3.2 Catecholamines

- Anatomically distinct neuronal systems use 3 different catecholamines in the brain:
  1. Norepinephrine
  2. Epinephrine
  3. Dopamine

- Present in relatively small amounts and initially thought not to be important in brain
- All synthesized from the essential amino acid tyrosine in a common pathway
- Microanatomy of a catecholamine-containing neuron is distinct
- A single neuron can give rise to long terminal branches with thousands of varicosities (swellings along the axon)
- Varicosities contain all machinery for neurotransmitter synthesis, storage and release
- Varicosities are points of synaptic contact
- **Arousal, mood, central regulation of blood pressure**
(3.2.1) Norepinephrine (NE)

- Formed in brain from tyrosine; NE biosynthesis varies with neuronal activity
- Stored in specialized vesicles thereby retarding diffusion out of the neuron
- Once in the synaptic cleft NE can act on alpha or beta adrenergic receptors
- Degredation of endogenous or exogenously administered catecholamines is much slower than the ACh-Acetylcholinesterase system
- Major enzymes in metabolic degradation of catecholamines:(1) monoamine oxidase (MAO) and (2) catechol-o-methyltransferase (COMT)
- MAO is more important as a means of terminating signal than in periphery
(A) Noradrenergic pathways

Local circuit neurons and two major clusters of NE-containing cell bodies from which axonal systems arise to innervate targets:

1) Locus ceruleus
   - A nucleus of the brain stem with ascending and descending projections
   - Targets are cerebral cortex, hippocampus, thalamic nuclei, cerebellum and spinal cord
   - Locus ceruleus projections to spinal cord are thought to be important in regulation and processing of perceptual information (e.g. pain)
   - Catecholaminergic drugs can influence pain perception

2) Lateral tegmental noradrenergic neurons
   - Part of the reticular activating system
   - Important for arousal
   - A site of importance in the action of antidepressant drugs
   - Amphetamine causes arousal in humans
(B) Pharmacology of central noradrenergic neurons

1) **Enzymatic synthesis** - Tyrosine hydroxylase is rate limiting

2) **Storage** -

3) **Release** - Ca²⁺-mediated exocytosis from presynaptic vesicles.

4) **Receptor interaction** - (a) presynaptic α₂ receptors, clonidine is a potent agonist at autoreceptors and (b) postsynaptic receptors

5) **Reuptake** - by a specific membrane transporter. Terminates the action of NE in the synapse.

6) **Monoamine oxidase (MAO)** - Free NE or DA in the presynaptic terminal can be degraded by MAO. MAO is bound to mitochondria and is abundant in neurons

7) **Catechol-o-methyltransferase (COMT)** - NE can be degraded by COMT. Widespread in neuronal and non-neuronal tissue
(C) Adrenergic receptors
- G protein coupled; 7 transmembrane domains
- Multiple receptor subtypes - pre and postsynaptic receptors
- Differ in the effectors to which they are coupled and this is the basis of the most current receptor classification:
  - As in the periphery, three main families have been described: α₁, α₂ and β-adrenoceptors
  - α₁ receptors stimulate phospholipase C producing IP₃ and DAG as second messengers, α₂ receptors inhibit adenylate cyclase (thus decrease cAMP formation) through coupling to G₁, β receptors in brain stimulate adenylate cyclase through coupling to Gₛ

(D) Norepinephrine transporter (NET)
- Located on presynaptic terminal (distinct from vesicular transporter)
- NET has 12 transmembrane domains
- Sympathomimetic effects of cocaine related to inhibition of NE (serotonin and dopamine) transport
- Target of the tricyclic antidepressants
(3.2.2) Epinephrine
- Neurons were identified much later in the CNS
- Still limited understanding but thought to play a role in neuroendocrine mechanisms and blood pressure control

(3.2.3) Dopamine (DA)
- Originally thought to be an intermediate in biosynthesis of NE
- Distribution does not parallel that of NE
- Once DA is released into the synaptic cleft it acts at specific DA receptors
- Approx. half catecholaminergic neurons are dopaminergic
- Motor control, Behavioural control, Endocrine

(A) Dopaminergic Pathways
Divided into 3 categories based on the length of the efferent dopamine fibers:

1) long
2) intermediate
3) short

1) Two important nuclei in the midbrain with long axon projections:
a) Substantia nigra
- Dopamine containing neurons project to the striatum
- This pathway degenerates in Parkinson’s disease
- Led to discovery of therapeutic effects of L-dopa in Parkinson’s disease
  Side effects of antipsychotic drugs mimic symptoms of Parkinson’s disease
b) Ventral tegmental area (VTA)

- Projects to the limbic structures and cerebral cortex (A10)
- Sometimes known as the mesolimbic and mesocortical pathways
- Doesn’t seem to degenerate in Parkinson’s disease
- Before these pathways were mapped they were known as the medial forebrain bundle
- We now know a limbic structure, the **nucleus accumbens**, is the reward center in the brain
- Nucleus accumbens plays an important role as a target for drugs of abuse (amphetamine and cocaine)
2) Dopaminergic nuclei with intermediate axon projections:
   a) arcuate nucleus of hypothalamus to the intermediate lobe of the pituitary
      • DA is a key regulator of prolactin
      • Prolactin inhibitory factor (PIF) is DA
      • DA is inhibitory to the release of prolactin
      • characteristic effect of antipsychotic drugs (which are DA antagonists) is that will increase prolactin levels in the serum and this can be used to titrate the dose of an antipsychotic
   b) hypothalamic nuclei to median eminance
   c) posterior hypothalamus to anterior hypothalamus

3) Dopaminergic nuclei with short axon projections:
   a) retina
   b) olfactory bulb
1) **Enzymatic synthesis** - Tyrosine hydroxylase is rate limiting.

2) **Storage** - Reserpine and tetrabenzene interfere with uptake into storage vesicles.

3) **Release** - Ca$^{2+}$-mediated exocytosis from vesicles.

4) **Receptor interaction** - (a) presynaptic dopamine autoreceptors, (b) postsynaptic dopamine receptors.

5) **Reuptake** - by a specific protein or transporter. Terminates the action of DA in the synapse. Amphetamine inhibits reuptake.

6) **Monoamine oxidase** (MAO) - Free DA in the presynaptic terminal can be degraded by MAO.

7) **Catechol-o-methyltransferase** (COMT) - degrades DA.

8) Primary **metabolites** - homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC). Accumulation in CSF can be used as an index of the functional activity of dopaminergic neurons.
(C) Dopamine receptors

- G protein coupled; 7 transmembrane domains
- Multiple receptor subtypes (D₁ D₂ D₃ D₄ Dₛ)
- D₁ inc. adenylate cyclase through coupling to Gₛ (D₃ is D₁-like)
- D₂ dec. adenylate cyclase through coupling to G₁ (D₃ D₄ are D₂-like)
- Early classification schemes divided receptors into pre and postsynaptic receptors and this is still useful

<table>
<thead>
<tr>
<th>Agonists</th>
<th>D₁ like</th>
<th>D₂ like</th>
</tr>
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<tbody>
<tr>
<td>Dopamine</td>
<td>Agonist (low potency)</td>
<td>Agonist (high potency)</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>Partial Agonist (low potency)</td>
<td>Agonist (high potency)</td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>Partial Agonist (low potency)</td>
<td>Agonist (high potency)</td>
</tr>
</tbody>
</table>

Examples of Antagonists
Chlorpromazine, Haloperidol, Spiperone, Sulpiride, Clozapine

(D) Dopamine transporter (DAT)

- Located on presynaptic terminal.
- 12 transmembrane domains.
- Target for cocaine (especially at the level of the nucleus accumbens).
- MPP⁺ (neurotoxin that produces a Parkinson’s Disease-like syndrome) is a substrate for this transporter.
- Amphetamine inhibits reuptake at this site.
3.3 Serotonin (5-Hydroxytryptamine or 5-HT)

- Found in many non-neuronal cells, only 1-2% of serotonin in the body is found in brain. It cannot cross the blood brain barrier so neurons must synthesize it.
- Found in serum (serotonin) and gut (enteramine) and in brain in the 1950s
- Proposed in the 1950s that the hallucinogenic effects of LSD are caused by action upon 5-HT pathways

**Hallucinations, behavioural changes, sleep, wakefulness, mood, feeding behaviour, control of sensory transmission (startle, avoidance, nociception), regulation of temperature, blood pressure, sexual function**

**(A) Serotonergic pathways**

- Nine nuclei clustered in or near the midline or raphe regions of the pons and upper brain stem
- These serotonin-containing cell bodies send ascending projections to the forebrain especially limbic structures and cerebral cortex
- More like that of NE, remember DA containing neurons were more restricted
- Part of the efficacy of antidepressant drugs that influence serotonergic neurotransmission may derive from their ability to interact with serotonergic synapses in the cerebral cortex

(B) Pharmacology of serotonin containing neurons

1) **Enzymatic synthesis** - precursor for synthesis is dietary tryptophan, with tryptophan hydroxylase representing the rate limiting step in the biosynthetic pathways

2) **Storage** - Reserpine and tetrabenzene interfere with uptake into vesicles.

3) **Release** - Normally via Ca\(^{2+}\)-mediated exocytosis from vesicles. LSD causes a net decrease in release.
4) **Receptor interaction** - (a) presynaptic autoreceptors, (b) postsynaptic receptors.

5) **Reuptake** - by a specific protein or transporter. Terminates the action of 5-HT in the synapse. There are clinically important inhibitors of this mechanism.

6) **Monoamine oxidase (MAO)** - Free 5-HT in the presynaptic terminal can be degraded by MAO. Then oxidized further to 5-hydroxyindoleacetic acid (5-HIAA)

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**(C) Serotonin receptors**

- Multiple subtypes: 7 classes have evolved to recognize serotonin (5HT₁-5HT₇) all G protein coupled except 5-HT₃
- There are expressed **pre and postsynaptically**
- Autoreceptors: 1) decrease vascular release of 5-HT  
  2) decrease 5-HT synthesis
- 5-HT₁, 5-HT₂, and 5-HT₃ are important in CNS
- 5-HT₃ is a ligand-gated cation channel, oligomer composed of several subunits assembled in a pentameric structure
- In vivo 5-HT₃ agonists induce nausea and vomiting, this is thought to underlie the emetic side effects of cancer chemotherapy
- 5-HT₃ receptor is the target for antiemetic compounds (e.g. **ondansetron** is a selective 5-HT₃ antagonist)

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**(D) Serotonin transporter**

- Located on presynaptic terminal, belongs to the family of biogenic amine transporters (like NE and DA)
- 12 transmembrane domains
- Cocaine has high affinity for the serotonin transporter
- Target of some classes of antidepressant drugs (e.g. fluoxetine, imipramine)
Serotonin, Dopamine, Norepinephrine and Epinephrine are sometimes grouped as a class known as **biogenic amines**. All are amines structurally and are in low abundance in brain relative to amino acid transmitters

### 3.4 Amino acids

- Brain levels are very high relative to biogenic amines and neuropeptide transmitters (µmoles/g compared with nmols/g)
- Brain levels are a reflection of the abundance of neurons utilizing amino acid transmitters
- Cell bodies are located throughout the brain (there is also a discrete distribution)
- Very rapid signaling (milliseconds compared with sec to min) mediated by ligand gated ion channels. Final response of a neuron depends excitatory and inhibitory inputs that converge upon it:
  - **GABA** (γ-aminobutyric acid) and **Glycine** are inhibitory neurotransmitters
  - **Glutamate** is an excitatory neurotransmitter

### 3.4.1 GABA

- Identified in 1950 as a component of normal brain tissue
- **Major inhibitory neurotransmitter in brain** (whereas glycine is important in the spinal cord and brainstem). Only trace amounts of GABA are found in the periphery.
- Antagonists of inhibitory neurotransmitters cause convulsions
- Glycine receptors in the spinal cord and brainstem are also ligand-gated chloride channels. Strychnine is a glycine antagonist, causes lethal convulsions.
- At least 30% of all neuronal synapses utilize GABA as a neurotransmitter
• Two types of GABAergic neurons:
  (1) Short interneurons predominate
  (2) Long GABA-ergic projection neurons go to cerebellum and striatum

(A) Pharmacology of GABA containing neurons

1) **Enzymatic synthesis** - in a single step at the terminal by \( \alpha \)-decarboxylation of L-glutamic acid by **glutamic acid decarboxylase** (GAD) expressed in GABAergic neurons

2) **Storage** - in synaptic vesicles.

3) **Release** - Normally via \( \text{Ca}^{2+} \)-dependent manner from vesicles.

4) **Receptor interaction** - (a) presynaptic autoreceptors, (b) postsynaptic GABA receptors.

5) **Reuptake** - by a specific protein or transporter. Primarily responsible for terminating the signal in the synaptic cleft. Glial cells also have a GABA transporter (different from the neuronal transporter)

6) **Metabolism** - primarily by **GABA transaminase** which is expressed pre and postsynaptically and appears to be localized in mitochondria

(B) GABA receptors

• GABA\(_A\) receptor is a ligand gated ion channel

• GABA\(_B\) receptor is G-protein coupled, to date functionally less important, negatively coupled to adenylyl cyclase. Baclofen is a GABA\(_B\) receptor agonist, introduced in 1972 to treat spasticity.

• GABA\(_C\) originally described a bicuculline and and baclofen-insensitive \(^3\)H\)GABA binding site on cerebellar membranes. Pharmacological and electrophysiological properties distinguish it from GABA\(_A\). Most studied in the retina.

**GABA\(_A\) receptor**
- Is a member of the superfamily of **ligand-gated ion channels**
- Heterooligomeric protein, probably composed of 5 subunits based on homology with nicotinic ACh receptor
- Subunits span the cell membrane to form a chloride channel
- Multiple subtypes of each subunit have been identified: α, β, γ, δ, families (2 α, 2 β and a γ or δ ~ 300 combinations, depending on the isoform present)
- Receptor assembly differs in various brain regions and can change (e.g. development, tolerance to benzodiazepines)
- GABA binds to the extracellular surface → channel opens allowing Cl- ions flow down their concentration gradient → hyperpolarization of postsynaptic neuronal membrane
Pharmacology of the GABA<sub>A</sub> receptor

1) **GABA recognition site.**

2) Ligand-gated ion channels open and close as a function of occupancy of the ligand binding site (e.g. GABA or GABA agonists)

3) **Muscimol** is a potent GABA<sub>A</sub> receptor agonist, **bicuculline** is a GABA antagonist.

4) **Benzodiazepine binding site** on the receptor complex ⇒ inc. frequency of channel opening. Drugs that bind to this site and enhance the effects of GABA are agonists, compounds that bind to this site but decrease the effects off GABA are known as **inverse agonists.**

5) **Barbiturate binding site** ⇒ prolong duration of channel opening.

6) **Steroid binding site** ⇒ benzodiazepene-like effect.

7) Convulsants such as the natural toxin **picrotoxin** & products of polyurethane combustion, are ion channel blockers.

► Schematic Illustration of a GABA<sub>A</sub> Receptor, with Its Binding Sites
• Transmitter recognition site (GABA)
• Allosteric modulatory sites (benzodiazepines, barbiturates, neurosteroids)
  o Four known classes of BDZ bind to the Benzodiazepene recognition site:
    ▪ full agonists/positive modulators
    ▪ full inverse agonists/negative modulators antagonists
    ▪ partial agonists and partial inverse agonists
• The ion channel (chloride channel)

(C) GABA transporter
• 12 transmembrane domains; Na\(^{2+}\)-dependent
• Specifically clears GABA from the synapse
• Glial transporter is distinct from that found in neurons

3.4.2 Glycine is also an Inhibitory Neurotransmitter
• High concentrations in the spinal cord and brain stem
• Inhibitory neurotransmitter
• Acts at its own receptors which functionally resemble GABA
• Inhibitory glycine receptor is a postsynaptic ligand-gated chloride channel
• Oligomeric transmembrane protein comprised of 5 subunits (three \(\alpha\) and 2 \(\beta\) subunits)
• 4 different genes encode \(\alpha\) subunits 1-4, \(\beta\) subunit is essential for targeting the receptor to the synapse
• Strychnine is a competitive glycine antagonist (powerful convulsant)
• Strychnine-sensitive inhibitory glycine receptor
3.4.3 Glutamate

- Major excitatory neurotransmitter in the brain.
- Ubiquitous distribution in brain but not an equal distribution
- 25-30% of neurons utilize glutamate as a neurotransmitter.

Figure 10-2. Glutamate and GABA Synthesis and Metabolism. A. Glutamate synthesis and metabolism is intertwined with GABA synthesis and metabolism. In one pathway for glutamate synthesis, α-ketoglutarate produced by the Krebs cycle serves as a substrate for the enzyme GABA transaminase (GABA-T), which reductively transaminates intraneuronal α-ketoglutarate to glutamate. The same enzyme, GABA transaminase, also converts succinic semialdehyde to GABA. Alternatively, glutamate is converted to GABA by the enzyme glutamic acid decarboxylase (GAD), changing the major excitatory neurotransmitter to the major inhibitory transmitter. GABA-T: GABA transaminase; SSADH: succinic semialdehyde dehydrogenase; GAD: glutamic acid decarboxylase. B. Glutamate transporters present in neurons [Gt(n)] and glial cells [Gt(g)] sequester glutamate (Glut) from the synaptic cleft into their respective cells. In the glial cell, the enzyme glutamine synthetase transforms glutamate into glutamine (Gln). Glutamine is then transferred to the neuron, which converts it back to glutamate via mitochondria-associated glutaminase.
(A) Pharmacology of glutamate containing neuron

1) **Enzymatic synthesis** - main sources of glutamate is from Kreb’s cycle.
2) **Storage** - in synaptic vesicles.
3) **Release** - Normally via Ca^{2+}-dependent manner from vesicles.
4) **Receptor interaction** - presynaptic and postsynaptic receptors.
5) **Reuptake** - glutamate transporters are primarily responsible for terminating the signal in the synaptic cleft. Glial cells also have glutamate transporters.
6) **Metabolism** - decarboxylated to GABA or transaminated to glycine

(B) Glutamate receptors

Two main families based on their signal transduction pathways:

1. Ionotropic glutamate receptors (ligand-gated ion channels)
2. Metabotropic receptors (G-protein coupled receptors)
   - All utilize glutamate as their endogenous neurotransmitter
   - Metabotropic receptors work on a slower time scale (sec to min compared with milliseconds)

**Ionotropic glutamate receptors**

- Originally classified pharmacologically based upon activation by synthetic glutamate analogues:
  1. NMDA (N-methyl-D-aspartate)
  2. non-NMDA:
     - Non-NMDA are subdivided into:
       1. AMPA-preferring (GluR1-GluR4)
       2. Kainate preferring (GluR5-7 and KA1-2)
Domoic acid is a potent agonist at KA receptors (3 times more potent than KA). Contaminated shellfish \(\Rightarrow\) amnesic shellfish poisoning in humans

- All 3 types of ionotropic glutamate receptor are composed of multiple subunits that assemble to form a central cation channel.
- Each subunit is encoded by its own gene.
- Receptors are excited by an influx of cations (\(\text{Na}^{2+}, \text{K}^{+}\) and \(\text{Ca}^{2+}\)) producing a rapid depolarization of the postsynaptic neuron.

**NMDA subtype of ionotropic glutamate receptor**

- Often coexists with AMPA/Kainate receptors on the same neuron.
- Two major families of subunits: NMDAR1 and NMDAR2(A-D).
- A functional receptor contains at least one copy of NMDAR1 and some combination of NMDAR2 (A,B,C or D).
- Like GABA\(_A\) receptors, this kind of structure allows great diversity.
- These receptors are essential for normal neurotransmission and that associated with learning and memory.

  **Synaptic plasticity** is a change in the strength of connections between neurons.

  Long-lasting enhancement of synaptic transmission is known as **long-term potentiation** – thought to underlie learning and memory.

- Overactivation of these receptors causes \(\text{Ca}^{2+}\)-mediated cell death and this mechanism is responsible for the morbidity associated with stroke (this phenomenon is also known as **excitotoxicity**).
Pharmacology of the NMDA receptor

- Schematic illustration of an NMDA Receptor, with its Binding Sites

- **Glutamate binding site**, to which NMDA also binds
- **Glycine** is a co-agonist, referred to as the strychnine-insensitive glycine binding site to distinguish it from the glycine receptor found in the spinal cord.
- Glycine and glutamate must be present for the channel to open.
- Integral cation channel is highly permeable to \( \text{Ca}^{2+} \)
- Under normal physiological conditions the channel is blocked by \( \text{Mg}^{2+} \). This is an unique property of NMDA receptors. This blockage is voltage dependent and is relieved during depolarization.
- NMDA receptor is known as a **coincidence detector**, many events have to occur simultaneously for the NMDA receptor to allow \( \text{Ca}^{2+} \) into the cell.
- A large family of compounds are known as **channel blockers**. These compounds bind within the ion channel e.g. ketamine, phencyclidine (PCP).
(C) Glutamate transporter

- 12 transmembrane domains
- Specifically clears glutamate from the synapse
- Many subtypes exist (this seems to be true of GABA and glycine transporters as well)
3.5 Neuroactive peptides

- Differ from monoamine and amino acid transmitters in that they arise from prohormones which are cleaved to biologically active peptides.
- Synthesized from mRNA, translated into an amino acid sequence and packaged into vesicles in s.e.r., then transported to nerve terminals.
- Function alone but also coexist with other neurotransmitters.
- No reuptake mechanisms have been described.
- Homology in peptide sequences has led to organization into families. e.g. Opioids (enkephalins, dynorphins), Neurohypophyseal hormones (vasopressin, oxytocin), Tachykinins (Substance P), Insulin (insulin, insulin-like growth factors).

Opioid peptides and Opioid Receptors

Opioid receptors are activated by the products of 3 endogenous opioid peptide genes:

1. Proenkephalin yields: Met and Leu Enkephalin
2. Prodynorphin yields dynorphin
3. Proopiomelanocortin (POMC) yields β-endorphin

- Three well-characterized subtypes of opioid receptor: **μ** (μ), **δ** (δ) and **κ** (κ).
- Found in spinal cord, peripheral sensory and autonomic nerves and widely distributed in brain.
- Mu-receptors are largely responsible for the analgesic and unwanted effects of morphine (a selective μ opioid receptor agonist).

All are G-protein coupled to inhibition of adenylate cyclase.
3.6 Histamine

Present in brain in relatively small amounts (compare peripheral tissue)
Synthesized in brain, neurons are mainly confined to hypothalamus
Released in a circadian rhythm (active during waking hours)
$H_1$, $H_2$ and $H_3$ receptors – G protein coupled widely expressed in brain
$H_1$ antagonists are strongly sedating (and antiemetic)
$H_2$ antagonists like cimetidine, ranitidine have poor penetration of the
BBB – low CNS side effects

3.7 Adenosine

Four adenosine receptors: $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$ receptors – all G protein
coupled
Nonselective antagonists = xanthine, theophylline, caffeine all have
low micromolar affinity
Agonists under development for arrhythmia, cardio and cerebro-
protection, hypotension and anti-psychotics
Antagonists under development for asthma, Parkinson’s Disease, renal
disease, inflammation, cognition