Events at the synapse

- Arrival of electrical potential
- Opening of voltage-gate Ca++ channels = influx of Ca++
- Interaction between Ca++ and vesicular proteins leading to exocytosis
- Release of neurotransmitter into the synaptic cleft
- Binding of neurotransmitter on the post-synaptic cell resulting in an inhibitory or excitatory effect
More on Exocytosis

1 Opening of voltage gate Ca++ channels (arrows) causes synaptic vesicles to be pulled into contact with the presynaptic membrane by actin filaments. Matching pairs of fusion protein macromolecules (FPMs) in the vesicle and presynaptic membrane are aligned.

2 The FPMs separate (outward arrows), permitting transmitter molecules to enter the synaptic cleft.
Events at the synapse

3 Vesicle membrane is incorporated into the presynaptic membrane while transmitter is activating the specific receptors.

4 Clathrin molecules assist inward movement of vesicle membrane. Dynamin molecules *(green)* assist approximation of FPM pairs *(inward arrows)* and pinch the neck of the emerging vesicle.

5 The vesicle is now free for recycling.
Neurotransmission and metabolism

Each neurotransmitter binds to a different receptor type that has an affinity for that ligand. Ligand metabolism can occur via enzymatic breakdown, reuptake or diffusion.
Post-synaptic excitation and inhibition

- Pre-synaptic cells can be inhibitory (eg GABA) or excitatory (eg Glutamate)
- Post-synaptic cells can have numerous pre-synaptic cells (inhibitory and excitatory)
- Inhibitory neurotransmitters cause influx of Cl⁻ and efflux of K⁺
- Excitatory neurotransmitters cause the influx of Na⁺ or sometimes Ca²⁺

  - Local depolarization is an excitatory postsynaptic potential (EPSP)
  - Local hyperpolarization is an inhibitory postsynaptic potential (IPSP)
Presynaptic Facilitation and Inhibition

1: Interneuron
2: Presynaptic neuron
3: Postsynaptic neuron

**A** shows when an interneuron acts to facilitate the presynaptic neuron thus an increase in neurotransmitter is released into the synapse that will act on the postsynaptic neuron.

**B** shows when an interneuron acts to inhibit the presynaptic neuron thus decreasing its release of neurotransmitter into the synapse.
Membrane bound receptors

- Receptors are typically named for the transmitter/modulator to which they bind
- Produce either direct or indirect actions:
  - Act directly: when the receptor and ion channel make up a single functional unit
  - Act indirectly: using a cascade of intracellular molecules to activate ion channels or cause other changes within the postsynaptic neuron
- Postsynaptic receptors use three mechanisms to transduce signals: Directly opens ion channels (fast synaptic transmission) or indirectly: opens ion channels (slow synaptic transmission). Activates a cascade of intracellular events, including activating genes (slow synaptic transmission).
- Types include ligand gated (ionotropic) and G-protein coupled (metabotropic)
G Protein vs Ligand Gated

- **Receptor channels**
  - Briefly opens ion channels that span membrane
  - Local depolarization or hyperpolarization of membrane

- **G-protein mediated receptors**
  - By α chain or second messenger
  - Prolonged opening of ion channels that span membrane
  - Local depolarization or hyperpolarization of membrane
  - Increase rate of synthesis of specific cellular products
  - Affect cell metabolism and other processes

- **Second messenger**
  - Activate genes
  - Modulate calcium levels inside the cell

Image: (Lundy-Ekman, 2013)
Intracellular Events – GPCRs

A. Neuromodulator binds to receptor, activating a G-protein-coupled receptor (GPCR).

B. Adenyl cyclase is activated, converting GDP to GTP. Bound receptor and G-protein.

C. GTP-bound alpha subunit activates adenyl cyclase, converting ATP to cAMP.

First messenger + receptor and G-protein → Effector enzyme → Second messenger → Effect
Preparation Questions for Quiz

With the following questions use your textbook and read chapter 2 and 3 to find the answers

Retrograde transport:
A. Recycles substances from the axon back to the soma.
B. Moves neurotransmitters from the dendrites to the cell body.
C. Moves substances from the soma toward the axon terminal.
D. Moves neurotransmitters across the synaptic cleft.
E. Moves information from astrocyte to astrocyte.

Afferent neurons convey information:
A. Between interneurons.
B. From the CNS to skeletal muscles.
C. From peripheral receptors to the CNS.
D. Between the soma and presynaptic terminal.
E. From the CNS to smooth muscles.

The resting membrane potential is:
A. The same as the membrane equilibrium potential.
B. The voltage difference across a neuron's cell membrane, maintained by an unequal distribution of one specific ion.
C. Maintained by active transport of sodium ions (Na+) and potassium ions (K+) and passive diffusion of Na+, K+, and chloride ions (Cl−) through the cell membrane.
D. Typically measured at +70 millivolts (mV) because the intracellular environment is more positively charged than the extracellular environment.
E. Created by a more negative charge inside the membrane than outside because Na+ is continuously moved inside the cell membrane by an active transport pump.
Depolarization occurs when:
A. The membrane potential becomes less negative than the resting membrane potential.
B. The membrane potential becomes more negative than the resting membrane potential.
C. Cl\(^-\) influx hyperpolarizes the membrane.
D. The presynaptic terminal of a neuron is inhibited by another neuron.
E. All membrane channels are closed, preventing the influx of Na\(^+\).

Local potentials:
A. Are either receptor or synaptic potentials.
B. Spread passively only a short distance along the cell membrane.
C. Result from stimulation of sensory receptors or from the binding of a neurotransmitter with chemical receptor sites on a postsynaptic membrane.
D. Both A and B
E. A, B, and C

Which of the following change the electrical potential across the cell membrane?
A. Activation and opening of ligand-gated K\(^+\) channels.
B. Activation and opening of modality-gated Na\(^+\) channels.
C. Activation and opening of voltage-gated Cl\(^-\) channels.
D. Leak channels, which allow continuous diffusion of small ions.
E. All of the above

Propagation of an action potential along an axon is dependent on a(n):
A. Complete myelination of the axon by glial cells.
B. Anterograde diffusion of the electric potential with active generation of new potentials.
C. Rapid repolarization associated with passive diffusion of Cl\(^-\).
D. Retrograde diffusion of the electrical potential.
E. Na\(^+\)/K\(^+\) pump moving sufficient quantities of Na\(^+\) into of the cell and K\(^+\) out of the cell.

The nodes of Ranvier:
A. Are distributed approximately every 1 to 2 millimeters (mm) along the membrane of the cell axon.
B. Contain a high density of modality-gated K\(^+\) channels for rapid depolarization of the membrane.
C. Contain a high density of voltage-gated Na\(^+\) channels for rapid repolarization of the membrane.
D. Have low membrane capacitance, preventing the accumulation of electrical charge.
E. Are heavily myelinated, which allows for rapid diffusion of an electrical potential.
Neurotransmitters vs Neuromodulators

Neurotransmitters
• Chemicals that convey information among neurons.
• Released by a presynaptic neuron and acts directly on postsynaptic ion channels or activates proteins inside the postsynaptic neuron.

Neuromodulators
• Released into extracellular fluid and adjust the activity of many neurons.
• Alter neural function by acting at a distance away from the synaptic cleft.
• Effects manifest more slowly and usually last longer than those of neurotransmitters, which happen in seconds; the effects last from minutes to days.
Types of Neurotransmitters and Neuromodulators

Fast-acting:
- Acetylcholine: usually excitatory
- Amino acids
  - Glutamate: excitatory
  - Glycine and g-aminobutyric acid (GABA): inhibitory

Slow-acting:
- Amines: dopamine, norepinephrine, serotonin, histamine
- Peptides: substance P, calcitonin gene-related peptide, galanin, enkephalin
- Nitric oxide: diffusible transmitter
Glutamate – The “on switch” for Brain Neurons

The main fast acting excitatory neurotransmitter of the CNS.
Involved in learning and memory

Receptor types:

- **N-methyl-D-aspartate (NMDA)** – ionotropic – involved in long term potentiation (covered in session 3) – Glycine also binds
- **α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)** – ionotropic – excitatory postsynaptically
- **Kainate (KAR)** – ionotropic – excitatory postsynaptically, but can be inhibitory presynaptically
- **Metabotropic receptors** – increases or decreases NMDA activity

(Lundy-Ekman, 2013; Purves et al, 2012; Beck, 2008; Kandel et al, 2013)
Glutamate metabolism

- Glutamine is converted into glutamate via glutaminase
- Excitatory Amino Acid Transporters (EATT in image) transport glutamate into the glial cell and glutamine back into the presynaptic terminal.
- Vesicular glutamate transporters transport glutamate into synaptic vesicles
Glutamate – The “on switch” for Brain Neurons

- Plays a major role in brain development, neuronal migration, differentiation and axon development.
- In the mature nervous system, glutamate is required for stimulus-dependant modifications of synapses such as long term potentiation.
- Neural injury results in the release of high amounts of glutamate into the extracellular space and is toxic to neurons.
- The development of Huntington’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis and stroke related cell death due to persistent stimulation of glutamate receptors leading to neuronal degeneration/necrosis/apoptosis – Glutamate mediated excitotoxicity
Excitotoxicity refers to the ability of glutamate and associated chemicals to destroy neurons by prolonged excitatory neurotransmission – this occurs when glutamate stays for prolonged periods of time in the synaptic cleft.

A) The NMDA receptor is usually blocked by the Mg²⁺ ion. Positive ions are unable to rush in even if glutamate binds to NMDA unless the Mg²⁺ ion is removed by an increase in the cell voltage.

B) The non-NMDA receptor opens as soon as glutamate binds to it. Opening of the non-NMDA receptor allows the entry of positive ions into the cell.

As glutamate continually binds to non-NMDA receptors and allows the entry of positive ions, the cell’s voltage rises. Ultimately, the voltage reaches a certain value that causes the Mg²⁺ ion to be removed from the opening of the NMDA receptor. Ca²⁺ flows through the open NMDA receptor and causes various activities to occur that lead to cell death.
γ-Aminobutyric acid (GABA) –
The “Chillout” Inhibitory Neurotransmitter

- The main fast acting inhibitory neurotransmitter of the CNS
- Involved in sedation, antianxiety, antiseizure, sleep inducing
- Receptor types:
  - $\text{GABA}_A$ – expressed in most neurons – ionotropic Cl$^-$ channels – anti-convulsives and antianxiety drugs are agonists – when activated by a drug this gives the person a sense of euphoria
  - $\text{GABA}_B$ – metabotropic slow acting – linked to ion channels via secondary messenger systems
  - Expressed in neurons in the hypothalamus, cerebellum and spinal cord
- Antiepileptic drugs increase GABA levels – inhibiting the effect of Glutamate
- Valium increases the action of GABA – causing muscle relaxation
- Alcohol increases the effect of GABA – causing disturbances in the regulation of coordination and balance
GABA – The “Chillout” Inhibitory Neurotransmitter

- GABA is synthesized from glutamate by the enzyme glutamic acid decarboxylase, which requires cofactor pyridoxal phosphate (derived from Vitamin B6).
- **Glucose** is the precursor to GABA synthesis as glutamate is produced via the citric acid cycle (*pyruvate* or *glutamine* can also be precursors).
- **B6 deficiency** leads to reduced GABA in the brain and seizures.
- GABA removal is similar to glutamate with GAT1 removing GABA from synaptic cleft
- Once back in the neuron, GABA mainly gets converted to succinate – metabolised back into citric acid cycle. This degradation occurs via GABA aminotransferase and succinic semialdehyde dehydrogenase (mitochondrial enzymes)
Glycine – 2nd Most Common Inhibitory Neurotransmitter

- Mainly inhibitory action via spinal interneurons
- Ionotropic receptor type (GlyR)
- Also binds to NMDA receptor (different action to GlyR)
- Glycine is derived from serine in mitochondria via mitochondrial serine transhydroxymethylase
- Glycine production in mitochondria is regulated by SHMT2\textsuperscript{17} and GCAT\textsuperscript{18}
Acetylcholine (ACh) – The Memory and Motor Neurotransmitter

• Excitatory neurotransmitter of preganglionic and postganglionic parasympathetic neurons and preganglionic sympathetic neurons.
• Inhibitory neurotransmitter in some postganglionic cells.
• Excitatory neurotransmitter of alpha motor neurons at the neuromuscular junction.
• In the CNS, it is involved in memory formation, cortical processing, arousal and maintaining attention.
• Receptor types:
  – **Nicotinic** – ionotropic Na⁺ channel – neuromuscular junction, preganglionic autonomic neurons and some in the CNS.
  – **Muscarinic** – metabotropic slow acting – linked to ion channels via secondary messenger systems - parasympathetic autonomically innervated visceral organs, on the sweat glands and piloerector muscles and both post-synaptically and pre-synaptically in the CNS.
Acetylcholine (ACh) – The Memory and Motor Neurotransmitter

**Effects of acetylcholine**
- Initiates skeletal muscle contraction – alpha motor neurons
- Slows heart rate – Vagus nerve (sinoatrial node)
- Causes bronchoconstriction – Vagus nerve (Reissessen smooth muscle cells contraction)
- Pupillary constriction – oculomotor nerve (Edinger-Wesphal nucleus to ciliary ganglia = pupil constriction via iris sphincter muscle)
- Arousal and sleep/wake cycle – Pedunculopontine and laterodorsal tegmental pathways
- Reward system – basal forebrain to prefrontal cortex and brainstem to ventral tegmental area
- Involved in learning and memory – hippocampal pathways

**NOTE:** will be discussed more in motor and autonomic nervous system sessions
Acetylcholine (ACh) – The Memory and Motor Neurotransmitter

• ACh is synthesised by combining **Acetyl Coenzyme A** (via mitochondria) and **Choline** (via choline transporters) and catalysed by choline acetyltransferase (CAT)

• Choline can also be synthesised by the break down of **phosphatidylcholine** (derived from the inner cell membrane)

• Acetylcholine is degraded to acetate and choline via **acetylcholinesterase**

*Image: (Purves et al, 2012)*
Dopamine – The Motivation and Addiction Neuromodulator

- Main functions are involved in movement coordination, motivation, reward and reinforcement.
- Main locations of action – ventral striatum, hippocampus, amygdala, basal ganglia and frontal lobe.
- Main projections start at the ventral tegmental area and substantia nigra.
- Receptor types:
  - **D1** – Excitatory GPCR – Knockout receptor = hyperlocomotion and aphagia – locations: caudate/putamen, nucleus accumbens, olfactory tubercle and cortex.
  - **D2** – Inhibitory GPCR – main antipsychotic receptor – GPCR – Knockout receptor = Parkinsonian-like motor impairments – expressed in caudate/putamen, nucleus accumbens and midbrain.
  - **D3** – Inhibitory GPCR – Knockout receptor = Mild hyperactivity – expressed in olfactory tubercle and hypothalamus.
  - **D4** – Inhibitory GPCR – Knockout receptor = Reduced spontaneous activity and reduced response to novelty – expressed in frontal cortex, medulla, midbrain and nucleus accumbens.
  - **D5** – Excitatory GPCR – Knockout receptor = hyperlocomotion and high blood pressure – expressed in hippocampus, hypothalamus and cortex.

(Lundy-Ekman, 2013; Purves et al, 2012; Beck, 2008; Kandel et al, 2013)
Dopamine – The Motivation and Addiction Neuromodulator

• Areas of action
  – Limbic System: feelings of pleasure and reinforcement of behaviours
  – Caudate head: decision making and goal orientated behaviour
  – Putamen: control of movement
  – Frontal lobe: cognition, particularly planning

• Agonist drugs: amphetamines directly bind to receptors, cocaine blocks reuptake

• Antagonist drugs: antipsychotics (decrease hallucinations, disorganised thought and delusions) block D2 receptors

(Lundy-Ekman, 2013; Purves et al, 2012; Beck, 2008; Kandel et al, 2013)

Image: (Purves et al, 2012)
Dopamine – The Motivation and Addiction Neuromodulator

• Synthesis
  – L-Tyrosine to L-DOPA via Tyrosine hydroxylase
  – L-DOPA to Dopamine via DOPA decarboxylase

• Transport into vesicles via vesicular monoamine transporter (VMAT)

• Removal via active transport into glia or presynaptic cell through Na+ dependant dopamine co-transporters (DAT) – Cocaine and amphetamines inhibit DAT activity

• Glia and neurons can both degrade dopamine via monoamine oxidase (MAO) and catechol Õ-methyltransferase (COMT) – some antidepressants inhibit these enzymes

(Lundy-Ekman, 2013; Purves et al, 2012; Beck, 2008; Kandel et al, 2013)
Noradrenaline – Stress and Focus

Primary excitatory neurotransmitter of the sympathetic nervous system and neuromodulatory effect on the cortex

Main functions:
- Fight or flight response
  - Increase heart rate and contraction force, bronchiole dilation, inhibition of peristalsis, activates sweat glands, pupil dilation and activates HPA axis
- Mood regulation and maintaining attention to sensory stimuli

Receptor types (all GPCR):
- α1 (excitatory) – heart, liver, cerebellum, cortex, blood vessels, spleen kidney and aorta
- α2 (inhibitory) – pancreas, small intestine, locus ceruleus, hippocampus, liver, thalamus, heart, lung, aorta and olfactory bulb
- β 1-3 (excitatory) – heart, kidney, liver, cortex, smooth muscle and hypothalamus

Synthesis: L-Tyrosine to L-DOPA via Tyrosine hydroxylase then L-DOPA to Dopamine via DOPA decarboxylase then Dopamine to Norepinephrine via Dopamine β-hydroxylase

(Lundy-Ekman, 2013; Purves et al, 2012; Beck, 2008; Kandel et al, 2013)
Serotonin – 5-Hydroxytryptamine (5-HT)

- A main modulatory neurotransmitter of the CNS and PNS
- Antinociceptive in the CNS and pronociceptive in the PNS
- Involved in regulation of sleep, mood, arousal and appetite
- Acts on neurons in a majority grey matter in the brain and in the spinal cord

- 5-HT is involved in the hypothalamic control of the anterior pituitary gland – particularly increasing the blood levels of adrenocorticotrophin and corticotrophin-releasing hormone
- Increase synthesis of 5-HT induces an increase in prolactin and growth hormone in plasma
- Serotonin appears to be involved in the regulation of circadian rhythms
- Receptors 5-HT1-3

Image: (Purves et al, 2012)
Neuromodulatory Control of Consciousness
## Distribution of Neuromodulators in the Brain

<table>
<thead>
<tr>
<th>Transmitter</th>
<th>Serotonin</th>
<th>Norepinephrine</th>
<th>Acetylcholine</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>Raphe nuclei</td>
<td>Locus coerules and medial reticular zone</td>
<td>Pedunculopontine nucleus</td>
<td>Substantia nigra and ventral tegmental area</td>
</tr>
<tr>
<td>Limbic</td>
<td>Amygdala</td>
<td></td>
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<tr>
<td></td>
<td>ventral striatum septal area</td>
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<tr>
<td>Basal forebrain</td>
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<tr>
<td>Neocortex</td>
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<tr>
<td>Thalamus</td>
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<tr>
<td>Striatum</td>
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<tr>
<td>Cerebellar cortex</td>
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</tbody>
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Image: (Lundy-Ekman, 2013)
Neuropeptides

• Endorphins – Opioid Neuropeptides
  – Modulate nociceptive signalling
• Substance P
  – Stimulates nerve endings at the site of injury and then in the CNS.
  – Acts as a neurotransmitter carrying information from the spinal cord to the brain.
  – Strongly implicated as a neuromodulator in the pathophysiologic response to pain syndromes, which involves the perception of normally innocuous stimuli as painful.
# Neurotransmitter Cycles

<table>
<thead>
<tr>
<th>Small-molecule</th>
<th>Neuropeptides</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Synthesis</strong></td>
<td>In cell bodies in the form of pre-(pro)-peptide and later cleaved into active transmitter</td>
</tr>
<tr>
<td>In presynaptic terminal by specific enzymes</td>
<td></td>
</tr>
<tr>
<td><strong>Packaging and release</strong></td>
<td>Large vesicles away from active zone</td>
</tr>
<tr>
<td>Small vesicles near active zone in pre-synaptic terminal.  Low frequency of neural activity sufficient for release. Docked vesicles released with influx of even low levels of Ca++ Fast acting</td>
<td>High frequency of neural activity required for release. Vesicles only released with influx of high levels of Ca++ Slow acting</td>
</tr>
<tr>
<td><strong>Removal</strong></td>
<td>Passive diffusion</td>
</tr>
<tr>
<td>Passive diffusion</td>
<td>Enzymatic degradation</td>
</tr>
<tr>
<td>Reuptake into presynaptic terminal</td>
<td></td>
</tr>
<tr>
<td>Uptake into astrocytes</td>
<td></td>
</tr>
<tr>
<td>Enzymatic degradation</td>
<td></td>
</tr>
</tbody>
</table>

Image: (Lundy-Ekman, 2013)
References

Image References

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