Neurotransmitter Systems II
Receptors

Reading:
BCP Chapter 6
Neurotransmitter Systems

Normal function of the human brain requires an orderly set of chemical reactions. Some of the most important chemical reactions are those associated with synaptic transmission.

Identification and Distribution
- Criteria
- Localization/function

Receptors
- Subtypes
- Activation

Neurochemistry
- Synthesis
- Cycling
Major Receptor Subtypes

Neurotransmitters (small and large) exert their post-synaptic effects by binding to receptors.

As a rule, no two neurotransmitters bind to the same receptor; however, one neurotransmitter can bind to many different receptors.

Each of the different receptors a neurotransmitter binds to is called a receptor subtype.

There are two major subtypes of receptors:
- **Ionotropic**: ligand-activated ion channels (small NT)
- **Metabotropic**: signal proteins and G proteins
  
  G proteins may act directly on an ion channel … or act on an enzyme that generates an intracellular “second messenger” (which can e.g. affect ion channels, alter gene expression). Small NTs tend to affect ion channels; large NTs alter gene expression.
Like other channels (e.g., leakage, voltage-gated), **ionotropic channels** are proteins with *multiple membrane-spanning domains* called *subunits* (e.g., five for ACh; four for glutamate receptors). Subunits organize to form the *channel pore*.

**Ligand Binding Site(s):**
One or more subunits has a binding site for a specific neurotransmitter. The more binding sites occupied with transmitter, the longer the channel is open.

**Selectivity filter:**
Loop segments located in the pore restrict channel to specific ion(s).
Ionotropic Channels 2

The primary structures of transmitter-gated channels in the brain show clear similarities. In particular, the subunits of most such receptors contain four regions termed M1 to M4, which are segments where the polypeptides will coil into alpha helices that span the membrane.

Variations among channel structures account for their differences. For example, different transmitter binding sites let one channel respond to ACh while another responds to Glu. The amino acids along the ion pore confer the selectivity of ion passage.
Most G-protein-coupled receptors are simple variations on a common plan, consisting of a single polypeptide containing seven membrane-spanning alpha helices.

Two of the extracellular loops of the polypeptide form the transmitter binding sites. Structural variations in this region determine which neurotransmitter will bind to the receptor.

Two of the intracellular loops can bind to and activate G-proteins. Structural variations here determine which G-proteins, and consequently, which effector systems are activated in response to the transmitter binding.
Metabotropic Channels 2

Most G-proteins have the same mode of operation:

- **inactive**: 3 subunits — $\alpha$, $\beta$, and $\gamma$ — “float” in membrane (GDP bound to $\alpha$ subunit)
- **active**: G-protein bumps into an activated receptor and exchanges GDP for GTP
- activated GTP-bound G-protein splits into two parts: $G_{\alpha}$-GTP and $G_{\beta\gamma}$ — both parts can produce effects in the cell by binding to effector proteins
- $G_{\alpha}$ inactivates by slowly converting GTP to GDP.
- $G_{\alpha}$-GDP and $G_{\beta\gamma}$ recombine to start the cycle again.
Metabotropic Channels 3

G-protein exert their effects in one of two ways: binding to G-protein-gated ion channels; or binding to G-protein activated enzymes.

Because the effects do not involve any other chemical intermediaries, the first route is sometimes called the shortcut pathway.

Nearby ion channels can be opened directly by $G_{\beta \gamma}$ subunits, with the fastest response times on the order of 30ms.
In addition to opening directly ion channels, G-proteins exert their effects by activating certain enzymes, which in turn activate other “downstream” enzymes. The whole process is called a second messenger cascade.

There are two well-known cascades initiated by $G_\alpha$ subunits:

• adenylyl cyclase $\rightarrow$ cAMP $\rightarrow$ protein kinase A
  - stimulate: $G_{\alpha S}$
  - inhibit: $G_{\alpha i}$

• phospholipase C $\rightarrow$ protein kinase C + Ca$^{2+}$
  - stimulate: $G_{\alpha q}$

Protein kinases phosphorylate other proteins including ion channels and transcription factors, altering their activity. The effects of kinases are cell specific (as different kinases affect different proteins and cells express unique sets of proteins) and regulated (by the activity of protein phosphatase enzymes that de-phosphorylate).
Synaptic transmission using transmitter-gated channels is simple and fast. Transmission involving G-protein-coupled receptors is complex and slow.

What are the advantages? One advantage is signal amplification. That is, the activation of one G-protein-coupled receptor can lead (via the $G_\alpha$ second messenger cascade) to the altered activation of not one, but many, ion channels.

For example, affecting leakage channels could alter overall cell excitability, whereas altering calcium channels could effect release of neurotransmitter.
Much of what we know about the function of receptors was first learned using neuropharmacological analysis (i.e., measuring postsynaptic responses to the application of various drugs).

Receptor subtypes are distinguished by the drugs that selectively (or maximally) mimic (agonize) or block (antagonize) the activity of the natural neurotransmitter. Receptor subtypes are named based on their agonists (e.g., nicotinic or muscarinic acetylcholine receptors).

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Receptor Subtype</th>
<th>Agonist</th>
<th>Antagonist</th>
<th>Major subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine (ACh)</td>
<td>Nicotinic receptor</td>
<td>Nicotine</td>
<td>Curare</td>
<td>Ionotropic</td>
</tr>
<tr>
<td></td>
<td>Muscarinic receptor</td>
<td>Muscarine</td>
<td>Atropine</td>
<td>Metabotropic</td>
</tr>
<tr>
<td>Norepinephrine (NE)</td>
<td>α receptor</td>
<td>Phenylephrine</td>
<td>Phenoxybenzamine</td>
<td>Metabotropic</td>
</tr>
<tr>
<td></td>
<td>β receptor</td>
<td>Isoproterenol</td>
<td>Propranolol</td>
<td>Metabotropic</td>
</tr>
<tr>
<td>Glutamate (Glu)</td>
<td>AMPA</td>
<td>AMPA</td>
<td>CNQX</td>
<td>Ionotropic</td>
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<tr>
<td></td>
<td>NMDA</td>
<td>NMDA</td>
<td>AP5</td>
<td>Ionotropic</td>
</tr>
<tr>
<td>GABA</td>
<td>GABA_A</td>
<td>Muscimol</td>
<td>Bicuculline</td>
<td>Ionotropic</td>
</tr>
<tr>
<td></td>
<td>GABA_B</td>
<td>Baclofen</td>
<td>Phaclofen</td>
<td>Metabotropic</td>
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</tbody>
</table>
Many chemicals may mimic (agonize) or block (antagonize) the activity of natural neurotransmitters. The relative strength of these compounds can be assessed with ligand-binding methods.

The potency of agonists is measured using a stimulator assay, in which various concentrations of the drug are applied and a response parameter (e.g. size of PSP, second messenger) is recorded.

The \( \text{EC}_{50} \) of a graded dose response curve represents the concentration of the compound where 50% of its maximal effect is observed. The higher the potency (affinity) of the drug, the lower the concentration needed to produce the half-maximal response.
The potency of antagonists is measured using a competition assay. In this assay, a baseline response is established using an agonist. Then, the effects of applying various concentrations of the antagonist are observed.

The $\text{IC}_{50}$ of a *graded* dose response curve represents the concentration of the compound where the agonist response is reduced by 50%. The higher the potency (affinity) of the antagonist, the lower the concentration of the drug needed to reduce the response in half.
Pharmacological analysis suggests that neurotransmitters bind to a large number of different receptor subtypes, including multiple kinds of ionotropic and metabotropic receptors.

Subtypes may be differentially distributed in the nervous system or located on the same neuron. For example, nicotinic acetylcholine receptors predominate in the PNS, whereas muscarinic receptors are the primary receptor type in the CNS. On the other hand, NMDA glutamate receptors are usually co-localized with AMPA receptors in the brain.
Receptor subtypes differ (as expected) in their molecular structure. For example, GABA\textsubscript{A} receptors are transmitter-gated ion channels, whereas GABA\textsubscript{B} receptors are G-protein coupled (metabotropic) receptors.

Unexpectedly, the number of receptor subtypes is likely much larger than that suggested by pharmacological data. For example, GABA\textsubscript{A} receptors are composed of five subunits, and multiple polypeptides can substitute for one another at each subunit. While some possible subunit combinations may not be manufactured, it is clear that receptor subtypes can show a wide diversity of structure and thus function.