channel at the neuromuscular junction and the non-NMDA glutamate receptor channel in the CNS are permeable to both Na and K. So the reversal potential should be somewhere between the reversal potentials of Na and K, right? But where? Where depends on the relative permeability of the channel to Na and K. You have already done calculations with the GHK equation. If the permeabilities to Na and K are about equal, the GHK equation can be solved to show the reversal potential is 0 mV.

When we talked about voltage-dependent conductances, we saw that channels were either open or closed and that the mean open time depended on the closing rate, and the mean closed time depended on the mean opening rate, given here as alpha and beta, for the reaction $C \leftrightarrow O$ or closed to open with forward rate beta and backward rate alpha. Over many channels we can come up with an average or mean open time, but for single channels, open times may be quite variable.

If we were to sum the currents from all of the channels exposed to NT at a synapse, we might see something like that in this Fig (16.4B) (if $C \leftrightarrow O$ is valid and opening occurs instantly with NT release). This represents the synaptic current. Earlier in the course I gave synaptic currents as on-off steps as a simplification in our computations. Even

the instantaneous on with exponential decay here is a simplification as we shall see a bit later. From this synaptic current, we can do curve fitting (fit $A \cdot \exp(-t/\tau)$ to find the time constant of decay, τ . From this we can get the mean open time, which turns out to be $1/\alpha$.

(If the synaptic current did have an instantaneous on, and an exponential decay time course, what would the EPSP look like? The EPSP time course would be different. How will it be different? It would be slower with a final decay at the rate of the membrane time constant.)

OH 3 (OPTIONAL: MOST SHOULD SKIP THIS) How can we get the mean open time? Consider $C \xrightarrow{\alpha} O$.

$$\begin{aligned}\text{Prob}(\text{closed at } t + \Delta t \mid \text{open at } t) &= \alpha * \Delta t \\ \text{Prob}(\text{open at } t + \Delta t \mid \text{open at } t) &= 1 - \alpha * \Delta t\end{aligned}$$

Now,

$$\text{Prob}(\text{open at } t + \Delta t) = \text{Prob}(\text{open at } t) \text{Prob}(\text{open at } t + \Delta t \mid \text{open at } t)$$

$$P_o(t + \Delta t) = P_o(t) (1 - \alpha \Delta t) = P_o(t) - \alpha \Delta t P_o(t)$$

$$\{P_o(t + \Delta t) - P_o(t)\} / \Delta t = -\alpha P_o(t) \text{ or } dP_o/dt = -\alpha P_o(t)$$

Solving gives $P_o(t) = \exp(-\alpha t)$ or an expression for the prob of being open at t

$$\begin{aligned}\text{Prob}(\text{being closed at time } t) &= 1 - \exp(-\alpha t) \\ \text{this is the cumulative distribution function (or distribution function)}\end{aligned}$$

How do we get the mean time of closing? In the discrete case we might have for example, 5 closings at 1 ms, 2 closings at 2 ms and 1 closing at 3 ms. The mean time of closing = $\sum (\text{prob} * \text{number}) = 5/8 * 1 + 2/8 * 2 + 1/8 * 3 = 12/8$ or 1.5 ms

For a continuous random variable we need the integral of $t * \text{the probability density function (pdf)}$. The pdf is equal to the derivative of the distribution function or $\alpha \cdot \exp(-\alpha t)$. Integrating t times this with limits from 0 to infinity gives $1/\alpha$ for the mean time to closing which we prefer to call the mean open time. So when we do curve fitting $\tau = 1/\alpha$ and we can get the mean open time.

START READING AGAIN HERE

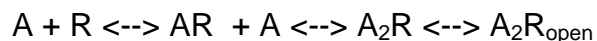
Why is this important? The mean open time gives us one parameter for the kinetic scheme for channel opening.

OH 4 Now the real situation is actually a bit more complicated than $C \leftrightarrow O$. Back in 1972/3 Katz and Miledi postulated that at the neuromuscular junction the reaction was



where acetylcholine binds to the acetylcholine receptor which then undergoes an allosteric transformation to the open state (changes shape to allow channel opening).

Today we believe that two ACh molecules are needed to open a channel or



However in many synapses, the experimental data do not always fit to such a simple scheme. We want to know how a scheme works. For example we may have a number of desensitized states or more than one open state. An example is shown here for non-NMDA channels. Sometimes this leads to bursts of openings as mentioned earlier in the course. I won't describe the mathematical approaches used to get the mean open time from these more complicated kinetic schemes. Often parameter fitting is done with a number of different postulated kinetic schemes to determine which one provides the best fit to the data.

What does this mean for the synaptic current? The synaptic current is not a simple on-off step (although individual channels are on-off) and usually the current is not instantaneous on with exponential decay, but some other shape with a definite rise time and a decay time that may be best described by a number of exponential terms. Analysis of the rise and decay can tell us which kinetic scheme is valid.

Things would be relatively simple if channel openings could be simply described by the kinetic schemes we have looked at. However at some synapses, there are multiple types of channels responsive to the neurotransmitter.

OH 5 In the CNS, we often see non-NMDA (AMPA) and NMDA channels present together at a synapse. Both types of channels are sensitive to glutamate. Near the resting potential, release of glutamate will cause AMPA channels to become open, but NMDA channels will be largely blocked by Mg. Nevertheless it is possible to observe the time course of the two components with the use of blockers. Here are some recordings done by Forsythe and Westbrook in zero Mg, showing the recorded EPSP, the resulting EPSP when the NMDA channels are blocked (leaving the AMPA component) and the NMDA component of the EPSP obtained by subtraction. (Why can't we use an AMPA blocker and leave the NMDA component? Because the NMDA component depends on the voltage from the AMPA component, at least when Mg is present—however many experiments are done in zero Mg)

What we see here is that the time course of the NMDA component of the EPSP is much longer lasting than the 5-10 ms time course of the AMPA component, as shown here. In fact the decay of the NMDA EPSP takes longer than what would be expected from the membrane time constant. (Why might this happen? Glutamate may unbind slowly-- glutamate has a higher affinity for NMDA receptors primarily because of the slow unbinding rate).

There may be different proportions of NMDA and AMPA channels at different synapses and this could have functional consequences. The recording at the bottom of this overhead shows an example of a synapse with just an AMPA component.

Let's review the differences between the NMDA and AMPA components of the EPSP. 1) NMDA has slower on-off kinetics, 2) the NMDA is permeable to Ca, and 3) NMDA has voltage dependent block by Magnesium as you know.

OH 6 A dual component of the IPSP is sometimes seen as illustrated in this overhead. The IPSP has two components, a fast component and a slow component. The fast component is due to the opening of chloride channels at GABA_A receptor channels. The slow component is due to the opening of K channels at GABA_B receptor channels. Both types of receptors are sensitive to GABA. You can see the differences in the reversal potentials for these two components in the figure on the right and differences in the time courses on the left. (Why is the response to GABA_B so much slower? It must act through a G-protein receptor modifying a K channel).

OH 7 METABOTROPIC RECEPTORS. A second class of receptors is the metabotropic receptors. The action of neurotransmitter at these receptors does not open ion channels directly as with the ionotropic receptors. The effects on the channel are indirect and can occur through an intermediate protein, i.e. a G protein, or through some 2nd messenger system. The metabotropic receptors may coexist with ionotropic receptors at synapses. This is certainly true of glutamate metabotropic receptors in the CNS.

Because the metabotropic receptors affect channels indirectly, 1) the time course of action is much slower than that of ionotropic receptors, and 2) the duration may be much longer. The duration can be seconds to minutes because 2nd messenger systems are slow. For example, cAMP takes a while to be synthesized and then levels remain elevated for some time. The duration of the change via phosphorylation will last until some phosphatase comes along to reverse the process.

Two examples--one specifically mentioned in the book and one just vaguely mentioned.

Example--Aplysia. Transmitter 5HT binds to receptor which activates a G protein which increases cAMP. Cyclic AMP activates PKA which phosphorylates a channel protein leading to a change (decrease) in ionic K conductance (via conformation change)

There are other cases where metabotropic receptors also mediate a conductance decrease. For example the muscarinic ACh and metabotropic glutamate receptors lead to a decrease in a voltage-dependent K conductance in part by releasing intracellular Ca from stores.

OH 8 Here is a table of ionotropic and metabotropic neurotransmitter candidates, not for memorization, but to give you an appreciation of the number of candidates identified and their effects.

CHAP 17 Information processing in complex dendrites

- Processing of information without axons or action potentials
 - dendrodendritic synapses in the olfactory granule cell
- Cells with dendrites and axons
 - can distal inputs be effective? Historical view of this question focusing on R_m estimates
 - Possible means of boosting distal inputs
 - Higher g_{syn} in distal dendrites? Evidence for this
 - Voltage-gated channels in dendrites as a means to boost EPSPs
 - Example of voltage-gated channels in dendrites—CA1 hippocampal pyramidal neuron comparison to other cell types
 - Backpropagating action potential—where is the action potential generated?
 - Dendritic action potentials?
 - Role of backpropagating action potential in plasticity
 - STDP—Spike timing dependent plasticity
 - Independent dendrites as functional units
- Dendritic Spines

The traditional view of how information flows in neurons is that inputs occur in the dendrite, they get summed at the axon hillock and the cell fires an action potential. A signal going down an axon is a relatively simple straight forward process. Dendrites on the other hand have turned out to be very complicated. 10-15 years ago or even 30 years ago we were pretty confident we know what dendrites did, but now we aren't so sure.

OH 9 There are a number of neurons, often sensory, that do operations in the absence of axons or if they have axons, sometimes do operations without action potentials. Some well studied examples are from the visual system (photoreceptors, horizontal cells, amacrine cells, bipolar cells) or from invertebrate preparations (lobster STG).

OLFACTORY GRANULE CELL This cell has no axon. The peripheral dendrites have spines which receive excitation from mitral cells and which inhibit mitral and tufted cell dendrites. (so it inhibits itself!). Specifically, as shown here, the mitral cell releases glutamate which excites the granule cell and the granule cell releases GABA which inhibits the mitral cell.

I believe this example was the first demonstration of dendro-dendritic synapses. The Output from the spines does not depend on a signal from the soma even though the cell does produce impulses. So the granule cell spine forms a local I/O compartment independent of neighboring spines to some extent. Dendritic output is specific.

These examples show that our traditional view of inputs summing in dendrites and the O/P being via axons has some significant exceptions which allow for specific local processing of signals.

OH 10 Even within traditional cells, the function of dendrites and the role in integration can be confusing.

First the old question--what do distal dendrites do? Up to 1960, in large part due to Eccles, the view was distal dendrites did not do much. However, we know that in many cells specific pathways terminate on distal dendrites. The large pyramidal cells of cortex with their extensive dendrites receive specific input on their most distal parts. Dentate granule cells have a layered input. The middle third receives input from the medial entorhinal cortex (with auditory information) and the distal third receives input from the lateral entorhinal cortex (with olfactory information).

The modeling work of Rall demonstrated however, that distal inputs could be effective, although less so than proximal inputs. We saw earlier that voltage spread depends on λ , the space constant and that, by applying the work of Rall, dendritic trees had an electrotonic length of 1-2 space constants. However if R_m were much higher, then the electrotonic length of dendritic trees could be much smaller i.e., 0.3 maybe, and distal inputs could be nearly as close electrotonically as proximal inputs.

So what is R_m in dendrites? In the 1970s, a hypothesis was floated that R_m was higher in dendrites than at the soma. In the late 1980s as the whole cell patch clamp technique became more widespread, it was accepted that intracellular recordings introduce an electrode shunt and that this has held down R_m estimates. Estimates of R_m with the best intracellular recordings seemed to peak at about 10-20,000 ohms-cm². With whole cell patch recordings, estimates rose to 100,000 ohms-cm² or higher in some cases. There has been debate about the validity of the higher readings and recordings with the perforated (nystatin) patch method have provided estimates of R_m in between the intracellular electrode and whole cell patch recordings.

Today it seems that R_m is higher than previously believed, but is still low enough that distal inputs should be smaller, even much smaller than proximal inputs.

Is R_m higher in the dendrites? Although an electrode shunt might make it seem that soma R_m is lower than dendritic R_m , this does not mean that R_m increases as you go out distally in the dendrites. In some cells, as we shall see soon, some voltage-dependent conductances increase in density in distal dendrites which could make R_m actually lower in distal dendrites than at the soma.

OH 11 In 1983, Redman and Walmsley proposed that neurons compensate for electrotonic decay by having larger inputs in distal dendrites by having a **larger synaptic conductance**. (How could this occur?--more receptors, more neurotransmitter, more channels open). This idea had been lingering in the literature for a long time until 2000, when Magee and Cook proposed the same thing for hippocampal pyramidal cells. Cook and Magee were able to stimulate and record both proximally and distally, and inject current proximally and distally. Both proximal and distal inputs produced similar EPSP amplitudes at the soma. They seem to have ruled out all other possible explanations for these results other than an increase in gsyn. (OH-Magee Fig. 1).

OH 12 Another way that distal inputs may be boosted is if there are **voltage dependent conductances in the dendrites**. The patch clamp technique has allowed dendrites to be patched and currents recorded. It has been found that there are lots of Na, K, and Ca channels in dendrites. The book describes this situation in a few cell types including Purkinje cells and hippocampal pyramidal cells.

I would like to concentrate on the hippocampal pyramidal cell, first because I am most familiar with it and second, the description in the book is incomplete.

First of all, the soma and dendrites seem to have similar densities of voltage dependent channels. These are much lower than at the nodes of Ranvier in the axon and so the action potential, under most circumstances, is generated at the first node rather than in the cell body or the dendrites or even the early part of the axon.

Regarding **Na**--the distribution of channels in the soma and dendrites is similar, but there seems to be some differences in the biophysical properties of the channels, in activation kinetics.

As for **Ca** channels--the densities are similar, soma and dendrites, but the types of channels are different. In the soma region we see primarily L and N calcium channels (the high threshold Ca channels that might be activated by an AP), whereas in the dendrites you find more R (high threshold) and T calcium channels (lower threshold)

As for **K** channels the Delayed Rectifier (HH) type K is distributed uniformly, but the transient A current channel density increases 5X with distance from the soma.

Also the mixed Na/K hyperpolarization activated current, the **I_H** current, increases 7X with distance from the soma.

So what do these voltage dependent channels on dendrites do for EPSPs?

As for single inputs, not much. The studies from Magee and Cook show this as well.

For multiple inputs the situation may change. In cortical pyramidal cells, it has been shown that you can get an action potential in distal dendrites (local "Hot spots" in the dendrites) and this may boost EPSPs. In other cells, strong input may move the site of action potential initiation from the first node back to the soma or proximal dendrites.

In the hippocampal pyramidal cells (CA1), if you block K you can move the site of AP initiation to the dendrites. If you block distal K you can convert the cell to a burster.

OH 13 Here we have graphs of the relative densities of various voltage gated conductances in the dendrites for a number of different cell types. The function of these different distributions of voltage-gated channels is an area of active study. T means total in the plot for Ca (not calcium t-type) and T means transient for K (the A current).

OH 14 One place where voltage dependent conductances in dendrites may be important is with mediating what happens during **action potential backpropagation**. The action potential is generated at the Initial segment or at the first node in the axon in most cases, because this is the area where threshold is lowest. Once the action potential is generated in the first node, for example, it propagates down the axon, but also back to the soma and dendrites. It may eventually die out in the dendrites and the usual situation is that the voltage-dependent conductances in the dendrites cannot sustain the action potential all the way to the distal end.

Do Action potentials occur in dendrites? Sometimes yes, particularly if you block K channels as noted earlier. Also strong dendritic input may generate action potentials at dendritic "hot spots".

OH 15 Now, suppose you pair an EPSP with a back propagating action potential. What happens? The EPSP may activate the K conductances active near the resting potential and these K conductances may become inactivated. (For example A-type K conductances). If the action potential backpropagates during this time of inactivation, it will generally be bigger and may go back all the way with little decrement in amplitude.

This back-propagating action potential is thought to play a role in learning and memory or in coincidence detection.

In fact the relative timing of the EPSP with the back-propagating action potential is thought to be important. Plasticity (called **spike timing dependent plasticity** or **STDP**) occurs only if the EPSP is activated in a brief time window before the action potential.

If the action potential arrives before the EPSP, then there is no change in calcium influx through NMDA receptors, because the action potential is over by the time the NMDA channels open. This situation may cause no change in synaptic weight or else may result in depression of the synaptic weight. If the action potential arrives shortly after the EPSP, then NMDA channels, which are still open, are opened further (or unblocked by Mg actually) by the action potential and this increases Ca influx and can lead to

potentiation of the synaptic weight. If the action potential arrives a while after the EPSP then the NMDA channels have closed and there is no change. It is only during a brief window of time that a back-propagating action potential will increase calcium influx and affect plasticity.

Voltage dependent conductances may also allow individual dendritic branches to behave independently.

OH 16 Finally, much of the input to many neurons in the brain occurs on **dendritic spines**. These are short protrusions that stick out from dendrites and have a variety of shapes. Why should synapses be made on spines?

It is possible to imagine that spines might play a function in augmenting EPSP amplitude, particularly if there are voltage-dependent Na or Ca channels on spines, and many studies have been done about this. However for spines to play an electric role in signaling would require very high spine stem resistances—much higher than thought to exist.

The current thinking is that spines provide an isolated environment where calcium levels can become locally quite elevated without causing cell death. Calcium entering a spine head can initiate biochemical cascades that can lead to learning and memory.