Neurobiology SS22 Abstract

What is a connectome?
Opposed to genomes, which stand for all genetic information of an organism, connectome is all neuronal information of an organism. *A connectome is a comprehensive map of neural connections in the brain, and may be thought of as its "wiring diagram". The foundation of human cognition lies in the pattern of dynamic interactions shaped by the connectome.* Therefore, it is the goal of all scientists among us to learn as much as possible about our connectomes, as knowing more of our connectomes means knowing more of ourselves.

This is a collection of multiple concepts and foundations from the Neuroscience realm, according to the lecture Neurobiology SS22 at the University of Vienna. Direct citations in these lecture notes are written in italic.

Lecture Description: *Neurobiologische Forschung bietet faszinierende Einblicke in die vielfältigen Aspekte der Gehirnfunktionen. Das Ziel der Vorlesung ist es, den Studierenden im Masterprogramm einen Überblick über die Organisation, Entwicklung und Funktion des Nervensystems zu geben. Beginnend mit einen historischen Überblick zur Gehirnforschung sowie einer Besprechung des Aufbaus von Gehirnen und ihren zellulären Einheiten, liegt der erste Schwerpunkt auf der Generierung, Weitergabe und Verarbeitung neuronaler Information (z.B Membraneigenschaften, Ruhe- und Aktionspotential, Elektrische-/Chemische Synapse, Ionenkanäle, Neurotransmitter und Second Messenger). Aufbauend auf diese zellulären Grundlagen werden dann komplexere Phänomene der neuronalen Plastizität und Mechanismen bei Lern- und Erinnerungsprozessen besprochen. In einem zweiten Teil sollen die molekularen Grundlagen der Gehirnentwicklung sowie ursächliche Zusammenhänge bei neuronalen Erkrankungen vorgestellt werden. Ausgehend von Entwicklungsprozessen zur Generierung und Spezifizierung der unterschiedlichen Nervenzellen im zentralen und peripheren Nervensystem wird die Frage diskutiert, wie sich einzelne Neurone zu funktionellen Netzwerken verbinden. Weiterführend sollen therapeutische Ansätze für die Regeneration des Nervensystems sowie verschiedener Arten neurodegenerativer Erkrankungen (z.B. Alzheimer, Parkinsons Disease) erläutert werden. Abschließend werden neuere Erkenntnisse zu der zentralen Frage vorgestellt, wie neuronale Schaltkreise komplexe Verhaltensantworten generieren.*

Created in collaboration <3 as all things should be

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1 Neuron Morphology

1.1 Introduction

Our brain and nervous system constantly process electrical or chemical signals, which essentially capture information obtained from senses and from the outside world. These are passed from neuron to neuron over synapses, a structure formed of pre- and post-synaptic residing most often between the axon terminals of one neuron and the dendrites of another neuron.

There are broadly three different types of neurons based on their functions[1]:

- **Sensory neurons**: respond to stimuli from sensory organs (eyes, ears, skin,..) and send those signals to brain and/or spinal cord.
- **Motor neurons**: respond to signals from brain and/or spinal cord to controls muscles and glands
- **Interneurons**: connect neurons to other neurons within the same region in the brain and/or spinal cord.

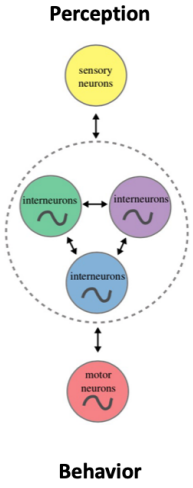

Figure 1.1: Neuron Types

A neural circuit is a network consisting of multiple connected neurons. Even though no two neurons are alike[2], they all share a similar morphology which is the topic of this chapter. We will discuss the general structure of the neuron and also the function of each component.

A neuron consists of a Soma (cell body), Dendrites and an Axon with the Growth Cone where the Axon Terminals are located.

1.2 Soma

Soma is the cell body or the non-process portion of a neuron. It contains the nucleus, which is the source of most of the RNA that is produced in the neuron. This is important to know as most proteins, that the neuron needs, are synthesized by mRNA, which means that if the axon is very long the proteins have to travel a long distance from the nucleus to e.g. the axon terminals.

[1] To understand fundamental principles of brain functions, neuroscientists can label neurons with a fluorescent protein, such as GFP - Green-Fluorescent-Protein - that reports neuronal activity.

[2] From Scientific American: *"[...] somatic mosaicism [which is that not all somas in an organism have the same genotype] is the rule, not the exception, with every neuron potentially having a different genome than those to which it is connected. A primary cause of somatic mutations has to do with errors during the DNA replication that occurs when cells divide"*

1.2.1 Axon Hillocks

Axon Hillocks are the part of the Soma that connects to the Axon. *The axon hillock is the last site in the soma where membrane potentials propagated from synaptic inputs are summated before being transmitted to the axon. The axon hillock has the greatest density of voltage-dependent sodium channels* (what exactly this means we will cover later). *The action potential initiation zone (trigger zone) is thought to be right between the axon hillock and the initial (unmyelinated) segment of the axon.*

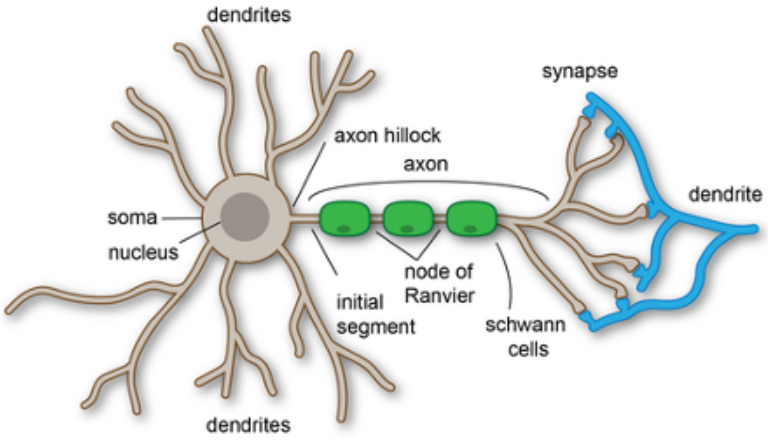

Figure 1.2: Structure of a Neuron

1.3 Dendrites

Dendrites extrude from the Soma kinda like a slime mold in branches. They are filaments (fadenförmige Zellstrukturen, consisting of proteins). Dendrites are the metaphorical ears

of a neuron as they receive information from another neuron. They play a critical role in integrating synaptic inputs and in determining the extent to which action potentials are produced by the neuron as the postsynaptic part of synapses is often located on dendrites.

Dendritic arborization, also known as dendritic branching, is a multi-step biological process by which neurons form new dendritic trees and branches to create new synapses. The morphology of dendrites such as branch density and grouping patterns are highly correlated to the function of the neuron.

Dendrites tend to get narrower with distance to the soma, which means that the fibre diameter at the end (=distal dendrite) is smaller and as such the resistance higher, the capacitance lower, the velocity of the conductance being lower and the voltage lower. On the other hand proximal dendrites have a lower resistance and higher capacitance.

1.3.1 Tiling

Neuronal tiling is a phenomenon in which multiple arbors of neurons innervate the same surface/tissue in a nonredundant and tiled pattern that maximizes coverage of the surface while minimizing overlap between neighbouring arbors. Hence, dendrites of the same neuron spread out by avoiding one another (selfavoidance). Moreover, dendrites of certain

types of neurons such as class III and class IV dendritic arborization neurons avoid dendrites of neighbouring neurons of the same type (tiling)[3], whereas dendrites of different neuronal types can cover the same territory (coexistence).

1.4 Axons

Axons (nerve fibres) are essentially long cylinders filled with axoplasm which is the fluid containing ions.

1.4.1 Myelin Sheath

Axons are myelinated in mammals, which leads to a better Action Potential conduction. But not all neurons need it. For neurons that are short or if the fibre is thin, the myelin does not make a difference. But for long distances, myelin contribute to a fast information transfer without loss of signal

Glia cells, so-called Schwanzellen, wrap the myelin only at the end of the axon when the axon is growing that's why the myelin cannot be reproduced when damaged.[4]

1.4.2 Nodes of Ranvier

Gaps in the myelin sheath are called nodes of Ranvier.

1.4.3 Growth Cone

The growth cone drives axon growth. *The morphology of the growth cone can be easily described by using the hand as an analogy. The fine extensions of the growth cone are pointed filopodia known as microspikes. The filopodia are like the "fingers" of the growth cone; they contain bundles of actin filaments (F-actin) that give them shape and support. Filopodia are the dominant structures in growth cones, and they appear as narrow cylindrical extensions which can extend several micrometres beyond the edge of the growth cone. The filopodia are bound by a membrane which contains receptors, and cell adhesion molecules that are important for axon growth and guidance.*

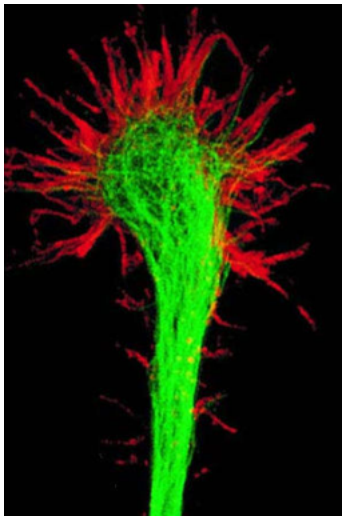

Figure 1.3: fluorescently-labeled growth cone. F-actin (red) and microtubules (green)

We will dedicate an entire chapter (2) to Axonal Growth and Axonal Guidance + Pathfinding.

[3] this tiling mechanism can be tested experimentally by removing/killing the neuron
[4] People with MS multiple sclerosis don't have that myelination due to inflammation.

1.4.4 Axon Terminals

The axon terminal is found at the end of the axon farthest from the soma and contains synapses. Synaptic boutons are specialized structures where neurotransmitter chemicals are released to communicate with target neurons.

1.4.5 Axonal Branching

1.4.6 Axonal Transport and Microtubules

As we established in the discussion of the nucleus, the traveling distance for the proteins synthesized by mRNA from the nucleus to a desired destination in the axon can be very long, which is why these proteins are usually carried by motor proteins, which can move freely from Soma to Axon Terminals along a structure called Microtubule. This microtubule, made out of tubulin, covers the length of the axon.

1.5 Synapses

When we talk about synapses we usually mean chemical synapses. A synapse usually consists of a presynaptic part, which is located at the axon terminals of one neuron, and a postsynaptic part, which is located on the dendrites of another neuron. Synapses are the microscopic gaps between two connected/communicating neurons. One such gap is sometimes also called the synaptic cleft.

1.6 Membrane

The entire neuron is separated from the exterior environment by a plasma membrane. The membrane consists of lipids which are great for insulating, meaning that electrical current cannot flow freely in the membrane. However, at some positions there are transmembrane proteins that allow electrically charged ions to flow across it (e.g. from the extracellular to the cytoplasmic side).

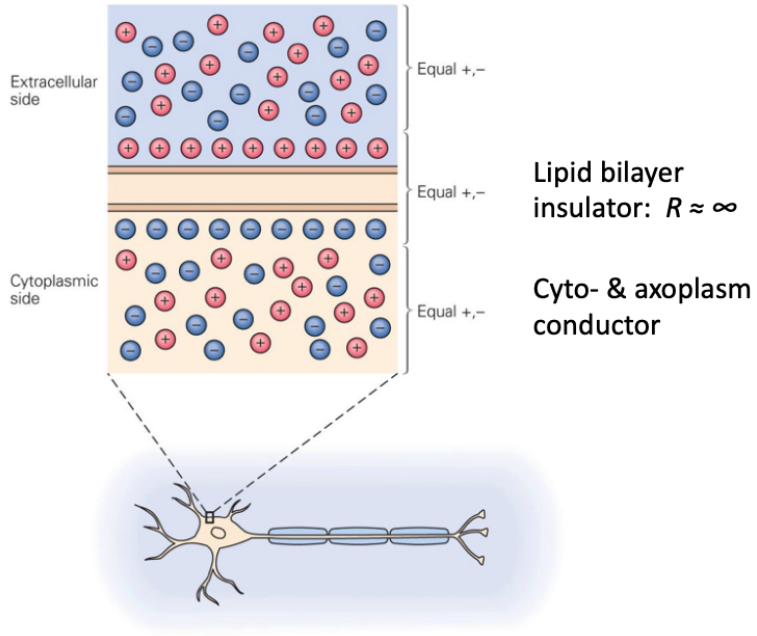

Figure 1.4: Ionic Gradients across Membrane

These structures can be:

- **Ion Pumps** which are ion transporters, transmembrane proteins that **actively** push ions across the membrane.
- **Ion Channels** which are pore-like proteins in the membrane allowing ions **passively** to go through them.[5]

Ion transporters (pumps) differ significantly from ion channels. Channels are pores that run through the membrane, whereas transporters are proteins that must change shape to switch which side of the membrane it is open to, because of this transporters are much slower at moving molecules than channels.

Before we delve into the anatomy of ion channels, let´s define some terms we will need:

1.6.1 Membrane as Electrical Circuit

From an electrotechnical perspective, a membrane can be simulated by an electric circuit consisting of a voltage source, capacity (capacitance) and a nonlinear resistance.
We measure an electrical potential (V) by the amount of energy needed to move a charge (Q) against it. Current(I) is the net movement of charge (Q) per unit time. Conductance (g) or resistance (R) is the ease/difficulty with which an electric current passes through a conductor. The insulator with the conducting material wrapped around it is equivalent to the lipid bi-layer which is the membrane.

[5] Although ion channels are often seen as pores, they are more than a simple pore as ions also interact with the channels

Membrane Potential

The membrane potential is the voltage between the extracellular and the cytoplasmic side, as there are different ionic concentrations on both sides. This voltage exists even in a resting state (polarized) where no depolarization happens. In this case the interior is 70mV more negative than the extracellular fluid.[6]

Membrane Resistance

is highly nonlinear because of the activity of the ionic channels embedded in the membrane. We can see a membrane potential decay in dendrites or axons with distance as signals or currents are passed down due to (axial) resistance.

Membrane Capacitance

The capacitance of a cell membrane is essentially the amount charge that can be stored and depends on the membrane thickness. It is proportional to the cell surface area. Increased capacitance leads to an increase of charge required to depolarize the membrane. This slows conduction, meaning that the membrane capacitance determines the propagation speed of signals/information. We get a delay in the response to current stimulations/injections. This was proved with the patch clamp experiment.[7]

1.6.2 Ion Channels

Ions

An ion is an atom or molecule with an electrical charge. If the ion is in water, it has a hydration shell, which determines the effective size and charge of the ion.

Both on the extracellular and the intracellular side, which is fluid, lots of ions move around. Ions like sodium, potassium, chloride, calcium, magnesium and so on. These ions have **electrochemical gradients** which determine the direction and the speed of them moving across the membrane. The gradient consists of two parts, the chemical gradient and the electrical gradient (difference in charge across a membrane).

When there are unequal concentrations of an ion across a permeable membrane, the ion will move across the membrane from the area of higher concentration to the area of lower concentration through simple diffusion. Unless an ion pump does the transportation, this can only happen if the ion channels are open (opening the gate).

[6] This membrane potential can be calculated with the Nernst Potential (Equation) if only one type of ion is involved. And for mixed ionic concentrations with the Goldman Equation -> for this sodium and potassium concentrations need to be known.

[7] Patch Clamp Experiment: In the 70s to get current recordings of single channels, the patch clamp technique was developed. A micropipette is pressed against the membrane of a cell creating a seal such that only few channels are open, and efflux/influx can be easier recorded. Also, through the pipette, different solutions containing ions can be in contact with the cell. These techniques were used to find both the open times and the resulting current strengths of single channels under the control of different intra- and extracellular solutions and under the influence of transmitters

Gating

Some ion channels only allow specific ions to pass based not only on size but also chemical composition of the pore. On a low-level the narrowest part of an open transmembrane protein/pore, which is called Selectivity Filter (SF), are lined with amino acid residues that interact with the passing ions. Whether a channel opens or closes depends on:

- Membrane potential/voltage. These are the **voltage-gated channels**
- Binding to a ligand or neurotransmitter. These are the **ligand-gated ion channels**
- changes in **temperature**
- **stretching** or deformation of the cell membrane
- **phosphorylation** - adding of phosphate group to the ion channel
- interaction of other molecules (**G-Protein**) in the cell

The gating dynamics all contribute to the overall conductivity of the neuron. In general, one can say that the opening and closing of a gate is a stochastic process. We will discuss in more detail how this ion flow and the transportation of signals works in Chapter 5.

Gap Junction

If two cells are connected, a gap junction forms the channel that lies over both cell membranes such that ions can pass from one cell to another. As such, gap junctions are basically the communication device (pore) between two cells. It is also known as an electrical synapse, as it works like a synapse but merely with ions.

The gap junction can be rectifying, meaning that outward flow for an ion is faster than inward flow, meaning we have an activation in one direction from pre-synaptic to post-synaptic and a co-inhibition in the other direction, or vice versa. The junction is non-selective, meaning all types of ions can pass, and the synaptic transmission is fast.

There are two types of gap junctions:

- homotypic: same channel composition on both sides
- heterotypic: different channel composition on both sides

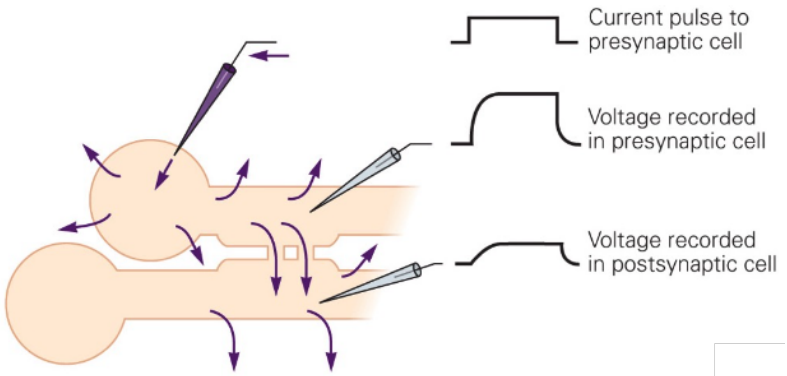

Figure 1.5: voltage differences between both gap junction sides

Depending on the compositions, upon passing a current from the pre-synaptic to the post-synaptic, the voltage can vary on both sides, as can be seen in Figure 1.5, being much lower in the postsynaptic cell, which is very ineffective. In some cases, the gap junction current might not evoke an action potential in the coupled cell.

2 Axon Guidance

Axonal Guidance or Pathfinding is the process in which neurons send out their axon or more specifically the axon growth cone to reach and connect the target neuron. This process happens mainly in neurogenesis - the production of neurons, as such it is a main topic in embryonic development, although neurogenesis can also happen later in life.

2.1 Introduction

An axon follow precise paths that are recognized by the axon growth cone which lies at the tip of the axon. For the growth cone there is no intrinsic programming of the choice where to go but at the growth cone lie many receptors that respond to signals - also called guidance cues - from the extracellular environment that instruct the growth cone in which direction to grow. These signals can be repulsive or attractive, meaning the growth cone grows towards the guidance cue or away from it.

The motility of the growth cone is achieved through the coordinated behaviors of microtubules and the actin cytoskeleton. Axon extension, retraction, and turning in response to signaling cues require changes in the distribution of microtubules within the growth cone. For the axon to turn, microtubules from the central domain must penetrate the transition zone to invade the peripheral domain, preferentially on the side of the growth cone in the direction of the turn.

As we saw in Chapter 1, the cytoskeleton of the growth cone consists of microtubules and actin. Together they can be seen as the "engine" in the context of Axonal Guidance. The "steering wheel" is given by the guidance cues that signal the microtubules and the actin at the choice points or the "intersections" in which direction they should grow.

2.2 Choice Points

Growth cone stretches out its filopodia and continues to grow in one direction, usually in the direction in which more filopodia have been formed. These "fingertips" contain actin. The microtubules accumulate at a "choice point" in a certain direction and extend there, their asymmetric arrangement suggests the direction. The filopodia follow the asymmetry of the microtubules.

2.3 Pioneer Neurons

It is thought that axons of pioneer neurons, pioneer axons, serve as a pathway for additional neurons that develop later in the embryo and project their axons to the appropriate target, thus pioneer neurons are followed by other neurons. If they stop growing all following neurons also stop, which means that the follow neurons are dependent on the pioneer

neurons. They also need to always find the right track as many neurons can be followed. A challenge for the pioneer neuron is that it always has too look for guidepost cells.

2.4 Guidepost Cells

Guidepost cells are cells which assist in the subcellular organization of both neural axon growth and migration. They act as intermediate targets for long and complex axonal growths by creating short and easy pathways, leading axon growth cones towards their target area.

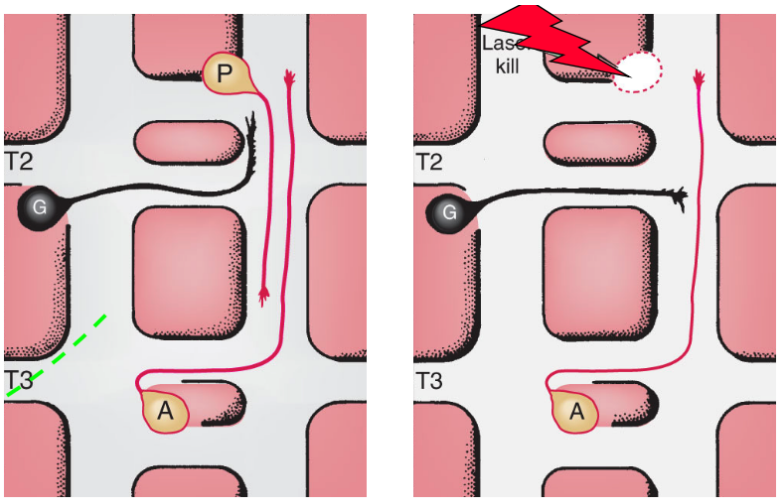

Figure 2.1: Labeled Pathway through Guideposts

To qualify as a guidepost cell, neurons hypothesized to be influenced by a guidance cell are examined during development. To test the guidance cell in question, neural axon growth and migration is first examined in the presence of the guidance cell. Then, the guidance cell is destroyed to further examine neural axon growth and migration in the absence of the guidance cell. If the neuronal axon extends towards the path in the presence of the guidance cell and loses its path in the absence of the guidance cell, it is qualified as a guidepost cell.

Another way would be to translocate the guidepost cell.

2.5 Guidance Cues

Growth cone receptors detect the presence of axon guidance molecules such as Netrin, Slit, Ephrins, and Semaphorins.

2.5.1 Commissural Midline Crossing

One choice point, or intersection, where an axon has to make the decision where to go is the commissural midline, where axons have to go from one brain hemisphere to the other.

[1]

This crossing is done via a Netrin-mediated attraction towards the floor plate, which is located on the ventral midline of the neural tube (the embryonic precursor of the CNS), where Netrin is released.[2] These Netrins are picked up by the receptors DCC on the growth cone of the pioneer neuron. After being attracted by the midline, the commissural neurons then need to leave the midline and go to the other side which is mediated by repulsive cues from the protein Slit. Slit at the midline activates Robo receptors on the axons, thereby repelling them out of the midline into distinct longitudinal tracts on the contralateral side of the CNS.

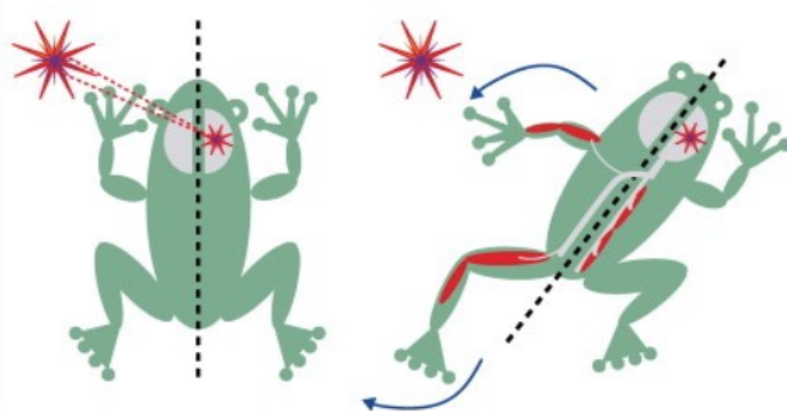

Figure 2.2: Limbed Vertebrate Escapee leveraging midline crossing

The need for midline crossing is suspected to stem from the fact that limbed vertebrates attempt to escape a left-side threat by extending left limbs, pushing on the ground to turn to the right, which can be seen in Figure 2.2. Here the coordination of left-right hemisphere is needed.

2.5.2 Visual Map Theory by Cajal

According to this theory, the function of the optic chiasm, which is the part where optic nerves cross, is to repair the retinal field image on the visual cortex. *The pupil in the vertebrates' eyes inverts the image on the retina, so that the visual periphery projects to the medial side of the retina. By the chiasmatic crossing, the visual periphery is again on the outside.*

[1] This midline crossing is important especially for human vision and motor control, where the left and right side need to be coordinated. Human Vision: The optic tract is composed of the left and the right optic tract, each of which conveys visual information exclusive to its respective contralateral (=the other side) half of the visual field. Motor control: If you move one side of your body, this midline crossing helps block the other side of the body/inhibit mirrored movement.

[2] Netrins are a class of proteins

2.5.3 Visual Axon Guidance

Rotating Retina

The retinal ganglion cell circuit was first investigated in frogs: Roger Sperry cut the optical nerve of the nerve of the eye and reinserted it twisted by 180°. In a normal frog eye the anterior axons in the retina innervate the posterior end of the tectum. In the course of the regeneration of the neurons, the associated neuron halves reconnected and the animals behaved the animals behaved as if their environment had been turned upside down. They did not learn to hunt to hunt successfully again, even after a long time. Thus, he could conclude that the neural connections between the retina and the optic tectum were rather subject to anatomical than to experiential rules. were subject to. He proposed that a biochemical gradient (or gradients), in the case Ephrins for is responsible for this distinctive space allocation of neurons. The retina in amphibians can regenerate, so the severed nerves can reconnect.

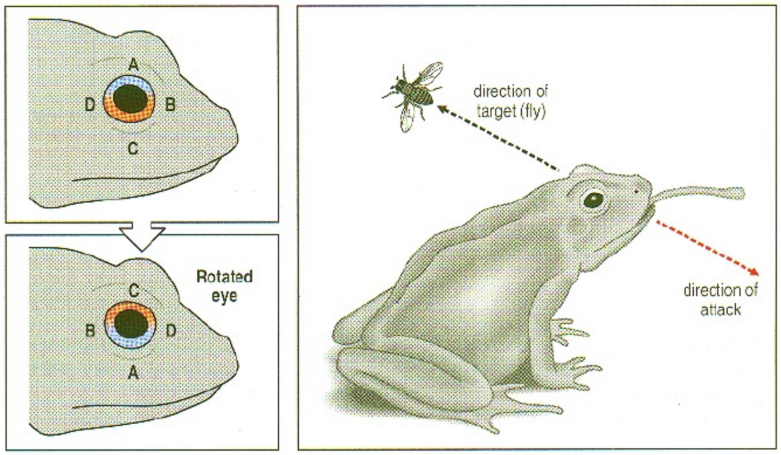

Figure 2.3: Sperry´s frog with rotated eye

Strip Assay

Membranes from the anterior and posterior parts of the tectum were transformed into a striped carpet. When retinal tissue is cultured on the carpet, it can be that temporal retinal axons, but not nasal axons, prefer to grow on the anterior tectum membrane. Once the posterior membranes are treated with heat, formaldehyde, etc., which removes all PI-linked membrane molecules, no preference could be detected anymore. This suggests that the relevant component here is a membrane-bound protein that is repulsive to temporal axons and to which nasal axons are insensitive. The more posteriorly the tectum membrane was sampled, the stronger this repulsive effect could be detected. Thus, a posteriorly increasing gradient of ephrins (ephrin A5 and A2) could be detected. When both ephrin gradients are removed by knockout, a more or less random arrangement of temporal axons is observed. However, since there is no complete chaos in the arrangement, it can be assumed that another factor must be involved.

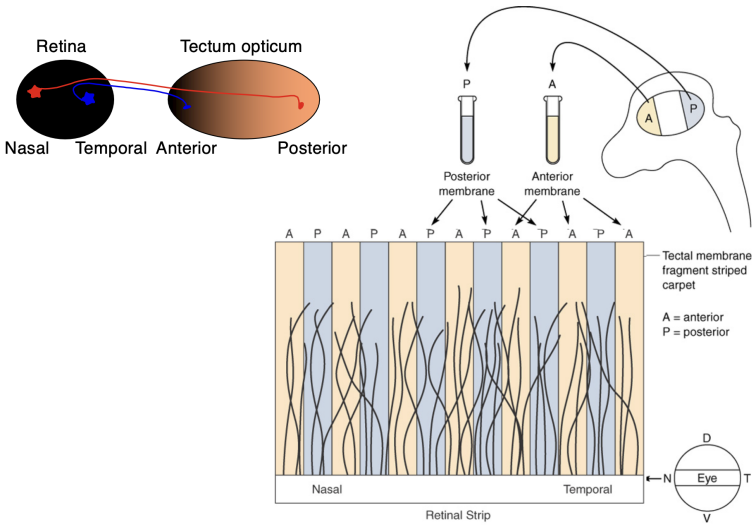

Figure 2.4: Topographic Visual Map & Strip Assay

Topographical Visual Map (Retinotopic Mapping)

There are two mechanisms that lead to the formation of the before mentioned topographic visual map:

- The interaction of the cue Ephrin and the receptor EphA
- Dynamic Growth (Dendritic Pruning)

Ephrin/EphA: The formation of an organized retinotopic map in the superior colliculus (SC) requires the proper migration of the axons of retinal ganglion cells (RGCs) from the retina to specific regions in the SC that is mediated by gradients of Eph and ephrin expression in both the SC and in migrating RGCs leaving the retina.

Dendritic Pruning/Dynamic Growth: One interesting hypothesis is that axons and dendrites are continuously extending and retracting their axons and dendrites. Several factors alter this dynamic growth including the Chemoaffinity Hypothesis, the presence of developed synapses, and neural activity. As the nervous system develops and more cells are added, this structural plasticity allows for axons to gradually refine their place within the retinotopy. This plasticity is not specific to retinal ganglion axons, rather it's been shown that dendritic arbors of tectal neurons and filopodial processes of radial glial cells are also highly dynamic

2.6 Local Translation

Usually proteins needed by the neuron are synthesized by mRNA in the soma and bind to motor proteins that move over the microtubule to the destined place. This can be slow, so what can be done is to send mRNA along the axon such that the proteins can be synthesized locally where they are needed.

3 Neuroplasticity

3.1 Long-Term Potentiation

Long-term potentiation (LTP) is a persistent strengthening of synapses based on recent patterns of activity. These are patterns of synaptic activity that produce a long-lasting increase in signal transmission between two neurons. The opposite of LTP is long-term depression, which produces a long-lasting decrease in synaptic strength.
It is one of several phenomena underlying synaptic plasticity, the ability of chemical synapses to change their strength. As memories are thought to be encoded by modification of synaptic strength, LTP is widely considered one of the major cellular mechanisms that underlies learning and memory.

A famous Hebbian learning rule is that coincident firing of neurons (or the lack thereof) changes synpatic physiology (Fire together, wire together). Synaptic plasticity can be associated with pre-, and –postsynaptic modification, as well as with growth processes

3.1.1 NMDA-dependent LTP

An example of an LTP mechanism is the NMDA receptor-dependent LTP. The NMDA is an ionotropic receptor that, when open, is cation- and especially calcium-permeable. To open the NMDA channel, not only glutamate has to bind but also Magnesium ions docked to the receptor need to get free: When a neuron is at rest, the NMDA receptor accepts a magnesium ion that blocks the channel, but when the neuron is depolarized, the magnesium is repelled and the channel becomes ion-permeable - more specifically - Calcium-permeable. Depolarization occurs by means of AMPA receptors, common excitatory glutamate receptors that are cation-selective channels. In a particular variant, they allow sodium and potassium to pass, but block calcium.

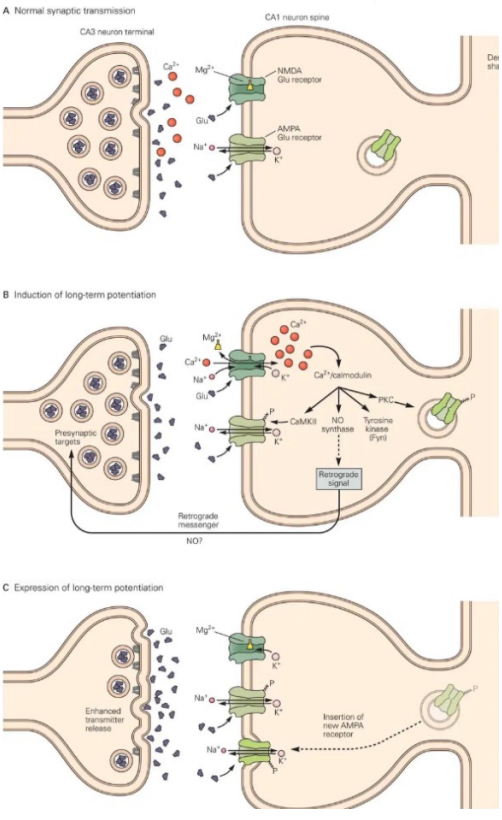

Figure 3.1: NMDA-dependent LTP

The selective calcium permeability of NMDA receptors is important because this activates

two mechanisms: Calcium acts as a second messenger to activate the protein kinase PKC among others which causes AMPA receptors to become phosphorylated and thus easier to open, an LTP mechanism. Second, the phosphorylation inserts more receptors to the postsynaptic membrane, which leads to stronger EPSPs.

3.1.2 Characteristics of LTP

- **Normal Synaptic Transmission:** Only small EPSP, weak ineffective synpatic transmisison. No action potential (AP). See last lecture.
- **Cooperativity:** Simultaneous activity of multiple inputs triggers AP and induces LTP at all synapses.
- **Associativity:** Pairing of a weak with a strong input causes LTP at both synapses.
- **Synapse Specificity:** Memories are formed selectively at active synapses.

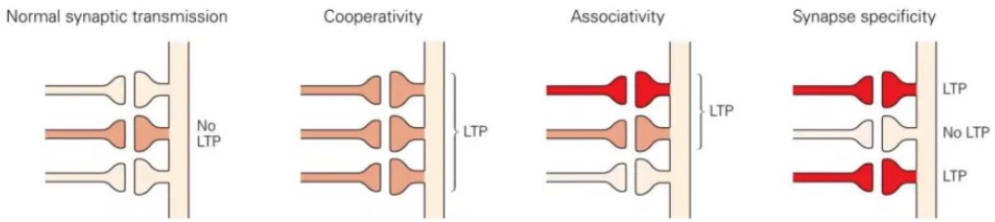

Figure 3.2: Features of LTP

3.1.3 Learning

Learning involves ensembles of neurons (engrams). An engram is a unit of cognitive information imprinted in a physical substance, theorized to be the means by which memories are stored as biophysical or biochemical changes in the brain or other biological tissue, in response to external stimuli.

The hippocampus contains neurons and neuronal ensembles corresponding to spatial maps and sequential trajectories for navigation. And these representations are modified by learning.

Synchronous (co-incident) activity across the brain serve as a model for information transfer and memory consolidation

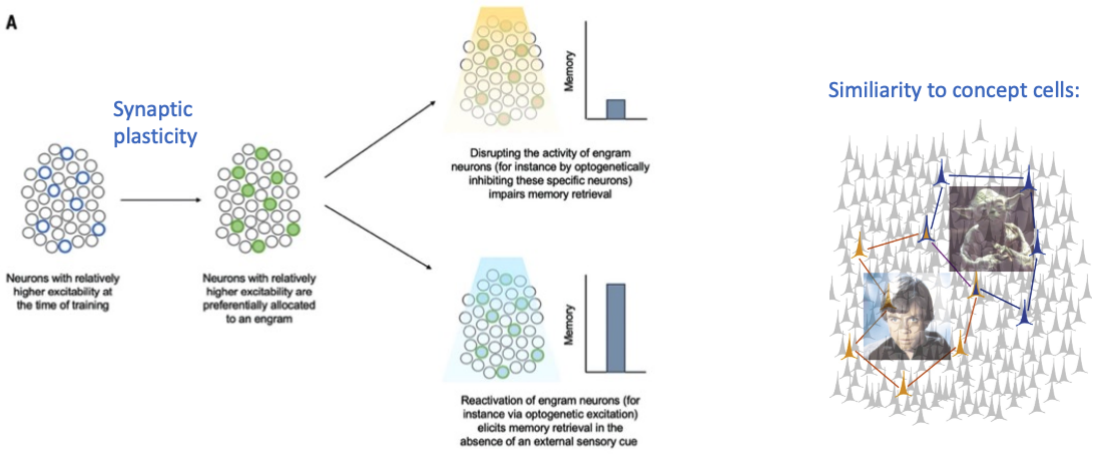

Figure 3.3: Neuronal Engrams

4 Neural Patterning

4.1 Introduction

The vertebrate central nervous system (CNS) is derived from the ectoderm—the outermost germ layer of the embryo. A part of the dorsal ectoderm becomes specified to neural ectoderm – neuroectoderm that forms the neural plate along the dorsal side of the embryo. This is a part of the early patterning of the embryo (including the invertebrate embryo) that also establishes an anterior-posterior axis. The neural plate is the source of the majority of neurons and glial cells of the CNS.

During the folding of the neural plate, the neural tube is formed. The notochord, among other structures, forms from the underlying mesoderm. This structure triggers the formation of a floor plate in the neural tube by Shh. Dorsally, BMP4.7 secreted by the ectoderm forms the Roof Plate in the neural tube.

4.2 Neural Induction

Neural induction is the process by which embryonic cells in the ectoderm make a decision to acquire a neural fate (to form the neural plate) rather than give rise to other structures such as epidermis or mesoderm.

Neural induction represents the earliest step in the determination of ectodermal cell fates. In vertebrates, bone morphogenetic proteins (BMPs) act as signals of epidermal induction. The inhibition of the BMP signalling pathway by Noggin or Chordin in the ectoderm is the hallmark of neural-fate acquisition, and forms the basis of the default model of neural induction.[1]

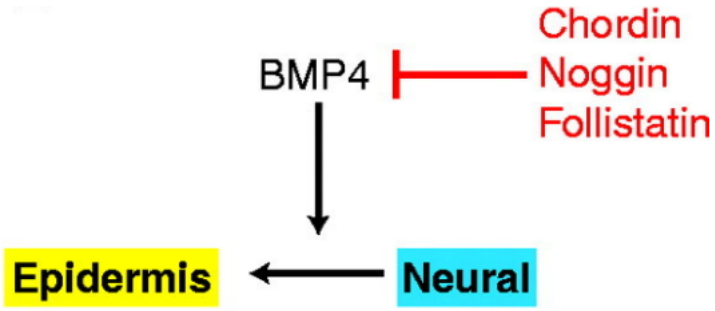

Figure 4.1: Double Inhibition: Inhibition of BMP stops the patterning of Epidermis

From these precursor cells, again only some differentiate into neuroblasts. This is seen

[1] Additionally, BMP regulates the embryonic dorsoventral asymmetry. Vertebrates have dorsal nervous system, invertebrates have NS at the front. Absence of noggin leads to misformations; e.g. spina bifida (open defect)

as the neural fate specification:

4.2.1 Neural Fate Specification

For cells that have the potential to adopt the same "fate", lateral inhibition specifies the development of some cells into a Neuroblast (NB) or into a GMC cell.

Lateral Inhibition: out of the precursor cells only some neuroblast cells emerge. Cells which prime more Delta than their neighboring cells, hinder the neighbors through binding to the receptor Notch to produce Asc+. These cells can't have a neural fate and will be epidermic cells.[2]

As such, Notch signaling is important for the neuron cell differentiation.

Apart from that, Notch signaling is important in embryogenesis: The Notch signaling pathway plays an important role in cell-cell communication, and further regulates embryonic development. Early studies in C. elegans indicate that Notch signaling has a major role in the induction of mesoderm.

4.2.2 Hox Genes

Hox Genes are a group of related genes which specify segment identity of tissue an embryo along the head-tail (anterior–posterior) axis. Depending on which combinations of Hox-Genes are expressed in each segment of the Hox-Code, the respective motor neurons will be formed. e.g. responsible for the correct wiring of the facial nerve Mutations in the Hox-Genes lead to a tissue developing into another part of the tissue.

4.2.3 Notochord

The notochord is responsible for the formation of the floor plate of the neural tube through Sonic Hedgehog (SHH). The floor plate itself starts producing SHH, once formed. One can test experimentally the role of notochord by looking at the spinal cord of embryo, if notochord is removed, no formation of the floor plate takes place.

4.2.4 Morphogens in Spinal Cord Development

Sonic hedgehog (Shh), along with members of the Wnt and bone morphogenetic protein (BMP) families, is a molecule that acts early as a morphogen to determine neuronal fate and later as an axon guidance factor to help direct the paths of developing neurons.[3]

[2] Testing by cell removal: Cells that have developed in a specific primary direction are removed. Cells with a secondary destination pathway replace the removed cells and now develop in the primary direction. Each cell takes into account information from surrounding cells: Delta-Notch pathway. Delta is the ligand, Notch the receptor. Delta expressed in certain cells activates Notch in neighboring cells, directing them in an alternative developmental direction -> regulates neuroblast formation in insects.

[3] Vertebrate commissural neurons of the dorsolateral spinal cord take a ventral trajectory toward the floor plate, cross the midline, and then turn, following along the floor plate, while moving anteriorly towards the brain. Shh, BMP, and Wnt are morphogens that help guide commissural axons along this path

5 Action Potentials

5.1 Introduction

Action Potentials (AP) are essentially propagations of signals/information. They are continuous depolarizations of the membrane potential. Meaning that there is a shift from a negative membrane potential to a more positive potential, such that the internal charge of the neuron becomes (more) positive, e.g. through an influx of cations (positively charged ions) like Sodium or an efflux of anions (negatively charged ions).

Opposed to depolarization, hyperpolarization is a change in the membrane potential that makes the potential more negative. This is often done via an efflux of Potassium (cation) and inhibited via an influx of Sodium (cation) through ion channels.

APs only travel in one direction from the soma along the axon towards to the axon termini. [1]

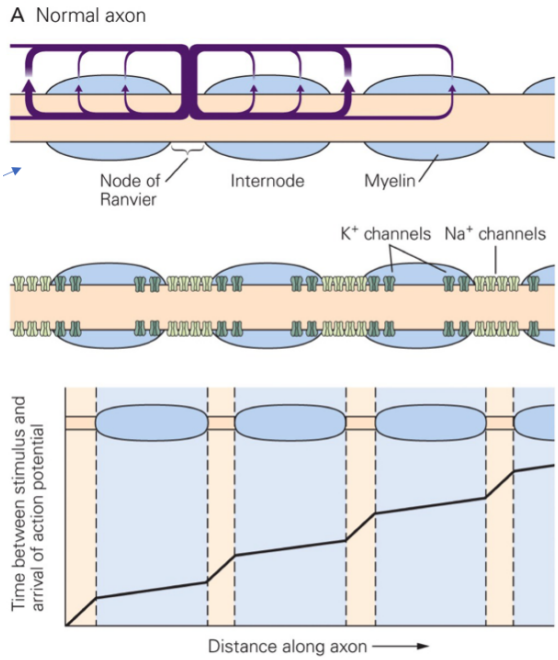

Figure 5.1: Propagation of an AP

The velocity of conducting an AP depends on fiber diameter of the axon, with velocity

[1] Propagation in the opposite direction—known as antidromic conduction—is very rare. However, if a axon is electrically stimulated in its middle (in an experiment e.g.), both halves of the axon are "fresh", i.e., unfired; then two action potentials will be generated, one traveling towards the axon hillock and the other traveling towards the synaptic knobs.

being low or "bad" with a small fibre diameter, and on insulation by the mentioned sheaths of myelin.

As seen in Figure 5.1, the AP jumps from one node of Ranvier to another over a long myelinated stretch of axon called the internode before recharging at the next node of Ranvier etc. until it reaches the axon terminal. This is also called saltatory conduction (latin: saltus - jump). Myelinated axons only allow action potentials to occur at the unmyelinated nodes of Ranvier that occur between the myelinated internodes. It is by this restriction that saltatory conduction propagates an action potential along the axon of a neuron at rates significantly higher than would be possible in unmyelinated axons.

5.1.1 Generation of an AP

The generation of an AP is enabled by an interplay of ligand-gated AND voltage-gated ion channels. The dendrites, that receive synaptic input, are characterized by a high concentration of ligand-gated ion channels, but the axon initial segment (trigger zone) that initiates the AP is characterized by a high concentration of voltage-gated ion (especially Sodium) channels.

The basic workflow of the generation of an AP:

The synapse on each dendrite of a neuron receives a signal from the pre-synaptic. This "signal" is usually a neurotransmitter molecule, released by the presynaptic neuron, that binds to the ionotropic receptors on the post-synaptic located on the dendrite. This binding opens the ligand-gated ion channels, which leads to a change in membrane potential.

This temporary depolarization from a single synapse is called EPSP (excitatory post-synaptic potential). IPSP (inhibitory postsynaptic potential) leads to the opposite effect (hyperpolarization).

All EPSPs (EPSPs from all synapses) are aggregated right after the axon hillock. If this membrane voltage reaches a certain threshold, an AP is fired and sent down the axon.

5.1.2 Characteristics of an AP

Positive Feedback Loops

APs are often considered to be regenerative because they are part of a positive feedback loop: The membrane potential controls the state of the voltage-gated ion channels, but the state of those ion channels also controls the membrane potential. Thus, in some(!) situations (depending on the permeabilities of the channels), a rise in the membrane potential can cause ion channels to open, thereby causing a further rise in the membrane potential. An action potential occurs when this positive feedback cycle (Hodgkin cycle) proceeds explosively.

Refractory Period

Each action potential is followed by a refractory period, which can be divided into

- *an absolute refractory period, during which it is impossible to evoke another action potential*
- *a relative refractory period, during which a stronger-than-usual stimulus is required*

Types of AP

- **Classical Action Potential**: invariant amplitude & waveform, self-terminating
- **Graded (Regenerative) Potential**: amplitude & waveform depend on stimulus, non-self-terminating

All-or-None

The amplitude of an action potential is independent of the amount of current that produced it. In other words, larger currents do not create larger action potentials. Therefore, action potentials are said to be all-or-none signals, since either they occur fully or they do not occur at all.

5.2 Flow of Ions - Mechanism

As we already established, there are two transmembrane proteins or protein structures that regulate or control the flow of ions from extracellular to cytoplasmic/intracellular: Ion pumps/transporters[2] and Ion channels. The Ion channels being either ligand-gated or voltage-gated.

5.2.1 Ion Transporters

We can generally distinguish two types of ion pumps: Primary and secondary transporters.

Primary transporters use energy to transport ions such as Na +, K+, and Ca2+ across a cells membrane and can create concentration gradients. The transporters use ATP (Adenosine triphosphate = energy currency of the cell) to transport an ion from a low concentration to a higher concentration.
Examples of proteins that use ATP are P-type ATPases that transfer Na +, K+, and Ca2+ ions by phosphorylation. This ATPase is essentially the transmembrane protein that catalyzes the decomposition of ATP into ADP and a free phosphate ion. This process of breaking a bond is called hydrolysis.[3] ADP can be further hydrolyzed to give energy, adenosine monophosphate (AMP).

The phosphate that broke free from the ATP binds to the transmembrane protein which causes the protein to change its shape (conformational change) and move sodium ions from the intracellular to the extracellular.

[2] "pump" and "transporter" will be used interchangeably here

[3] *ATP hydrolysis is the final link between the energy derived from food or sunlight and useful work such as muscle contraction, the establishment of electrochemical gradients across membranes, and biosynthetic processes necessary to maintain life.*

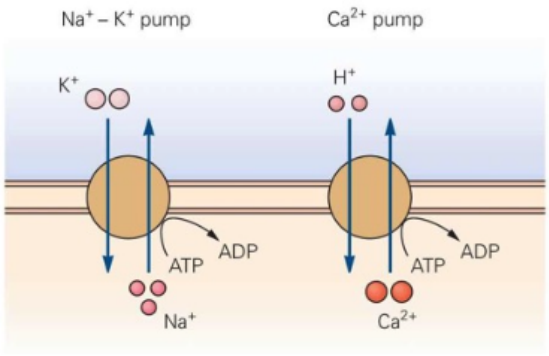

Figure 5.2: Primary active transport

Right afterwards, potassium ions bind to the protein, causing the phosphate to be released, the transmembrane protein to change its shape again and the potassium ions to be moved to the intracellular.[4]

5.2.2 Ligand-Gated Ion Channels - Ionotropic Receptors

As we already established, ligand-gated ion channels are mainly located in a high concentration on the dendrites, or more specifically - the membrane of the dendritic spine and they are waiting for an opening signal from another neuron such that an AP can be passed over.

Mechanism

Sitting inside each axon terminal, tethered to the membrane, are little balloons called vesicles - or more specifically **quanta**, filled with many copies of a chemical messenger, namely neurotransmitter.

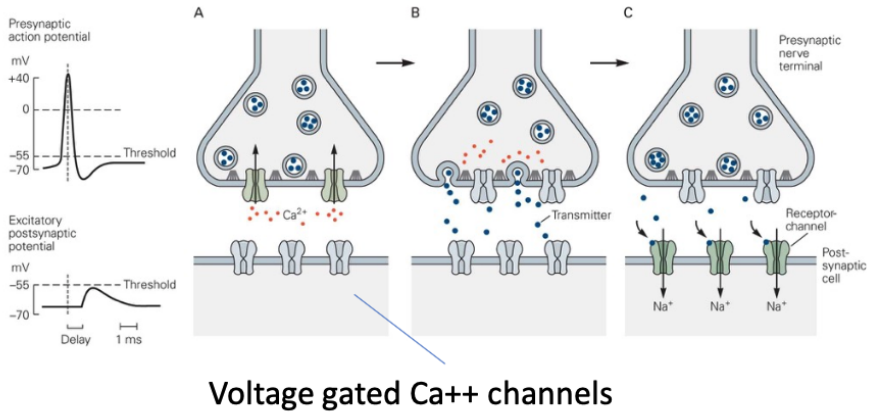

Figure 5.3: Conformation Change via Neurotransmitters

[4] *The sodium–potassium pump mechanism (which helps maintain a rest potential) moves 3 sodium ions out and moves 2 potassium ions in, thus, in total, removing one positive charge carrier from the intracellular space*

Along comes the AP that initiated in the trigger zone right after the axon hillock. It sweeps over the terminal and triggers the release of those neurotransmitters into the synapse, these bind to the (ionotropic) receptors - which are the ligand-gated ion channels - triggering them to change shape (conformational change) and open the channel for the ions to flow into/out of the neuron, as can be seen in Figure 5.3.

This process is also called a "transsynaptic" communication.

After the AP has passed, the locked neurotransmitters float off the receptors and then they are either

- taken up by the axon terminals from the presynaptic via "reuptake pumps", transporters, and being put into the vesicles of the presynaptic again
- degraded in the synapse by an enzyme with the breakdown products flushed into the extracellular environment

The Synaptic Vesicle Cycle

As mentioned, located on the axon terminals - or "terminal boutons", are lots of vesicles. Not all vesicles are located directly at the membrane and not all are incredibly mobile, actually there are three different groups of vesicles:

- **the readily releasable pool** vesicles are docked to the cell membrane and the first to be released on stimulation. The number of vesicles filled with neurotransmitters in this pool is rather low.
- **the recycling pool** is close to the cell membrane such that vesicles can be recycled quickly and are ready to be released. This pool of vesicles is larger than the readily releasable pool.
- **the reserve pool** is the largest pool of all but does not release neurotransmitters under normal conditions and the mobility is quite low.

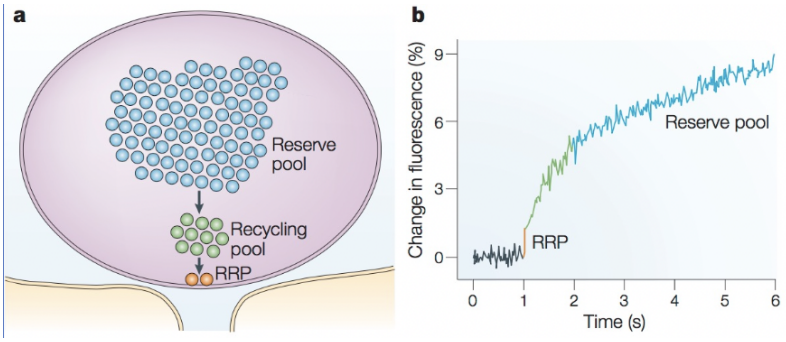

Figure 5.4: The three vesicle pools

In order for the vesicles to release their neurotransmitters to the synapse and finally to the postsynaptic they first have to fuse with the membrane.[5] This happens in following steps:

1. Neurotransmitter Uptake
Synaptic vesicles (SV) are loaded with a neurotransmitter. Loading of transmitter is an active process requiring a neurotransmitter transporter and a proton pump ATPase that provides an electrochemical gradient.

2. Reserve Pool
The formed vesicles are then part of the reserve pool where they are recruited from the readily releasable pool (RRP) to be docked at the active zone. There are about 2-10 RRP vesicles at the active zone.

3. Docking
The synapse vesicles (SV) from the RRP are bound to the molecule Synapsin which restricts the free movement of the vesicles. Upon an AP and undergoing a calcium influx, the Synapsin phosphorylates and releases the SVs which are then moved to the active zone. At the same time synaptotagmin has been recognized as the major sensor for Ca2+ within the cell.

4. Priming
Priming prepares the synaptic vesicle so that they are able to fuse rapidly in response to a calcium influx. This priming step is thought to involve the formation of partially assembled SNARE complexes.[6] The proteins Munc13, RIM, and RIM-BP participate in this event. Munc13 is thought to stimulate the change of the t-SNARE syntaxin from a closed conformation to an open conformation, which stimulates the assembly of v-SNARE /t-SNARE complexes.

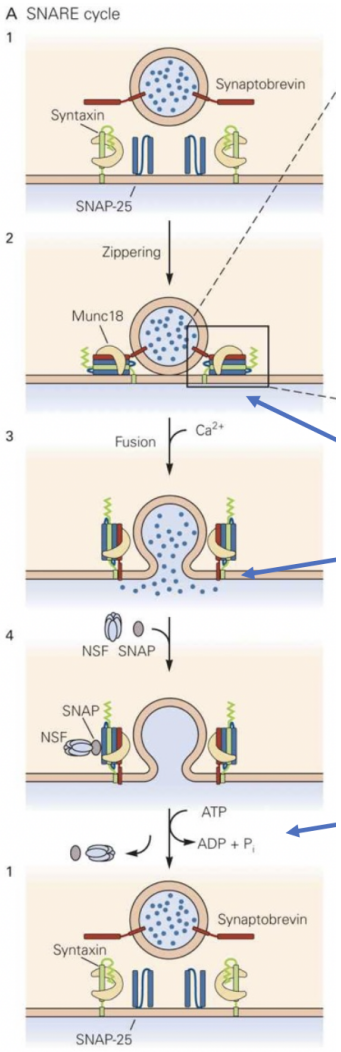

Figure 5.5: Fusion

5. Fusion
Syntaxin and SNAP-25 - on the extracellular side, interact with Synaptobrevin (also called VAMP or vesicle-associated membrane protein) - on the cytoplasmic side. SNAP-25 has two SNARE motifs, and Synaptobrevin and Syntaxin-1 each contain one SNARE motif. The three SNAREs assemble into a tight SNARE complex that brings the membranes into close proximity; this led to the fundamental notion that the energy released upon SNARE

[5] Many discoveries of the neurotransmitter release have taken place through experiments on the Calyx of Held: is a particularly large synapse in the mammalian auditory CNS.

[6] SNARE proteins are critical for most types of intracellular membrane traffic and are characterized by sequences called SNARE motifs that have high propensities to form coiled coils. The neuronal SNAREs that mediate synaptic exocytosis are the synaptic vesicle protein Synaptobrevin/VAMP (vesicle-associated membrane protein) and the plasma membrane proteins SNAP-25

complex assembly is used to induce membrane fusion, where vesicle-SNARE (v-SNARE; Synaptobrevin here) binds to the target membrane SNAREs (t-SNAREs;Syntaxin-1 and SNAP-25 here) to draw the membranes together and induce fusion. This process can be seen in Figure 5.5.
After fusion, the SNARE complex is disassembled by NSF, an ATPase, with the assistance of SNAPs to recycle the SNAREs for another round of fusion.

6.-8. Recycling - Endocytosis
After Ca2+-dependent fusion, the membrane and proteins of the synaptic vesicles found in the plasma membrane are recycled by endocytosis. Synaptic vesicles become re-filled with neurotransmitters and are ready for the next cycle of depolarization and fusion.

Two leading mechanisms of action are thought to be responsible for synaptic vesicle recycling: full collapse fusion, where the synaptic vesicle merges and becomes incorporated into the cell membrane, and the "kiss-and-run" method, where the synaptic vesicle "kisses" the cell membrane, opening a small pore for its neurotransmitter payload to be released through, then closes the pore and is recycled back into the cell.

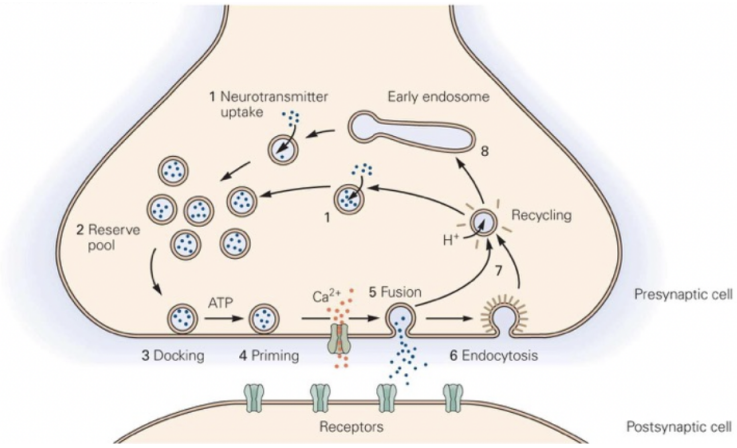

Figure 5.6: The Synaptic Vesicle Cycle

Stochastic Nature of Synaptic Communication

There are three important factors for the transsynaptic communication:

- the number of synaptic contacts
- the quantal size (the response to a single vesicle being released) = the size of the postsynaptic depolarization
- the probability of neurotransmitters being released at the synapse

Due to the varying numbers of molecules released by a quantum, varying numbers of vesicles at the terminals, the varying numbers of vesicles docked, etc., we get different combinations “size/concentration/position” which produces a post-synaptic response variability

of stochastic nature.

Moreover, the probability of release of a vesicle following a pre-synaptic spike is, usually, less than 1 and differs among brain areas. Vesicle release probability is another pre-synaptic stochastic factor affecting information transfer in the brain. As such, we can say that the aforementioned important pre-synaptic factors influencing the variability of the post-synaptic response are stochastic in nature.

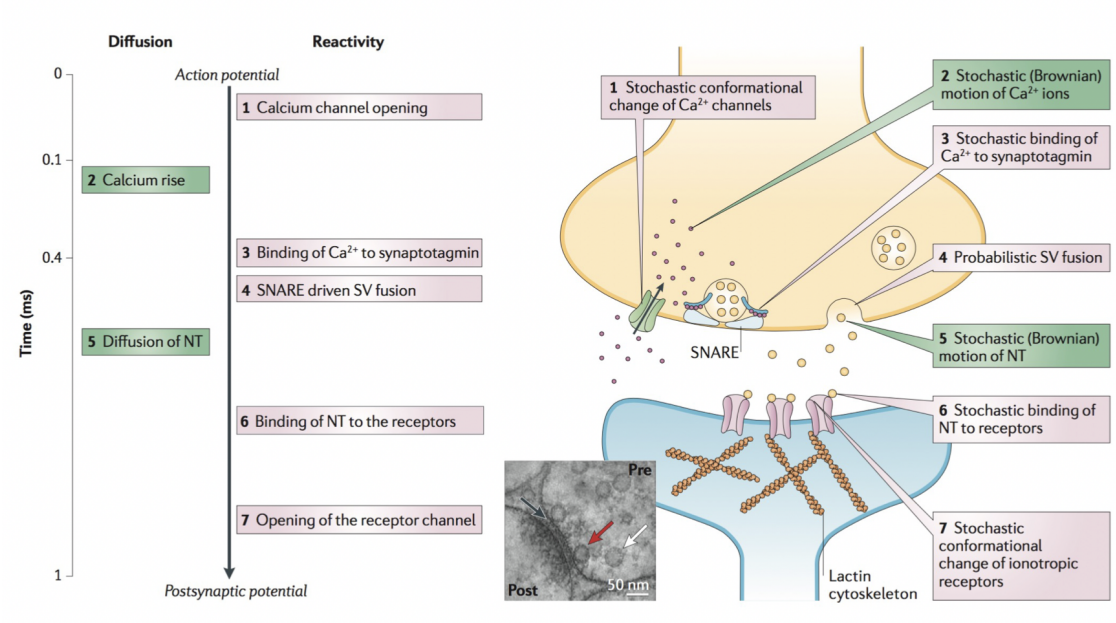

Figure 5.7: Stochastic events during neurotransmitter release and binding (neurotransmission)

Not only is the post-synaptic response stochastic, but also the presynaptic molecular processes that we discussed are probabilistic, as can be seen in Figure 5.7.

Neurotransmitters

Acetylcholine (ACh)

The ACh receptor is the prototypic non-selective ligand-gated ion channel. It has two binding sites for ACh, which is an excitatory neurotransmitter, and the binding leads to a stochastic opening and closing of the channel.

In Figure 5.8 we can see the transsynaptic communication in a neuromuscular junction - a gap junction between a motor neuron and a muscle fibre. On the presynaptic an action potential reaches the axon terminals which leads to voltage-

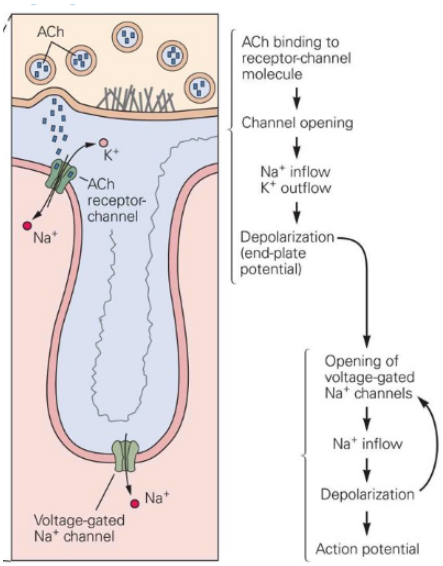

Figure 5.8: Transsynaptic Transfer of AP

gated calcium channels in the terminals to open, calcium ions
to flow into the terminal boutons and to kick-off the vesicle
fusion. On the postsynaptic, the muscle fibre, is the endplate with many ACh receptors to which the ACh molecules released from the presynaptic motor neuron bind. The receptors - aka ion channels - open and allow a Sodium inflow and Potassium outflow, leading to a depolarization - or endplate potential - which, as a reaction, opens the voltage-gated channels.[7]

Glutamate
Glutamate is the major excitatory neurotransmitter in the mammalian CNS. The agonists, the ionotropic receptors that Glutamate binds to, are AMPA and NMDA.

When it comes to the NMDA receptor, which has one binding spot for Glutamate, the binding of the ligand might not be enough to open the channel as it may be blocked by Magnesium ions which are only removed when the neuron is sufficiently depolarized. *Thus, the channel acts as a "coincidence detector" and only once both of these conditions are met, the channel opens and it allows positively charged ions (cations) to flow through the cell membrane.*[8]

GABA
y-Aminobutyric acid - GABA - is the major inhibitory neurotransmitter in the mammalian CNS. There are two known GABA receptors:

- $GABA_A$ which is a ionotropic receptor
- $GABA_B$ which is a metabotropic receptor (or GPCR)

5.2.3 Voltage-Gated Ion Channels

These transmembrane proteins react to changes of the membrane potential by changing their shape (conformational change) in a way that allows ions to pass the membrane.
These channels close within milliseconds after opening - they exhibit rapid inactivation mechanisms, the speed being determined by the ion-type, as the channels are ion-specific. These gating and inactivation kinetics shape the action potential.

Via a series of voltage clamp experiments conducted upon a giant squid axon, with varying extracellular sodium and potassium concentrations, Hodgkin and Huxley demonstrated by injecting currents that the change in current could be separated into two distinct components, a rapid inward current carried by Na+ ions, and a more slowly activating outward current carried by K+ ions. They concluded that these two currents result from independent permeability mechanisms for Na+ and K+ with conductances changing as a function of time and membrane potential.[9]

[7] The mammalian neuromuscular junction only passes a single neurotransmitter type which is the ACh, thus allows only excitatory inputs. The rest of the CNS works with multiple neurotransmitters and both excitatory and inhibitory inputs.

[8] *Extracellular magnesium (Mg2+) and zinc (Zn2+) ions can bind to specific sites on the receptor, blocking the passage of other cations through the open ion channel. Depolarization of the cell dislodges and repels the Mg2+ and Zn2+ ions from the pore, thus allowing a voltage-dependent flow of sodium (Na+) and calcium (Ca2+) ions into the cell and potassium (K+) out of the cell.*

[9] Upon conducting the voltage clamp experiments Hodgkin and Huxley developed the Hodgkin-Huxley Model: a set of nonlinear differential equations that approximates the electrical characteristics of excitable neurons.

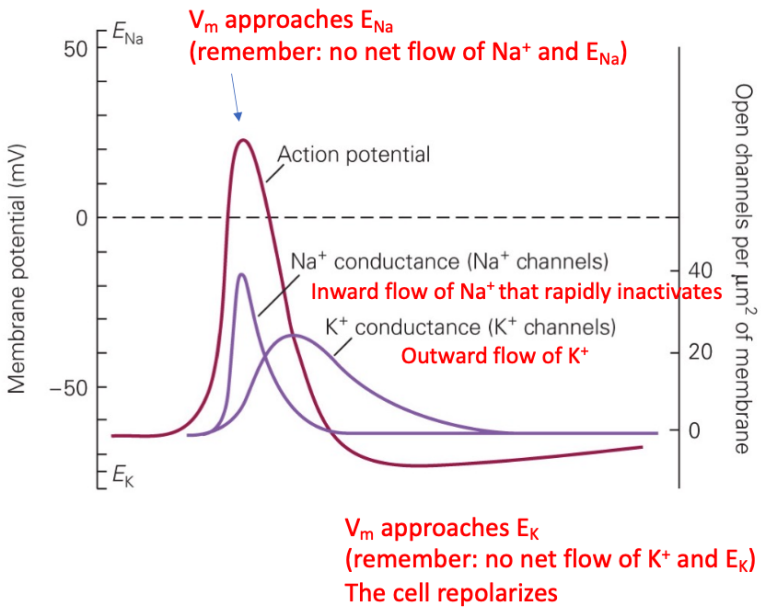

Figure 5.9: Conductances of Sodium and Potassium

By looking at Figure 5.9, which shows the different conductances of Sodium and Potassium, one can see that by the generation of an AP, the channels behave differently based on the ion type: opening/closing rapidly for Sodium channels and more slowly for Potassium channels.

Rectification

Some ion channels let ions flow inwardly (=into the cell) faster than outwardly, these are the inward-rectifier ion channels, outward-rectifier ion channels doing the opposite.

5.2.4 Other Gating - Metabotropic Receptors

The main difference between ionotropic and metabotropic receptors is that while the ionotropic receptors are the (transmembrane) ion channels, that open to let an influx of sodium happen, the metabotropic receptors *indirectly* open those ion channels via a signaling transduction cascade including so-called G-proteins. These metabotropic receptors are especially important in neuromodulatory circuits, where a neuron tries to modulate the interaction of other neurons, as neuromodulators typically bind to metabotropic receptors rather than ionotropic ones.

We will learn more about it in Chapter 7.

5.3 Postsynaptic Potentials

Postsynaptic Potentials are the potentials created at the tip of the dendritic spines, after having their ion channels opened by the neurotransmitters that were released from the

presynaptic. Depending on the ion channels that where opened (which depends on the type of presynaptic neuron), this potential is either an Excitatory Postsynaptic Potential (EPSP), which makes it more likely for the postsynaptic neuron to generate an AP, or an Inhibitory postsynaptic potential (IPSP), which has the opposite effect.[10] All potentials coming from the dendrites are aggregated at the trigger zone, right after the axon hillock.

5.3.1 Synaptic Input Summation

A neuron receives synaptic inputs on its dendrites. These synaptic inputs can stem from a single presynaptic neuron or from multiple presynaptic neurons. The inputs are then summated, in the case of the single presynaptic we call the summation **temporal** and in the case of multiple presynaptic neurons **spatial** summation.

5.3.2 IPSP

Inhibitory Postsynaptic Potentials, like those induced from GABA, have the effect, that they subtract or reduce any other Excitatory Postsynaptic Potentials coming from other synapses, so in the worst case they essentially prevent the EPSPs to reach the soma.

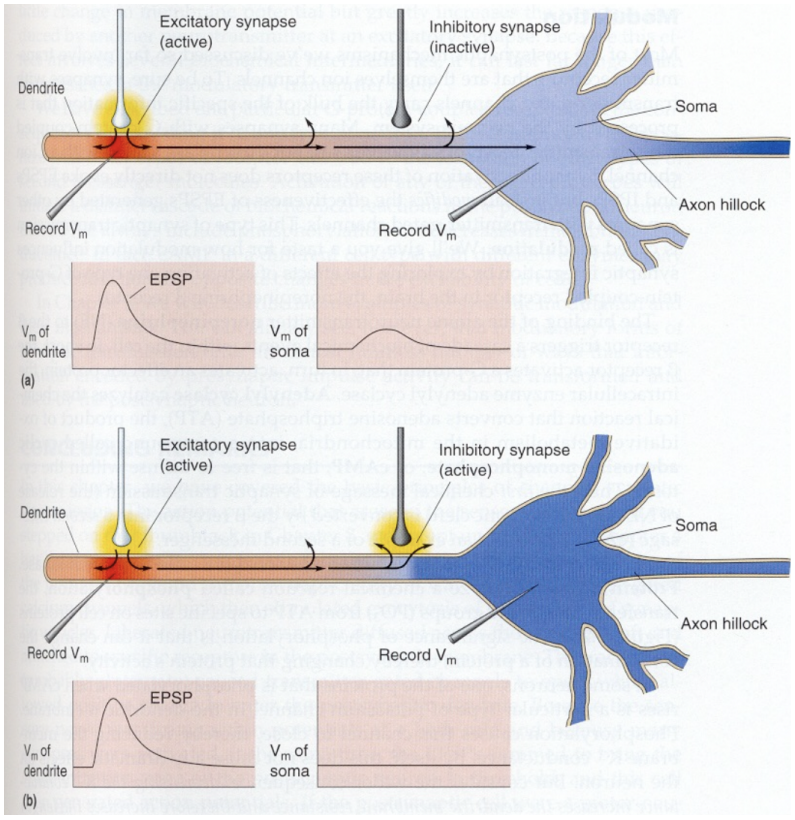

Figure 5.10: Effect of IPSP

[10] These potentials are called **graded** potentials, as opposed to normal action potentials that follow the "All-or-None"-law, they can vary in size and amplitude

In the case of GABA, or more specifically the ionotropic $GABA_A$ receptors, located at the postsynaptic dendritic spines, only let in chloride ions upon opening. These ions are anions, meaning the influx would lead to a hyperpolarization of the cell. This hyperpolarization can have two different effects:

- **simple inhibition**: hyperpolarization has a subtractive effect on the depolarization of the EPSPs
- **shunting inhibition**: hyperpolarization has a divisive effect on the depolarization of the EPSPs. This might happen in some neurons when the reversal potential (potential where current flow is 0) of the inhibitory synaptic is close or equal to the resting potential of the postsynaptic cell or if the input signals are small compared with the leak conductance of the postsynaptic cell.

5.3.3 Neural Backpropagation

Neural backpropagation is the phenomenon in which, after the action potential of a neuron creates a voltage spike down the axon (normal propagation), another impulse is generated from the soma and propagates towards the apical portions of the dendritic arbor or dendrites. There are two ways that this can happen:

- **echo of forward propagation**: During the initiation of an AP in the trigger zone, the soma becomes depolarized as well which spreads through the soma towards the dendritic tree.
- **retrograde signal**: Upon its initiation, the AP can send a retrograde signal in the opposite direction, which again leads to a depolarization of the soma.

5.3.4 Response Properties

The postsynaptic potential can have different properties:

- **delayed firing**
- **potential-dependent excitability**
- **spike accomodation**
- **bursting**: neuron repeatedly fires bursts of spikes

6 Neural Circuits

6.1 Brain Anatomy

6.1.1 Striatum

The (corpus) striatum is a bunch of neurons (nucleus) in the forebrain that is part of a group of nuclei called basal ganglia, which have a major role in motor movements and reward systems.

The striatum consists of two structures:

Dorsal Striatum

The dorsal striatum is composed of the caudate nucleus and the putamen.

The caudate nucleus has an important role in, apart from motor processes, procedural and associative learning and inhibitory control of action

Ventral Striatum

This consists of the **nucleus accumbens** and the olfactory tubercle, the latter being the central role in processing smell.

The nucleus accumbens plays an important role in processing stimuli that are rewarding and reinforcing. But it does not in instrumental learning unless it is a Pavlovian-instrumental transfer (PIT) where a stimuli is paired with a reward.

6.2 Microcircuits

Before we focused on the neurons individually and how the communication between two neurons generally works. Now we want to put all puzzles together and see how the interconnections of many many neurons within certain brain regions look like. How the neurons within a brain region are organized and how they interact with each other is defined as a **microcircuit**.

6.2.1 Striatum

Excitatory glutamatergic pathways lead from the cortex to the striatum and dopaminergic pathways that are excitatory in a direct fashion but inhibitory indirectly lead from other basal ganlgia structures to the striatum, in a way that the neurons in the striatum are excited and as a consquence inhibit the post-synaptic neurons right outside of the striatum. The striatum interacts with the cortex, receiving direct projections from and sending indirect projections to, but also directly with the global pallidus and indirectly with the thalamus. Additionally, the striatum receives inputs from the amygdala, the hippocampus

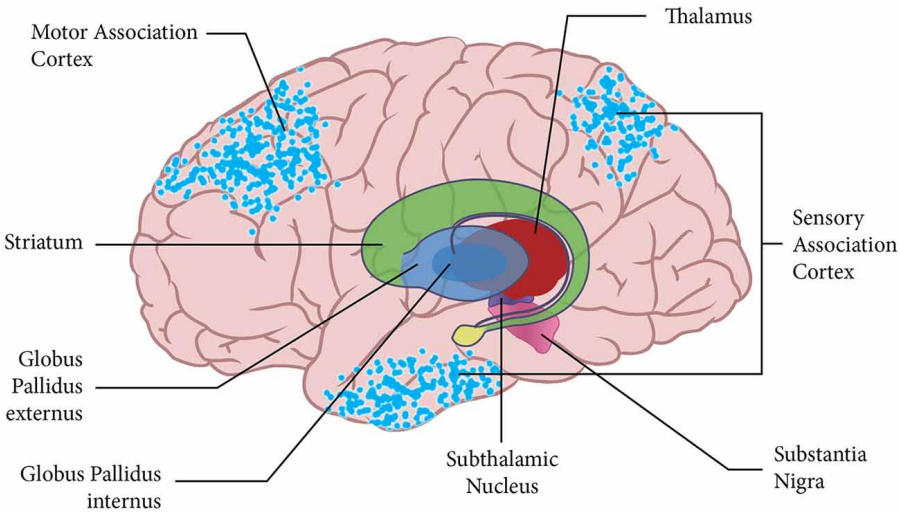

Figure 6.1: The Striatum

and the inferior temporal gyrus, among some other regions. The inferior temporal gyrus is associated with representation of objects, places, faces and colors.

6.2.2 Neocortex

The neocortex involved in higher-order brain functions such as sensory perception, cognition, generation of motor commands, spatial reasoning and language. About 80% of its neurons are excitatory and 20% inhibitory.

The neocortex consists of six horizontal layers segregated principally by cell type and neuronal connections:

Layer I, the molecular layer, is occupied by the dendrites of cells located in deeper layers and axons that travel through this layer to make connections in other areas of the cortex.
Layer I and II contain mainly small pyramidal shaped cells. Layer II, the external granular cell layer, is one of two layers that contain small spherical neurons. Layer III is called the external pyramidal cell layer. The axons of pyramidal neurons in layer II and III project locally to other neurons withing the same cortical area as well as to other cortical areas, thereby mediating intracortical communication.
Layer IV is the main recipient of sensory input from the thalamus and is most prominent in primary sensory areas. Layer V contains mainly pyramid cells that give rise to the major output pathways of the cortex, projecting to other cortical areas.
The neurons in Layer VI carry axons to and from

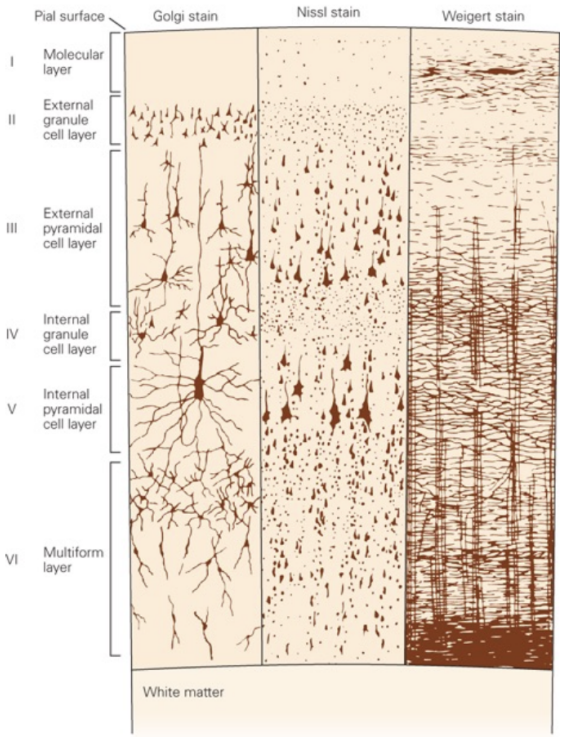

Figure 6.2: Layers of Neocortex

areas of the cortex.

Cortico-cortical connections that project from lower layers to higher layers, are called feedforward projections (ascending), whereas higher layer to lower layer connections are called feedback connection/projections (descending).

Types of Neurons in the Neocortex

The neurons are generally seperated into two groups:

- Principal or pyramidal neurons: pyramid-shaped cell that are mainly located in layers III, V and VI and are usually excitatory and make up 70-80% of the cortex.
- Local interneurons: There are GABAergic interneurons, that are inhibitory and make up 20-30% of the cortex.[1] They are located in all layers and are called local because they only make local computations. There are also excitatory interneurons, that are primarily located in layer IV and are the primary recipients of sensory information from the thalamus. Interneurons can feed into and control the input of pyramid cells.[2]

Pyramidal neurons and inhibitory interneurons constantly interact with each other which makes up the microcircuit. In Figure 6.3 we can e.g. see the different ways that interneurons can inhibit pyramidal neurons.[3]

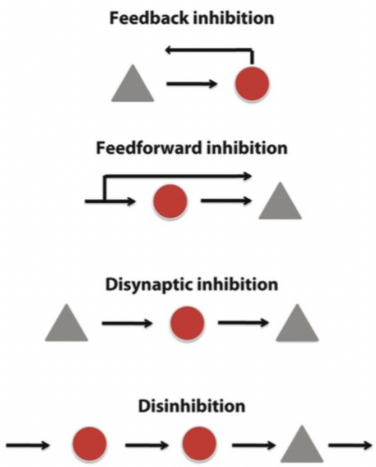

Figure 6.3: Interaction between interneurons (circle) and pyramidal cells (triangle)

Columnar Organization

The neocortex is often described as being arranged in vertical structures called cortical columns, patches of neocortex with a diameter of roughly 0.5 mm. These columns are often thought of as the basic repeating functional units of the neocortex.

[1] There are multiple sub-types of the inhibitory interneurons, such as basket cells, chandelier cells. The interneurons can also differ in spiking, eg. some tend to fast spiking, some to late spiking, etc.

[2] Pyramidal neurons are simply the most common excitatory neurons in the mammalian brain - in the cerebral cortex, the hippocampus, and the amygdala.

[3] AD Figure 6.3: Feedforward inhibition would lead to a narrowing of the window for temporal summation.

7 Neuromodulation

7.1 Introduction

Neuromodulation is the process where a selection of neurotransmitters have an influence on multiple neurons. They can regulate the population of those neurons.

The neurotransmitters that have a neuromodulatory effect usually bind to metabotropic receptors - or G-protein coupled receptors (GPCR), which are, as established in Chapter 5, receptors that can indirectly open or close ion channels on the cell membrane, via a second messenger signaling cascade.

As such, the "classic" neurotransmitters such as GABA and Glutamate or ACh are still being released by neuromodulatory neurons but they bind to different receptors (e.g. the GPCR $GABA_B$ instead of the ionotropic $GABA_A$). [1] But what happens when they bind to a GPCR?

7.2 G-Protein-Coupled receptors GPCR

A GPCR is bound to a G-protein complex. A G-protein is a trimer of alpha, beta and gamma subunits bound to GDP or GTP.[2] Now, when a ligand binds to the GPCR it causes a conformational change in the GPCR, making it an exchange factor (GEF), which in reaction exchanges the GDP bound to the G-protein for a GTP. The G protein's alpha subunit, together with the bound GTP, can then dissociate from the beta and gamma subunits to further affect intracellular signaling proteins. Both GTP-alpa and beta-gamma are active signaling molecules.

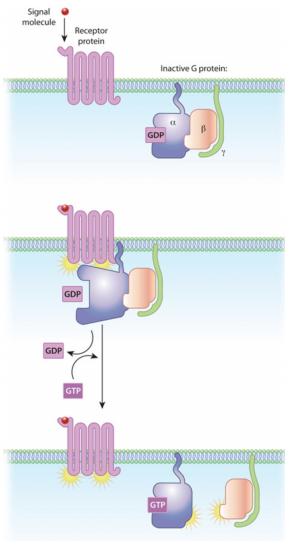

Figure 7.1: GDP-GTP

Following this activating one of two signaling cascades can be kick-offed:

- Cyclic AMP Pathway
- Phospholipase C Pathway

[1] There are other famous neuromodulators (neurotransmitters) that bind to metabotropic receptors: Dopamine - responsible for reward/craving as in mating, Serotonin - mood, cognition, reward, Adrenaline (Epinephrin) and Noradrenalin (Norepinephrin). Drugs that can influence these neurotransmitter systems are called monoaminergic.

[2] When G-proteins are bound to GTP, they are 'on', and, when they are bound to GDP, they are 'off'. G-proteins belong to the larger group of enzymes called GTPases.

7.2.1 Cyclic AMP Pathway cAMP

GPCRs are responsible for receiving signals in form of sugar, protein, etc. In this context they initiate the Cyclic AMP Pathway.

Cyclic AMP is involved in the regulation of glycogen, sugar, and lipid metabolism but it may affect brain function in many ways. In some cases, increase in levels of cAMP may result in an increase in the production of a neurotransmitter, contributing to an agonist effect.

Adenylyl cyclase (AC) being activated by the G-protein induces the conversion of ATP to cAMP. cAMP acts on the target protein kinase (PKA) which modulates a number of cellular substrates via phosphorylation, including ion channels.

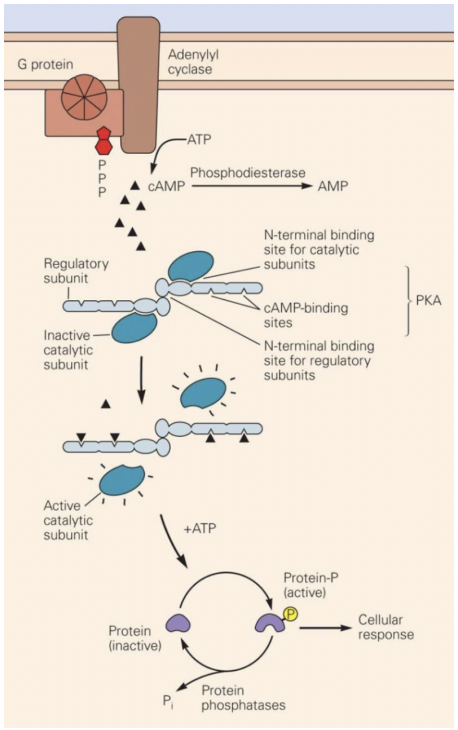

Figure 7.2: cAMP signaling cascade

7.2.2 Phospholipase C pathway PLC

Another signaling pathway is the one where Phospholipase C is activated which hydrolyzes PIP2 (component of the cell membrane) into the second messengers IP3 and DAG. IP3 binds with the IP3 receptor in the membrane of the endoplasmic reticulum to open Ca2+ channels. DAG and Ca2+ together activate protein kinase C (PKC) which also modulates ion channels via phosphorylation.

7.3 Properties of Neuromodulators

7.3.1 Extrasynaptic Transmission

Some neuromodulators can diffuse from the boutons through extracellular fluid and latch onto extrasynaptic receptors without any synaptic contact. This is called extrasynaptic or volume transmission.

7.3.2 Influence on Excitability of Neurons

Neuromodulators can indirectly modify synaptic interactions by changing the excitability of neurons.[3] Indirect effects include presynaptic modulation that can lead to changes in action potential shape, and postsynaptic modulation that for example increases voltage-gated inward currents to enhance EPSPs.

[3]Experiments on the STG = stomatogastric ganglion in crustaceans have shown that neuromodulators can even re-organize entire networks of neurons.

7.3.3 Neuropeptides

Neuropeptides are chemical messengers made up of small chains of amino acids that are synthesized and released by neurons. Neuropeptides typically bind to G protein-coupled receptors (GPCRs) to modulate neural activity.

Neuropeptides are released by dense core vesicles (DCV) after depolarization of the cell. Some evidence shows that neuropeptides are released after high-frequency firing or bursts, distinguishing dense core vesicle from synaptic vesicle release. Neuropeptides utilize extrasynaptic transmission and are not reuptaken quickly, allowing diffusion across broad areas to reach targets. This is why neuropeptides are seen as slow-acting neurotransmitters.

Expression of neuropeptides in the nervous system is diverse. Neuropeptides are often co-released with other neuropeptides and neurotransmitters, yielding a diversity of effects depending on the combination of release.

7.3.4 Behavioral Functions of Neuromodulators

The neuropeptides oxytocin (OXT), which is released during birth and facilitates birth and breastfeeding, and vasopressin (AVP) have had key roles throughout mammalian evolution in the regulation of complex social cognition and behaviours, such as attachment, social exploration, recognition and aggression, as well as anxiety, fear conditioning and fear extinction.

An overexpression of the Vasopressin receptors leads to an expression of pair bonding.

Neuropeptides are also responsible for inducing appetite, upon being activated by the hunger hormone Ghrelin: NPY and AGRP promote food intake and decrease energy expenditure. The neuropeptides A-MSH/CART on the other hand reduce food intake and increase energy expenditure.

Acronyms

ACh Acetylcholine. 28, 37
AP Action Potential. 21, 23

cAMP Cyclic Adenosine Monophosphate. iv, 38

EPSP Excitatory postsynaptic potential. 31

GPCR G-Protein coupled receptors. iv, 37

IPSP Inhibitory postsynaptic potential. 31

PLC Phospholipase C pathway. iv, 38

SF Selectivity Filter. 7