Neurotransmitter systems

Glutamate and GABA
  Dopamine
  Noradrenaline
  Acetylcholine
  Serotonin
Catecholamines are synthesized in a multi-step process

Catecholamines:
- Dopamine (DA)
- Norepinephrine (NE)
- Epinephrine (EPI)

The rate-limiting enzyme is TH, which is regulated by the product (feedback) and by stress (up-regulated).
Vescicles are loaded with catecholamines by the enzyme Vescicular Monoamine Transporter (VMAT).

VMATS are blocked by the drug Reserpine which causes sedation in animals and depressive symptoms in humans.

Catecholamine release is inhibited by autoreceptors located on dopaminergic and noradrenergic neurons.

Autoreceptors reduce the amount of calcium that enters the terminal in response to a nerve impulse, therefore inhibiting catecholamine release.
Modulation of catecholamine action

1. Modulation of release

Release of catecholamines is dependent on neuronal cell firing.

Some drugs induce the release independently from nerve cell firing.

In animal models increase in catecholamine release produces increased locomotor activity and stereotyped behavior.

Psychostimulants such as amphetamine and methamphetamine in humans cause increased alertness, euphoria, insomnia.

2. Modulation of autoreceptors

Stimulation of autoreceptors inhibits catecholamine release.

Autoreceptor antagonists increase catecholamine release.
3. Modulation of reuptake

Dopaminergic (DA) and Noradrenergic (NE) neurons have specific transporters on their membranes for the reuptake of neurotransmitter. These transporters are different from the autoreceptors described in point 2.

Drugs that block the transporters increase the amount of neurotransmitter in the Synaptic cleft therefore potentiating catecholamine transmission.

Reboexitine is a drug that specifically blocks NE uptake
Cocaine blocks the transport of DA, NE and 5-HT

4. Modulation of metabolism

Inside the terminal the transmitters are also catabolized by 2 enzymes: Catechol-O-methyltransferase (COMT) and monoamido oxidase (MAO)
Degradation of catecholamines produces metabolites: homovanillic acid (HVA) for DA and 3-methoxy-4hydroxy-phenylglycol (MHPG) (CNS) and vanillymandelic acid (VMA) (PNS) for NE.
The levels of these metabolites in blood or urines gives important indications for the catecholaminergic activity and therefore contributes to the diagnosis of mental disorders
MAO inhibitors (phenelzine or tranylcypromine) have been used in the treatment of Depression. COMT inhibitors: entacapone (Comtan) and tolcapone (Tasmar) are used As supplememts in the treatment of Parkinson disease.
Transgenic animals are useful for the characterization of neurotransmitters action

Mutant mice lacking the dopamine Transporter DAT show an increase in locomotor activity
Dopaminergic pathways

Dopaminergic neurons are localized in the mesencephalon (midbrain)

Nigrostriatal tract:
cells from the substantia nigra project to the striatum in the forebrain

This pathway is affected in Parkinson disease. It is involved in control of movements.
Mesolimbic dopamine pathway:
Domapinergic cells in the Ventral Tegmental Area (VTA) in the mesencephalon
Project to structures of the limbic system: nucleus accumbens, septum, amygdala, hippocampus
Mesocortical dopamine pathway:
Domaminergic cells in the Ventral Tegmental Area (VTA) in the mesencephalon project to cerebral cortex

Mesolimbic and mesocortical pathways have been implicated in drug abuse and schizophrenia
Other dopaminergic neurones are present in the retina and in the hypothalamus these last control the secretion of prolactin
Localized application of the neurotoxin 6-hydroxydopamine (6-OHDA) is used to Examine the role of specific pathways in behavior.

This neurotoxin is similar to DA, and therefore is taken selectively by dopaminergic Neurons which are damaged and die.
Dopamine receptors are found in the afferent structures of dopaminergic neurons. There are 5 known receptors: D1-D5 and they are all metabotropic receptors.

D1 receptors stimulate cAMP production, while activation of D2 receptors inhibits the production of cAMP.

This happens through the stimulation of 2 different G-proteins (D1/Gs-D2/Gi).

In addition, D2 also activates potassium channels.
Dopamine receptors agonists and antagonists control dopaminergic-related functions

Apomorphine is an agonist of both D1 and D2 receptors

SFK38393 is an activator of D1 receptors and in mice it enhances self-grooming behavior

Quinprole affects D2 and D3 receptors. It causes an increase in locomotion and Sniffing behavior

Antagonists of dopaminergic receptors cause catalepsy: lack of spontaneous movements

Haloperidol causes catalepsy through D2 receptors, SCH acts through D1 receptors

After prolonged treatment with D receptor antagonists the animals develop behavioral supersensitivity, meaning that their reaction to dopaminergic stimulants is increased
Mice that lack D1 receptors are insensitive to locomotor stimulating effects induced by cocaine
## Drugs that affect the dopaminergic system

<table>
<thead>
<tr>
<th>Drug</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOPA</td>
<td>Converted to DA in the brain</td>
</tr>
<tr>
<td>Phenelzine</td>
<td>Increases catecholamine levels by inhibiting MAO</td>
</tr>
<tr>
<td>α-Methyl-para-tyrosine (AMPT)</td>
<td>Depletes catecholamines by inhibiting tyrosine hydroxylase</td>
</tr>
<tr>
<td>Reserpine</td>
<td>Depletes catecholamines by inhibiting vesicular uptake</td>
</tr>
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<td>6-Hydroxydopamine (6-OHDA)</td>
<td>Damages or destroys catecholaminergic neurons</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>Releases catecholamines</td>
</tr>
<tr>
<td>Cocaine and methylphenidate</td>
<td>Inhibit catecholamine reuptake</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>Stimulates DA receptors generally (agonist)</td>
</tr>
<tr>
<td>SKF 38393</td>
<td>Stimulates D₁ receptors (agonist)</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>Stimulates D₂ and D₃ receptors (agonist)</td>
</tr>
<tr>
<td>SCH 23390</td>
<td>Blocks D₁ receptors (antagonist)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>Blocks D₂ receptors (antagonist)</td>
</tr>
</tbody>
</table>

Figure by MIT OpenCourseWare.
Parkinson disease

The major symptoms include deficits in movement, but some patients also show Cognitive dysfunctions

Caused by death of dopaminergic neurons in the substantia nigra

Possible cause: oxyradical-induced oxidative stress that damages/kills DA neurons

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The autonomic Nervous System

Source: Grey's Anatomy. Courtesy of Wikipedia.
NE transmission and the autonomic nervous system

Figures by MIT OpenCourseWare.
The NE-containing neurons are localized mostly in the LOCUS COERULEUS (LC): an area in the pons (brain stem). These cells provide inputs to cords, cerebellum, and to several areas in the forebrain.

Also NE neurons are in the ganglia of the sympathetic branch of the autonomic nervous system, therefore playing an important role in PNS.
The NE neurons in the LC play an important role in the state of VIGILANCE: being alert to external stimuli.

In vivo electrophysiological recordings from the LC showed that the firing depends on the status of the animal and the external stimuli.

Image removed due to copyright restrictions. Figure 5.12 in Meyer, and Quenzer, *Psychopharmacology*, 2004.
The receptors of NE and EPI are called ADRENERGIC RECEPTORS and they are Metabotropic receptors. They are distinguished in alpha (\(\alpha_1\) and \(\alpha_2\)) and beta (\(\beta_1\) and \(\beta_2\))

\(\beta_1\) and \(\beta_2\) receptors increase the levels of cAMP.
\(\alpha_2\) receptors inhibit adenilyl cyclase and increase K channels opening
\(\alpha_1\) receptors act with phosphoinositide as second messenger inducing an increase of Ca++ in the postsynaptic cell

The effects of drugs for the NE system are measured by looking at the vigilance: phenylephrine is \(\alpha_1\) receptor agonist, while isoproterenol is a \(\beta\) receptors agonist
**NE modulates:**

- vigilance
- anxiety
- pain
- hunger and eating behavior
- autonomic functions

It is important to consider the localization of receptor subunits in specific areas.
**Effects**

**Agonists:**

- $\alpha$ receptors stimulation leads to constriction of the blood vessels in the bronchial lining, this reducing congestion and edema. The agonist phenylephrine in the ingredient in Neosynephrine (a nasal spray)

- $\beta$ receptors stimulation induce relaxation of the bronchial muscles, therefore providing a wider airway. In fact albuterol in a very popular local medication in asthma

**Antagonists:**

- Prazosin is a $\alpha_1$ receptors antagonist and causes a relaxation of the blood vessels
- Propanolol is a $\beta$ receptors blocker that reduces the heart’s contractile force. They are both used as treatment for high blood pressure

Blockers are in general used to treat the symptoms of anxiety disorders: palpitation, tachycardia
## Location and physiological actions of peripheral $\alpha$- and $\beta$-adrenergic receptors

<table>
<thead>
<tr>
<th>Location</th>
<th>Action</th>
<th>Receptor subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>Increased rate and force of contraction</td>
<td>$\beta$</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>Constriction</td>
<td>$\alpha$</td>
</tr>
<tr>
<td></td>
<td>Dilation</td>
<td>$\beta$</td>
</tr>
<tr>
<td>Smooth muscle of the trachea and bronchi</td>
<td>Relaxation</td>
<td>$\beta$</td>
</tr>
<tr>
<td>Uterine smooth muscle</td>
<td>Contraction</td>
<td>$\alpha$</td>
</tr>
<tr>
<td>Bladder</td>
<td>Contraction</td>
<td>$\alpha$</td>
</tr>
<tr>
<td></td>
<td>Relaxation</td>
<td>$\beta$</td>
</tr>
<tr>
<td>Spleen</td>
<td>Contraction</td>
<td>$\alpha$</td>
</tr>
<tr>
<td></td>
<td>Relaxation</td>
<td>$\beta$</td>
</tr>
<tr>
<td>Iris</td>
<td>Pupil dilation</td>
<td>$\alpha$</td>
</tr>
<tr>
<td>Adipose (fat) tissue</td>
<td>Increased fat breakdown and release</td>
<td>$\beta$</td>
</tr>
</tbody>
</table>

Figure by MIT OpenCourseWare.
## Drugs that affect the Noradrenergic system

<table>
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<tr>
<th>Drug</th>
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<tr>
<td>Phenelzine</td>
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</tr>
<tr>
<td>Amphetamine</td>
<td>Releases catecholamines</td>
</tr>
<tr>
<td>Cocaine and methylphenidate</td>
<td>Inhibit catecholamine reuptake</td>
</tr>
<tr>
<td>Desipramine</td>
<td>Seletively inhibits NE reuptake</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>Stimulates α₁-receptors (agonist)</td>
</tr>
<tr>
<td>Clonidine</td>
<td>Stimulates α₂-receptors (agonist)</td>
</tr>
<tr>
<td>Albuterol</td>
<td>Stimulates β-receptors (partially selective for β₂)</td>
</tr>
<tr>
<td>Prazosin</td>
<td>Blocks α₁-receptors (antagonist)</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>Blocks α₂-receptors (antagonist)</td>
</tr>
<tr>
<td>Propranolol</td>
<td>Blocks β-receptors generally (antagonist)</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>Blocks β₁-receptors (antagonist)</td>
</tr>
</tbody>
</table>

Figure by MIT OpenCourseWare.
Acetylcholine

Acetylcholine is a neurotransmitter in:
- Neuromuscular junctions
- Peripheral Nervous System
- Central Nervous System

Factors that regulate Ach synthesis:
- Availability of reagents
- Firing rates

Since so far there are no drugs that control ChAT, the control of cholinergic system happen at different steps of transmission
Vesamicol is a drug that blocks the vesicular Ach transporter, therefore decreasing Ach transmission: Ach can only be released through vesicles.

A Choline transporter takes back Ach into the presynaptic terminal. If this transporter is blocked (hemicolinium-3 (HC-3)) the production of Ach declines. This suggests that there is recycling. HC-3 has to be administered locally.
Factors that modulate Ach release:

Toxin in the venom of the black widow spider

Induce a massive release of Ach, thereby causing: tremors, pain, vomiting, salivation, sweating

Botulinum toxins: blocks Ach release

The toxins are picked up by cholinergic neurons at the neuromuscular junction, thereby causing muscle paralysis.

Symptoms: blurred vision, difficulty speaking and swallowing, muscle weakness

At low dosage Botox is used also for therapeutic purposes:

Diseases causes by permanent muscle contraction: dystonias

Also used for reducing wrinkles
Acetylcholinesterase (AChE) degrades ACh

It is localized in the presynaptic terminal, in the membrane of the postsynaptic terminal, and at the neuromuscular junctions.

Drugs that block AChE increase the effects of Ach transmission.

Reversible inhibitors:

Physostigmine (Eserine) blocks AChE therefore causing: loss of reflexes, mental confusion, hallucination, convulsions, slurred speech. It crosses the BBB.

Neostigmine (Prostigmin) and pyrodostigmine (Mestinon) are analogous of Physostigmine that do not cross the BBB. They are used for the treatment of Myasthenia gravis: an autoimmune disorder where antibodies attack cholinergic Receptors at the neuromuscular junction, therefore in these patients the muscles are less sensitive to Ach.

These substances are used at low dosage also in pesticides.
Irreversible inhibitors of AChE:

Sarin and Soman: chemical gases developed in chemical warfare. They cause paralysis of the diaphragm, hence death.

Some analogous – but reversible- blockers of AChE are used as antidote.
Acetylcholine is fundamental in sympathetic and parasympathetic branches of the Autonomic nervous system.
Localization of cholinergic neurons in the CNS

Note that in the striatum (the target of dopaminergic neurons from substantia nigra), there are cholinergic interneurons. The control of movement depends on the balance between cholinergic and dopaminergic transmission. Therefore in Parkinson disease anticholinergic drugs are also used to improve the control of movements: orphenadrine (Norflex), benztropine mesylate (Cogentin), trihexyphenidyl (Artane).

Neurons of the BFCS are involved in cognitive functions
Role of cholinergic system in Alzheimer disease
Ach receptors

Nicotinic: ionotrophic, distributed mainly in neuromuscular junctions, autonomic nervous system, some neurons in the brain.

When they are activated the Na+ and Ca++ ions flow through the channel, thereby Mediating fast excitatory responses (in case of muscle, contraction)

The nicotinic receptor is made by different subunits

The composition at the neuronal and muscular synapses are different.

For example the effects of nicotine are different In the brain and in the muscles

A nicotinic receptor antagonist is D-tubocurarine, That is the main active ingredient of curare.

Curare blocks cholinergic transmission at the neuromuscular junction therefore causing respiratory paralysis.

Interestingly, treatment with neostigmine (anti AChE) overcomes the effects of curare.
Muscarinic receptors

Metabotropic receptors (M1-M5) that activate different second messenger pathways:
- Activation of phosphoinositide
- Decrease of cAMP
- Stimulation of K+ channels opening

These receptors play an important role in cognitive functions, and those in the striatum are involved in motor function.

M5 muscarine receptors are involved in morphine reward and dependence

![Figure by MIT OpenCourseWare.](image-url)
Muscarinic receptors outside the nervous system:

Cardiac muscle
Smooth muscles associated with several organs

They modulate heart rate and contractions, salivation, sweating, lacrimation

All these possible side effects need to be taken into account for the cognitive effects related to muscarinic receptors

Muscarinic receptor AGONISTS (muscarine, pilocarpine, arecoline):

Mime the effects of parasympathetic activation: lacrimation, salivation, sweating, Constriction of the iris, contraction of the smooth muscles of the viscera, diarrhea

Muscarinic receptor ANTAGONISTS (atropine, scopolamine)

Pupillary dilatation, reduction of secretions
### Drugs and Toxins that affect the Cholinergic System

<table>
<thead>
<tr>
<th>Drug</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesamicol</td>
<td>Depletes ACh by inhibiting vesicular uptake</td>
</tr>
<tr>
<td>Black widow spider venom</td>
<td>Stimulates ACh release</td>
</tr>
<tr>
<td>Botulinum toxin</td>
<td>Inhibits ACh release</td>
</tr>
<tr>
<td>Hemicholinium-3</td>
<td>Depletes ACh by inhibiting choline uptake by the nerve terminal</td>
</tr>
<tr>
<td>Physostigmine, neostigmine, and pyridostigmine</td>
<td>Increase ACh levels by inhibiting acetylcholinesterase reversibly</td>
</tr>
<tr>
<td>Sarin and Soman</td>
<td>Inhibit acetylcholinesterase irreversibly</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Stimulates nicotinic receptors (agonist)</td>
</tr>
<tr>
<td>Succinylcholine</td>
<td>Nicotinic receptor agonist that causes depolarization block</td>
</tr>
<tr>
<td>D-Tubocurarine</td>
<td>Blocks nicotinic receptors (antagonist)</td>
</tr>
<tr>
<td>Muscarine, pilocarpine, and arecoline</td>
<td>Stimulate muscarinic receptors (agonists)</td>
</tr>
<tr>
<td>Atropine and scopolamine</td>
<td>Block muscarinic receptors (antagonists)</td>
</tr>
</tbody>
</table>

Figure by MIT OpenCourseWare.
5-hydroxytryptamine (5-HT): Serotonin

Involved in depression, anxiety, obesity, aggression and drug addition

Starting reagent: tryptophan

Tryptophan hydroxylase is specific of Serotonergic neurons

The drug para-chlorophenylalanine (PCPA) selectively inhibits triptophan hydroxylase, Therefore blocking 5-HT synthesis

A diet rich in carbohydrates leads to the increase of insulin which facilitate glucose uptake, and also several other aa, but not tryptophan

Since it is the ratio between tryptophan and other aa that is important for crossing the BBB a carbo-rich diet would increase the uptake of tryptophan and eventually the production of serotonin

Figure by MIT OpenCourseWare.
Serotoninergic transmission is similar to DA and NE transmission

VMAT2 transport 5-HT into vesicles (reserpine blocker)
Presence of auto receptors that modulate firing rate - release
5-HT release can be stimulated by drugs with the structure of amphetamines

Once released, 5-HT is removed from the cleft by the e-HT transporter.
A blocker of the transporter is fluoxetine (Prozac) that potentiate 5-HT transmission

Monoamine oxidase (MAO) also catabolites 5-HT producing the metabolite
5-hydroxyindoleacetic acid (5-HIAA)
The majority of serotonergic nuclei are localized in the brainstem (medulla, pons and midbrain)

These nuclei are called the raphe nuclei and they are localized on the midline of the brainstem.

They project to all the forebrain regions.

Image removed due to copyright restrictions.
Figure 6.17 in Meyer, and Quenzer, *Psychopharmacology*, 2004.
The firing of the serotoninergic neurons is associated with the behavioral status of the animal: the firing slows down with sleep and shut off during REM sleep.

In general the firing is constant during repetitive movements, like chewing, and it suddenly stops when a new stimulus is presented.

Induced lesions of the serotoninergic system in animals show that it modulates food intake, reproductive behavior, pain sensitivity and learning and memory.
5-HT receptors

There are at least 15 receptor subtypes and they are all metabotropic, with the exception of 5-HT3, which is an excitatory ionotropic receptor.

5-HT1A is present in many brain areas, including the hippocampus and the amygdala.

It acts by inhibiting adenyl cyclase and by opening a K+ channel leading to membrane hyperpolarization.

Administration of 5-HT1A agonists produce hyperphagia.

The most studied antagonist (WAY-from the pharmaceutical company) produced decrease in body weight, but it was accompanied by side effects.

Image removed due to copyright restrictions.
Figure 6.19 (Part 1) in Meyer, and Quenzer, Psychopharmacology, 2004.
5-HT1A stimulation also produced reduction in anxiety, and this is what is used in the medication Buspar-commercial name for busiprone.

Another effect of 5-HT1A agonist is the inhibition of alcohol consumption.

**5-HT2A** receptors acts by activating protein kinase C.
They are present in cerebral cortex.

Agonist of this receptor cause Hallucinations, and this is supposed to be related to the effects of lysergic acid Diethylamide (LSD).
The best known agonist is DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane).

Known antagonists are ketanserin and ritanserin.

In general, these antagonists can be used for the treatment of schizophrenia.
Recently, drugs that act on both the DA and 5-HT system have shown the best results for the treatment of schizophrenia with lower side effects.

Image removed due to copyright restrictions.
Figure 6.19 (Part 2) in Meyer and Quenzer, *Psychopharmacology*, 2004.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>para</em>-Chlorophenylalanine</td>
<td>Depletes 5-HT by inhibiting tryptophan hydroxylase</td>
</tr>
<tr>
<td>Reserpine</td>
<td>Depletes 4-HT by inhibiting vesicular uptake</td>
</tr>
<tr>
<td><em>para</em>-Chloroamphetamine, fenfluramine, and MDMA</td>
<td>Release 5-HT from nerve terminals (MDMA and <em>para</em>-chloroamphetamine also have neurotoxic effects)</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Inhibits 5-HT reuptake</td>
</tr>
<tr>
<td>5,7-Dihydroxytryptamine</td>
<td>5-HT neurotoxin</td>
</tr>
<tr>
<td>Buspirone, ipsapirone, and 8-OH-DPAT</td>
<td>Stimulate 5-HT$_{1A}$ receptors (agonists)</td>
</tr>
<tr>
<td>WAY 100635</td>
<td>Blocks 5-HT$_{1A}$ receptors (antagonist)</td>
</tr>
<tr>
<td>DOI</td>
<td>Stimulates 5-HT$_{2A}$ receptors (agonist)</td>
</tr>
<tr>
<td>Ketanserin and ritanserin</td>
<td>Block 5-HT$_{2A}$ receptors (antagonists)</td>
</tr>
</tbody>
</table>