1. Acetylcholine
2. Glutamate
3. GABA
4. Neuropeptides

Acetylcholine (Figs. 5.4)

- The first identified NT (1926, Otto Loewi, from frog heart, vagus nerve of the parasympathetic nervous system), supporting the idea that neurons communicate with one another via chemicals.
- NT at the neuromuscular junction and in the visceral motor system, as well as in the CNS.
Acetylcholine Receptors

- Acetylcholine (ACh)

  - ACh receptors
    - Ionotropic receptors
    - Muscarinic receptors
    - Metabotropic receptors

  - N₁ or N₅
  - N₂ or N₄

- Neuromuscular junction
- Autonomic ganglia, CNS and adrenal medulla
- CNS
- Heart
- Smooth muscle
- CNS
- CNS

- Most NTs can activate multiple types of receptors (ionotropic or metabotropic), yielding many possible modes of synaptic signaling.
- Different ionotropic receptor subtypes may have different NT sensitivity, channel kinetics (activation, inactivation, sensitization), conductance, ion selectivity, Ca²⁺ permeability, drug sensitivity, etc.
- Different metabotropic receptor subtypes may have different downstream signaling partners (G proteins, effectors etc.) to mediate different intracellular events.
- The expression of NT receptor types is regulated by neuronal types, developmental stages, neuronal activity and internal physiological state, etc.
Nicotinic ACh Receptor: nAChR

- nAChR: ACh-gated nonselective cation channel ($E_{\text{rev}} = 0 \text{ mV}$, excitatory, permeable to $\text{Ca}^{2+}$) in the CNS, NMJ and the parasympathetic nervous system. Target of nicotine (nAChR agonist), and many neurotoxins (curare, $\alpha$-bungarotoxin, inhibitors of nAChR, causing paralysis and respiratory failure).

- Large protein complexes consisting of 5 subunits (pentameric). Each subunit has 4 transmembrane domains (TM) (a common feature for ligand-gated ion channel subunits).

- Channel composition (stoichiometry): homomeric (only one subunit type) or heteromeric (multiple subunit types).

Nicotinic ACh Receptor: nAChR (Fig. 6.3)

- Large protein complexes consisting of 5 subunits. Each subunit has 4 transmembrane domains (TM). Only the $\alpha$ subunits can bind to ACh (prerequisite subunit).

- The openings at either end of the channel pore are very large, and the pore narrows at the channel gate.

- Gating mechanism: binding of ACh causes a conformational change in part of the extracellular domain, which causes the pore-forming helices to move and open the gate.

- General arrangement for ionotropic NT receptors: several subunits coming together to form a ligand-gated ion channel ($\text{ligand binding domain + pore forming domain}$) is characteristic of all the ionotropic receptors at fast-acting synapses.
Muscarinic ACh Receptors: mAChR (Fig. 6.4)

- mAChR: ACh-activated G protein coupled receptor (GPCR), "metabotropic" AChR.
- Binding of ACh => mAChR conformation change => activating a G protein and subsequent downstream signaling events (e.g. activation of K⁺ channels, inhibitory effect or increasing Ca²⁺ conductance, excitatory effect).
- Five subtypes of mAChR are known and are coupled to different types of G proteins (different downstream signaling events and a variety of slow and/or longer-lasting postsynaptic response).
- Well known agonist: muscarine, carbachol; antagonist: atropine (in the eye drop for pupil dilation).

Major Neurotransmitters: Amino Acids

- **Glutamate** is the main excitatory neurotransmitter in the CNS of vertebrates. Most glutamate-gated ion channels allow Na⁺ influx (depolarization).
- **Gamma-aminobutyric acid (GABA)** is the neurotransmitter at most inhibitory synapses in the brain. Most GABA-gated ion channels allow Cl⁻ influx (Ecl-hyperpolarization).
- **Glycine** also acts at inhibitory synapses in the CNS that lies outside of the brain (spinal cord).
Glutamate Synthesis and Cycling between Neurons and Glia (Fig. 6.5)

- Glutamate: the major excitatory NT for vertebrate CNS; nearly all excitatory neurons in the CNS are glutamatergic; >50% of the central synapses.
- Local synthesis: from glutamine by the enzyme glutaminase within the mitochondrial compartment via a process named transamination.
- Uploaded by VGLUT into SVs.
- Termination: once released, glutamate is removed from the synaptic cleft by EAATs (Na+-dependent glutamate co-transporters) in glial cells, presynaptic or postsynaptic neurons.
- Glutamine synthetase: turning Glu (E) into Gln (Q) in glial cells.
- Glutamate-glutamine cycle: allows glial cells and presynaptic terminals to maintain an adequate supply of Glu for synaptic transmission and to terminate postsynaptic Glu response rapidly.

VGLUT: Vesicular Glutamate Transporter, powered by H⁺ gradient.
EAAT: Excitatory Amino Acid Transporter, powered by Na⁺ gradient. Multiple subtypes, some are glia-specific and some a neuron-specific.

Termination of Glutamatergic Response

- The concentration of glutamate released into the synaptic cleft rises to high levels (~1 mM), but remains at this concentration for only a few milliseconds.
- Main mechanisms:
  1. Diffusion
  2. Excitatory amino acid transporter (EAATs)
  3. Desensitization of glutamate receptors
- Excitotoxicity: In 1957, Lucas and Newhouse found that feeding sodium glutamate to infant mice destroys neurons in the retina. Glutamatergic synapses are over-activated by excess glutamate, likely due to high levels of Ca²⁺ influx, which activates enzymes to cause cell death. It may be involved in spinal cord injury, stroke, traumatic brain injury, etc.
Glutamate Receptors: Ionotropic & Metabotropic

Ion channel-associated

- AMPA
- Kainate
- NMDA

AMPA receptors are not permeable to Ca\(^{2+}\), except for the ones that do not contain GluR2 subunit.

G protein-coupled

- Metabotropic (mGluR)

Some mGluRs are expressed at presynaptic terminal to mediate presynaptic inhibition (inhibit VGCCs).

Structure of the AMPA & NMDA Receptor (Fig. 6.7)

- Large protein complexes consisting of 4 subunits (tetrameric). Each subunit has 3 transmembrane domains (TM).
- Most central excitatory synapses possess both AMPA and NMDA receptors.

GluN2 subunits bind glutamate, GluN1 and GluN3 bind glycine.
Different Ionotropic Glutamate Receptor Properties (Fig. 6.6)

- Different ionotropic glutamate receptor types have different ion channel properties.
- AMPA-R: fast onset, fast decay (rapid desensitization of AMPA-Rs), requires only glutamate for activation.
- Kainate-R: slower than AMPA-Rs
- NMDA-R: slower and longer-lasting. The long time course of NMDA-R provides opportunities for temporal and spatial summation of multiple inputs. Higher affinity to glutamate. Requires a co-agonist (glycine) in the extracellular fluid.
- NMDA-R: Ca²⁺ permeable; activation of NMDA-R can lead to a marked increase of intracellular Ca²⁺, an important 2nd messenger that can trigger multiple cellular signaling events critical for learning and memory.
- Voltage dependency of NMDA-R mediated EPSC: depolarization is required to remove Mg²⁺ blocker (coincidence detector).

Pharmacological Separation of Two Components of EPSC

- APV: NMDA receptor antagonist
- NMDA component: slower

Fig. 3. The effect of APV. A, the EPSC was recorded before and during the application of 50 μM APV at the indicated membrane potentials. B, peak current–voltage relations are shown before (●) and during (△) the application of APV. The current–voltage relation measured 25 ms after the peak of the EPSC (dotted line in A) before (●) and during (○) application of APV are also shown.

By S. HESTRIN*, R. A. NICOLL†, D. J. PERKEL† and P. SAH†
Pharmacological Separation of Two Components of EPSC

- CNQX: AMPA receptor antagonist
- NMDA component: slower

Fig. 4. The effect of CNQX. A, the EPSC was recorded before and during the application of 10 μM CNQX at the indicated membrane potentials. B, Peak current–voltage relation in control solution (△) and current–voltage relation measured 25 ms after the peak of the EPSC (dotted line in A) before (●) and during (○) application of CNQX.

By S. HESTRIN*, R. A. NICOLL†, D. J. PERKEL†, AND P. SAH†

Synthesis and Reuptake of the Inhibitory NTs: GABA (Fig. 6.8)

- GABA (γ-aminobutyric acid): the major inhibitory NT for vertebrate CNS; largely in the local interneurons; >1/3 of the synapses in the brain use GABA as the inhibitory NT.
- Local synthesis: from glutamate by the enzyme glutamic acid decarboxylase (GAD), a marker for GABAergic neurons.
- Uploaded by VIATT into SVs.
- Termination: once released, GABA is removed from the synaptic cleft by GAT (Na+-dependent GABA co-transporters) in glial cells or presynaptic neurons.
- Complete degradation of GABA requires enzymes in the mitochondria.
- Termination of signal: diffusion, reuptake and receptor desensitization.

GABA synthesis:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>Glutamic acid decarboxylase + pyridoxal phosphate</td>
</tr>
</tbody>
</table>

GABA degradation:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>Glutamic acid decarboxylase + pyridoxal phosphate</td>
</tr>
</tbody>
</table>

GABA transport:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA</td>
<td>GABA transporters</td>
</tr>
</tbody>
</table>

VIATT: Vesicular inhibitory amino acid transporter

Pyridoxal phosphate: A co-factor for GAD (derived from Vitamin B6)
**Ionotopic Receptors:** GABA<sub>A</sub> & GABA<sub>C</sub>
- Large protein complexes consisting of 5 subunits (pentamer). Each subunit has 4 transmembrane domains (TM). Similar to nAChRs.
- Ligand-gated Cl<sup>-</sup> channel (E<sub>Cl</sub> = -70 mV < threshold), leading to IPSCs.
- Intracellular Cl<sup>-</sup> is kept low in the adult neurons by a K<sup>+</sup>/Cl<sup>-</sup> transporter (KCC2) => opening of the channel results in Cl<sup>-</sup> influx
- What happens if intracellular Cl<sup>-</sup> is higher so that E<sub>Cl</sub> > threshold?

**Metabotropic Receptors:** GABA<sub>B</sub> (GPCR, 7-TM, heterodimer)
- Signaling events often leads to activation of a K<sup>+</sup> channel (E<sub>K</sub> = -90 mV < threshold) via G<sub>i/o</sub> (sensitive to pertussis toxin inhibition).
- Another mechanism: blocking voltage-sensitive Ca<sup>2+</sup> channel (if expressed at the presynaptic terminal, activation of GABA<sub>B</sub> could block SV release => presynaptic inhibition).

**Excitatory Actions of GABA in the Developing Brain (Box 6D)**

- In the developing brain, GABA is an *excitatory* neurotransmitter.
- Developmental changes in intracellular Cl<sup>-</sup> homeostasis. Different types of Cl<sup>-</sup> transporters are expressed. High in the immature neurons (E<sub>Cl</sub> > threshold), but low in the mature neurons (E<sub>Cl</sub> < threshold)
GABA<sub>A</sub> Receptors (Fig. 6.9)

- GABA<sub>A</sub> receptors contain two binding sites for GABA and numerous sites at which drugs bind to and modulate the receptors.
- **Agonist:** GABA, muscimol
- **Competitive antagonist:** bicuculline
- **Non-competitive antagonist:** picrotoxin
- **Allosteric modulator:** benzodiazepines (Valium, sedative), barbiturates (hypnotics, anesthetic), steroids, and ethanol.

Comparison of Key Ligand-Gated Channels

![Comparison of Key Ligand-Gated Channels](image)

Kandel et al., Principles of Neural Science, 5th Edition, Figure 10-7
Major Neurotransmitters: Neuropeptides (Fig. 6.17)

- Several **neuropeptides**, relatively short chains of amino acids, also function as neurotransmitters.
- Neuropeptides include **substance P** (excitatory) and **endorphins** (inhibitory), which both affect our perception of pain.
- Opiates (morphine, heroin) bind to the same receptors as **endorphins** and produce the same physiological effects (anesthetic effects).
- Many function as “neuromodulators”, which increase or decrease the excitability of the postsynaptic neurons.
- Receptors are GPCRs.

Proteolytic Processing of Pre-Propeptides (Fig. 6.16)

- Multi-step synthesis:
  1. Pre-proteins or pre-propeptides are synthesized in the cell body in the rough ER.
  2. Pro-peptides: after removal of the signal sequence (for secreted protein/peptide), the remaining peptide enters the Golgi apparatus and then is packaged into vesicles in the trans-Golgi network.
  3. Final processing occurs in the vesicles where pro-peptides are cleaved and processed into mature neuropeptides.
- Termination: degraded (not recycled) by peptidases, usually located on the extracellular surface of the plasma membrane.
- Propeptide precursors can give rise to more than one species of neuropeptide => multiple neuropeptides can be released from the same SV.
### Varieties of ionotropic NT receptors (Fig. 6.3)

<table>
<thead>
<tr>
<th>Receptor</th>
<th>nACh</th>
<th>AMPA</th>
<th>NMDA</th>
<th>Kainate</th>
<th>GABA</th>
<th>Glycine</th>
<th>Serotonin</th>
<th>Purines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subunits (combination of 4 or 5 required for each receptor type)</td>
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<td></td>
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<tr>
<td>α_{1–10}</td>
<td>GluA1</td>
<td>GluN1</td>
<td>GluK1</td>
<td>α_{1–6}</td>
<td>5-HT_{3A}</td>
<td>P2X_{1}</td>
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<tr>
<td>β_{1–4}</td>
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<td>GluN2A</td>
<td>GluK2</td>
<td>β_{1–3}</td>
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<td>P2X_{2}</td>
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<tr>
<td>γ</td>
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<td>GluN2B</td>
<td>GluK3</td>
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<td>5-HT_{3C}</td>
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<td>δ</td>
<td>GluA4</td>
<td>GluN2C</td>
<td>GluK4</td>
<td>δ</td>
<td>5-HT_{3D}</td>
<td>P2X_{4}</td>
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<td>GluK5</td>
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<td>GluN3A</td>
<td>GluN3B</td>
<td>θ</td>
<td>η</td>
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<td>θ</td>
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</table>

### Varieties of metabotropic NT receptors (Fig. 6.4)

<table>
<thead>
<tr>
<th>Receptor class</th>
<th>Muscarinic</th>
<th>Glutamate</th>
<th>GABA</th>
<th>Dopamine</th>
<th>Adrenergic</th>
<th>Histamine</th>
<th>Serotonin</th>
<th>Purines</th>
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<tr>
<td>Receptor subtype</td>
<td>M_{1}</td>
<td>mGlu_{1}</td>
<td>GABA_{B1}</td>
<td>D_{1}</td>
<td>Alpha</td>
<td>H_{1}</td>
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<td>M_{2}</td>
<td>mGlu_{2}</td>
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<tr>
<td></td>
<td>M_{3}</td>
<td>mGlu_{3}</td>
<td></td>
<td>D_{3}</td>
<td>α_{1B}</td>
<td>H_{3}</td>
<td>5-HT_{1D}</td>
<td>A_{2A}</td>
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</table>
TABLE 6.1 Functional Features of the Major Neurotransmitters

<table>
<thead>
<tr>
<th>NEUROTRANSMITTER</th>
<th>POSTSYNAPTIC EFFECT*</th>
<th>PRECURSOR(S)</th>
<th>RATE-LIMITING STEP IN SYNTHESIS</th>
<th>REMOVAL MECHANISM</th>
<th>TYPE OF VESICLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>Excitatory</td>
<td>Choline + acetyl CoA</td>
<td>CAT</td>
<td>AChEase</td>
<td>Small, clear</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Excitatory</td>
<td>Glutamine</td>
<td>Glutaminase</td>
<td>Transporters</td>
<td>Small, clear</td>
</tr>
<tr>
<td>GABA</td>
<td>Inhibitory</td>
<td>Glutamate</td>
<td>GAD</td>
<td>Transporters</td>
<td>Small, clear</td>
</tr>
<tr>
<td>Glycine</td>
<td>Inhibitory</td>
<td>Serine</td>
<td>Phosphoserine</td>
<td>Transporters</td>
<td>Small, clear</td>
</tr>
<tr>
<td>Catecholamines</td>
<td>Excitatory</td>
<td>Tyrosine</td>
<td>Tyrosine hydroxylase</td>
<td>Transporters, MAO, COMT</td>
<td>Small dense-core, or large irregular dense-core</td>
</tr>
<tr>
<td>(epinephrine, norepinephrine,</td>
<td></td>
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</tr>
<tr>
<td>dopamine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Large, dense-core</td>
</tr>
<tr>
<td>Serotonin (5 HT)</td>
<td>Excitatory</td>
<td>Tryptophan</td>
<td>Tryptophan hydroxylase</td>
<td>Transporters, MAO</td>
<td>Large, dense-core</td>
</tr>
<tr>
<td>Histamine</td>
<td>Excitatory</td>
<td>Histidine</td>
<td>Histidine decarboxylase</td>
<td>Transporters</td>
<td>Large, dense-core</td>
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<tr>
<td>ATP</td>
<td>Excitatory</td>
<td>ADP</td>
<td>Mitochondrial oxidative phosphorylation; glycolysis</td>
<td>Transporters</td>
<td>Small, clear</td>
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<tr>
<td>Neuropeptides</td>
<td>Excitatory and inhibitory</td>
<td>Amino acids (protein synthesis)</td>
<td>Synthesis and transport</td>
<td>Proteases</td>
<td>Large, dense-core</td>
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<td>Endocannabinoids</td>
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<td>Membrane lipids</td>
<td>Enzymatic modification of lipids</td>
<td>Hydrolysis by FAAH</td>
<td>None</td>
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<td>Nitric oxide</td>
<td>Excitatory and inhibitory</td>
<td>Arginine</td>
<td>Nitric oxide synthase</td>
<td>Spontaneous oxidation</td>
<td>None</td>
</tr>
</tbody>
</table>

*The most common postsynaptic effect is indicated; the same transmitter can elicit postsynaptic excitation or inhibition, depending on the nature of the receptors and ion channels activated by transmitter binding (see Chapter 5).

Sequential Interplay of Nicotinic and GABAergic Signaling Guides Neuronal Development

Zhaoping Liu, Robert A. Neff, Darwin K. Berg

GABA (γ-aminobutyric acid), the major inhibitory transmitter in the brain, goes through a transitory phase of excitation during development. The excitatory phase promotes neuronal growth and integration into circuits. We show here that spontaneous nicotinic cholinergic activity is responsible for terminating GABAergic excitation and initiating inhibition. It does so by changing chloride transporter levels, shifting the driving force on GABA-induced currents. The timing of the transition is critical, because the two phases of GABAergic signaling provide contrasting developmental instructions. Synergistic with nicotinic excitation, GABAergic inhibition constrains neuronal morphology and innervation. The results reveal a multilayered activity-dependent strategy controlling neuronal development.
**Background**: GABA, the main “inhibitory” transmitter in the brain, is actually excitatory during embryogenesis and early postnatal life because of a “reversed” Cl- gradient. The excitatory phase of GABA signaling is critical for proper neuronal development and integration into circuits, i.e. synapses forming onto the neuron. Key to the GABA switch from excitation to inhibition is the appearance of the “mature” Cl- transporter KCC2, which pumps Cl- out of the cell, and the loss of the “immature” transporter NKCC1, which pumps Cl- into the cell. The mature Cl- gradient then enables GABA to be inhibitory (Cl- rushes in when GABA<sub>A</sub> receptors are activated). What determines the timing of the transition?

- **Experiments**: Spontaneous nicotinic cholinergic signaling drives waves of excitation through the embryonic and early postnatal nervous system. Might this be related to the GABAergic switch? Test whether blocking nicotinic activity delays the developmental conversion of GABAergic transmission from excitation to inhibition. Methods: (1) In chick embryos block nicotinic activity receptor antagonists. (2) In mice block nicotinic activity by removing nicotinic receptor genes (knockouts). Easy test for GABA excitation: calcium fluor to “report” calcium influx (through VGCCs opened by the GABA excitation).

---

**Fig. 1.** Blocking nAChR extends the period of GABAergic excitation (pharmacology)

Chick ciliary ganglion: express both nAChRs and GABA<sub>A</sub>-R

E14 neuron (calcium imaging)

Before switch after switch

To block nAChRs: treating with various antagonists at E8

Equilibrium Potential: \( I = 0 \)

NKCC1: keeps intracellular Cl- high

Equilibrium Potential (relative to AP threshold)

Linking pharmacological manipulations with molecular mechanisms
Results: Endogenous nicotinic activity determines when GABAergic signaling converts from excitation to inhibition. Nicotinic activity does this by increasing KCC2 and decreasing NKCC1 to make a mature chloride gradient. Also shown (in other figures): (1) Preventing the depolarizing phase of GABA signaling causes the neurons to get less innervation. (2) Interestingly, even the initial inhibitory phase of GABAergic signaling has developmental instructions if, and only if, the neurons is also getting nicotinic excitation (integration is key).