Neurophysiology

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Contents

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The aim of this chapter is to introduce the interested reader to the neurophysiological processes that underlie the behavior of humans and most mammals. The communication of neurons is a basic prerequisite for us to perceive, process and react to environmental stimuli. If we touch a hot stove top, the stimulus is transmitted to the thalamus and cortex via nerve fibers in the peripheral and central nervous system. In these higher brain regions, the incoming information is processed and a conscious perception of the heat of the hot plate occurs, i.e. a sensation of pain in the case of a very hot plate. At the same time, a complex motor reaction is triggered by the activation of efferent nerve cells, and we pull our arm back. The association between touching the hot plate and the sensation of pain also sets in motion a learning process that is capable of changing our behavior in the long term. The sight of a hot plate, even one that is turned off, will signal danger to us in the future. Accordingly, we will exercise greater caution when dealing with hotplates and first check whether they are on or off before touching them. Motor skills, sensations and feelings involve the communication of neurons within one brain region, but also between different brain regions. The neurophysiological correlate for learning processes is a change in the strength of this communication.

Learning Objectives

After reading this chapter, the reader should understand the neurophysiological basis of neural communication and be aware of the major changes in this communication that underlie learning processes.

4.1 Membrane Potential

Membrane potential is the electrical voltage between the extracellular and intracellular space separated by the cell membrane (Hille 2001). All cells of an organism have a characteristic membrane potential. This is usually negative under resting conditions, meaning that the cell interior is more electrically negative than the extracellular space. Changes in this characteristic potential, which occur for example during the activity of an excitable cell, can be measured experimentally with the aid of microelectrodes. One measuring electrode is located in the extracellular space and a second in the cell or, in the *patch clamp technique*, directly on the cell membrane (◘ Fig. 4.1).

The characteristic membrane potential of excitable cells is mainly due to two conditions (Hille 2001):

1. Ions, i.e. electrically charged atoms or molecules, are unequally distributed intracellularly and extracellularly, and
2. the plasma membrane of the cell is permeable only for certain ions.

The most important charge carriers for the membrane potential of excitable cells are the positively charged cations sodium (Na^+) and potassium (K^+) and the negatively charged anion chloride (Cl^-). The concentration of positively charged Na^+ ions and negatively charged Cl^- ions is about 10–25 times higher extracellularly than intracellularly. In contrast, the intracellular concentration of K^+ ions is 30 times higher than extracellular (◘ Table 4.1). Intracellularly, anionic organic molecules are the most important carriers of negative charges.

The different distribution of ions is mainly due to the fact that proteins located in the cell membrane, so-called *pumps*, transport ions into or out of the cell against their concentration gradient. A prominent example of such a pump is the **sodium-potassium ATPase** (Skou 1957), for whose research the Scandinavian scientist Jens Christian Skou received the Nobel Prize in 1997. The sodium-potassium ATPase pumps three Na^+ ions out of the cell and two K^+ ions into the cell. It requires energy for this transport process, as it occurs in opposition to the concentration gradient of the two ions to be

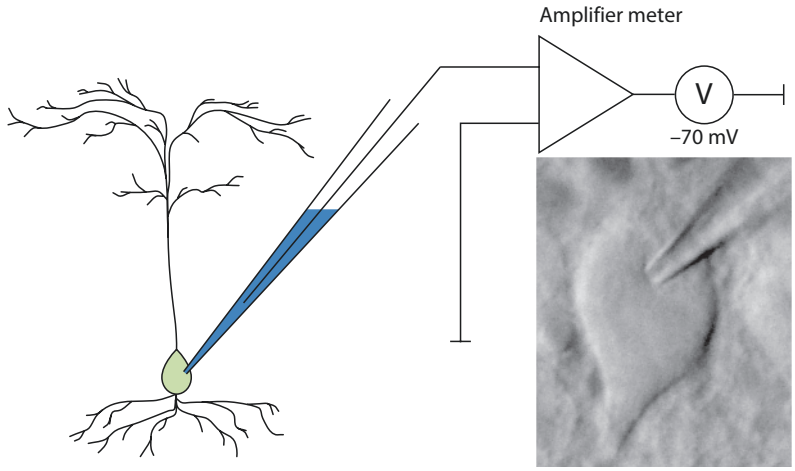

Fig. 4.1 Determination of the membrane potential of a neuron. To determine the potential of a cell membrane, a thin glass capillary with an opening of a few micrometers is positioned directly at the membrane. By sucking the cell membrane to the opening of the capillary, a high electrical seal is created. This is essential for measuring currents and potential changes in the pA or mV range that typically occur in nerve cells. Inside the glass capillary is a salt solution that is in contact with an electrode. Outside the cell there is another electrode. This setup allows the voltage to be measured. Due to the small size of the measured currents/voltages, they have to be electronically amplified before evaluation. In this example, the resting membrane potential of the neuron is −70 mV

Table 4.1 Intracellular and extracellular ion concentrations in humans

Ion	Concentration intracellular (mM)	Concentration extracellular (mM)	Equilibriumpotential (≈mV)
Na^+	15	145	+60
K^+	120	3	−95
Ca^{2+}	0.0001	1[a]	+120
Cl^-	7	120	−75
HCO_3^-	15	24	−13
A^{-b}	≈120–150	–	–

[a] The total Ca^{2+} ion concentration is about 2 mM, about half of which is unbound and ionized
[b] A^-: negatively charged organic molecules that cannot pass through the plasma membrane

transported. Due to the unequal ratios of this transport (the pump transports only two positively charged K^+ ions for three positively charged Na^+ ions), a slightly negative membrane potential is already generated by the activity of the sodium-potassium ATPase. Such potential-generating transport is called electrogenic (Fig. 4.2). As its name suggests, the sodium-potassium ATPase uses adenosine triphosphate (ATP) as an energy carrier. In this process, the hydrolysis of ATP to adenosine diphosphate (ADP) and phosphate by the phosphatate activity of the pump leads to the release of

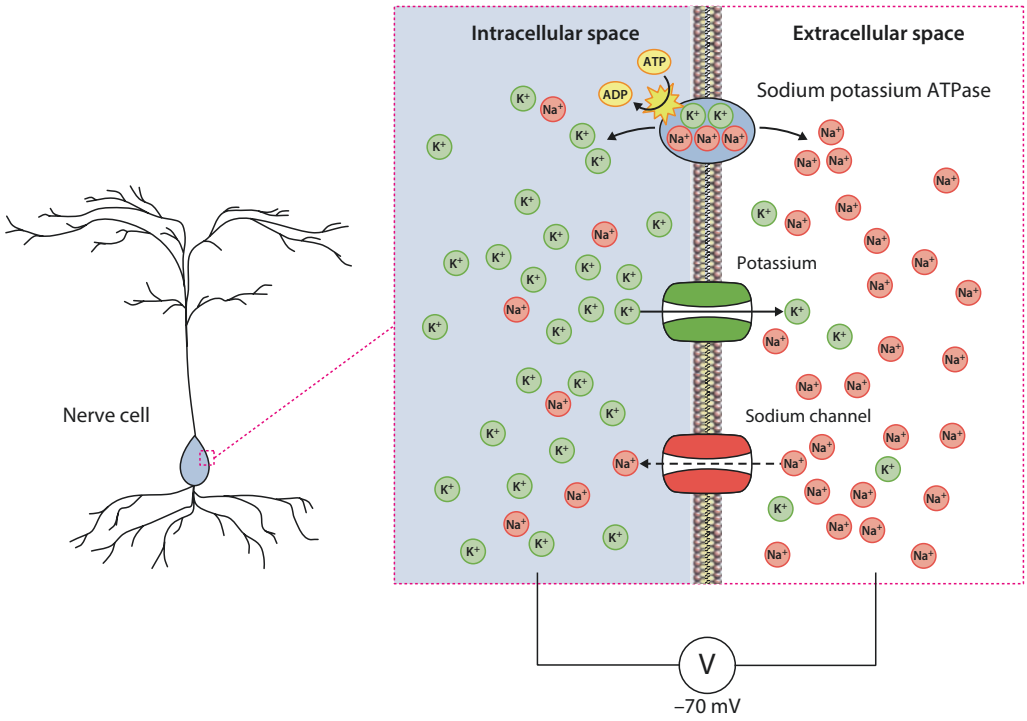

Fig. 4.2 Development of the negative membrane potential of a neuron. In order for the resting membrane potential of a nerve cell to settle at −70 mV, it requires various preconditions. First, there must be an unequal distribution of sodium and potassium ions intracellularly and extracellularly. This unequal distribution is generated and maintained under energy consumption (ATP → ADP) by the activity of the sodium-potassium ATPase (light blue). Second, the cell membrane must be particularly permeable to potassium ions and only slightly permeable to sodium ions. The relatively high potassium permeability under conditions of resting membrane potential is mediated by open potassium channels (green). Potassium continuously flows out of the cell into the extracellular space through these potassium channels. This constant flow of potassium causes the membrane potential of the cell to approach the equilibrium potential of potassium (Table 4.1). However, the equilibrium potential of potassium (about −90 mV) is usually lower than the resting membrane potential of a neuron (about −70 mV). This is because most neurons also have a low permeability to sodium ions under resting conditions. This permeability is mediated by a small number of open sodium channels (red). Sodium flux slightly shifts the membrane potential towards the sodium reversal potential (approx. +60 mV), causing the resting membrane potential to settle at values around −70 mV

the energy required for the transport process. This explains the observation that the negative resting membrane potential of excitable cells collapses when the energy carrier ATP is insufficiently available.

As described above, a basic requirement for the formation of the negative membrane potential of nerve cells is the unequal distribution of different ions between extracellular and intracellular space. In order to maintain this unequal distribution in a stable manner, an important property of the cell membrane is required. The cell membrane must have only a low *permeability* for ions, otherwise the extracellular and intracellular ion concentrations would equalize again

over time via diffusion. The permeability of the lipid bilayer of the cell membrane itself is very low. However, there are channel proteins in the membrane of neurons that are permeable to certain ions. While under resting conditions the permeability for ions such as Na^+ and Cl^- is low, there are K^+ channels in the cell membrane of neurons that are open even at negative membrane potential. K^+ ions can therefore flow out of the cell into the extracellular space through these open channels in the direction of the concentration gradient established by the sodium-potassium ATPase, even under resting conditions. In contrast, Na^+ ions can hardly flow into the cell under resting conditions because most Na^+ channels are closed. The membrane potential becomes more negative due to this shift of the positive charge carrier K^+ from intracellular to extracellular (Fig. 4.2). Accordingly, the electrogenic activity of sodium-potassium ATPase only partly explains the unequal charge distribution between the cell interior and the extracellular space. The negative resting membrane potential is additionally caused by a higher permeability for K^+ ions than, for example, for Na^+ or Cl^- ions. As we will see later, the permeability of the cell membrane to specific ions can change very rapidly. As a consequence of such a permeability change, the membrane potential shifts towards more positive or more negative values, depending on which ion flows more strongly across the membrane (Hille 2001).

Patch Clamp Technique

The technique is a measurement method used primarily in basic neuroscientific research, with the help of which scientists can trace the smallest currents and potential changes of living nerve cells. The technique was developed in the late 1970s and early 1980s by the two German electrophysiologists Bert Sakmann and Erwin Neher (Hamill et al. 1981). In order to be able to measure currents or potentials of a single nerve cell with as little interference as possible, a measuring electrode is brought into very close contact with the cell membrane of the cell. Neher and Sakmann succeeded in doing this by using a special glass electrode filled with a salt solution, which enabled them to suck the cell membrane onto the electrode by means of negative pressure. The currents or potential changes of the nerve cell can be derived via a wire in the glass electrode. The diameter of the tip of such a glass electrode is 1–2 µM, much smaller than the diameter of most neurons (about 20 µm for cortical pyramidal cells). Thus, only a small area (the eponymous *patch*) of the cell membrane is located under the glass electrode. In fact, the area of the cell membrane is so small that it often contains only one ion channel. The patch-clamp technique can therefore be used to study the activity of a single channel at very high resolution. One advantage of the patch-clamp technique is a strong reduction of background noise, which allows the derivation of even very small currents or voltage changes. This low noise of the patch clamp technique is a basic requirement when investigating the activity of individual channels. Thus, currents with amplitudes of a few picoamperes (1 pA = 10^{-12} A) can be easily resolved. At the same time, the patch clamp technique allows either the membrane voltage to be held constant (the eponymous clamp) when measuring currents or the current to be held constant when measuring voltage changes. This offers a great advantage in the controlled analysis of ion channel properties. However, the membrane under the pipette

can also be disrupted, so that in the *whole cell configuration* one can measure currents and voltage changes caused by the opening of ion channels in the membrane of the whole neuron. Since it is also possible to "patch" neurons in acute brain slices and even in vivo, the patch-clamp technique allows the analysis of the activity of a neuron that is in a neuronal network and receives information from other neurons. With the development of the patch-clamp technique, Bert Sakmann and Erwin Neher decisively advanced the understanding of the function of ion channels, which was honored with the award of the Nobel Prize in 1991.

4.2 Equilibrium Potential of an Ion

In ▶ Sect. 4.1 we have already discussed the two forces that are responsible for the movement of ions from intracellular to extracellular. Firstly, ions move in the direction of the concentration gradient. This chemical driving force leads to a movement of K^+ ions from intracellular to extracellular. On the other hand, ions also move along an electrical potential gradient. The negative membrane potential (i.e. the intracellular space is more negative than the extracellular space) thus causes K^+ ions to flow into the cell. However, chemical and electrical driving forces cannot be considered separately. If we take the movement of K^+ ions at a negative resting membrane potential of −70 mV as an example, the chemical driving force opposes the electrical one. Since at this potential the chemical driving force is greater than the electrical driving force, more K^+ ions will flow out of the cell than into it. Net, therefore, there is a K^+ efflux. In contrast, when Na^+ channels are opened, both driving forces will cause Na^+ ions to flow into the cell (Hille 2001).

The net movement of an ion is therefore dependent on the electrochemical driving force, in which both driving forces are combined. If the driving forces for an ion are opposite and equal, there is no net movement of the ion across the membrane. For example, this is the case for K^+ ions at about −95 mV. At this membrane potential, the chemical driving force is directed extracellularly and the electrical driving force is directed intracellularly. Since the driving forces are equal at about −95 mV, exactly as much K^+ ions flow out of the cell as into the cell. Thus, there is no net current flow. The potential of the cell at which this is true is therefore called the **equilibrium potential of** a given ion (◘ Fig. 4.3). If the intracellular and extracellular ion concentrations are known, the equilibrium potential of an ion can be calculated using the **Nernst equation** (Hille 2001):

$$E_{\text{ion}} = \frac{R \cdot T}{z \cdot F} * \ln \frac{\left[K^+\right]_a}{\left[K^+\right]_i} \qquad (4.1)$$

with E_{ion} = equilibrium potential, R = general gas constant, T = absolute temperature, F = Faraday constant, z = valence of ion.

In our body, the Na^+ ion concentration is usually much higher extracellularly than intracellularly ◘ Table 4.1). This explains why the equilibrium potential for Na^+ ions is positive (approx. +75 mV). The Cl^- ion concentration is also much higher extracellularly than intracellularly, but since Cl^- ions are negatively charged, the equilibrium potential is negative (approx. −50 mV).

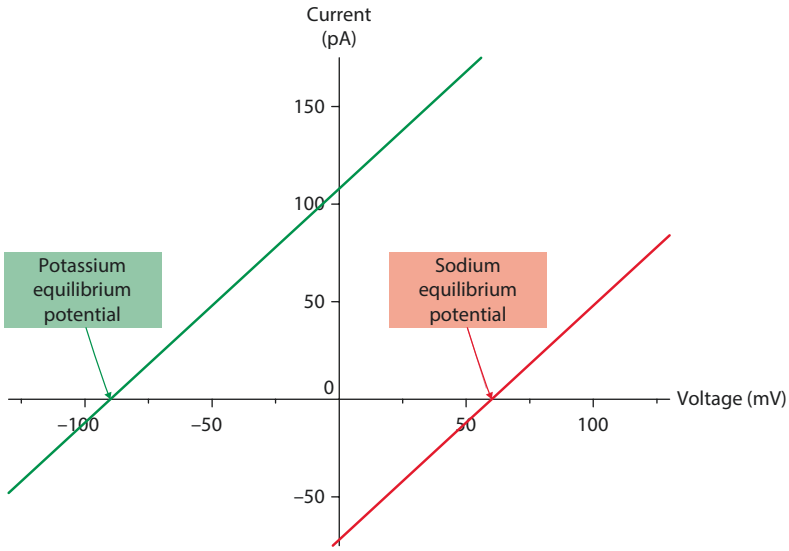

Fig. 4.3 Current-voltage characteristic of an ion channel. The passage of ions across the cell membrane occurs through ion channels along the electrochemical gradient. The magnitude and direction of the ion current depend on the membrane voltage and the concentration gradient. The current is zero when all driving forces cancel out (equilibrium potential, see Nernst Eq. 4.1). If, for example, the membrane voltage changes the magnitude and direction of the ion current will also change as a result. This in turn can be measured as an electric current. Due to the different charge and concentration distributions there is a specific relationship between current and voltage for each ion. The current-voltage curves shown here for idealized sodium and potassium channels are strictly linear. In nature, however, most channels show a divergent current-voltage curve. The channels change their permeability as a function of membrane voltage, thereby affecting the amplitude of potential ionic currents. Due to these properties, they are then referred to as inward or outward rectifying channels (cf. NMDA receptor channel, Fig. 4.12)

4.3 Resting Membrane Potential

The resting membrane potential is the membrane potential of a neuron at rest, i.e. when the neuron is not firing an action potential. In the brains of mammals, including humans, the resting membrane potential for most neurons is about −70 mV, which is close to the equilibrium potential for K^+ ions (about −95 mV). This can be explained by the fact that the cell membrane has a high permeability for K^+ ions under resting membrane conditions. Na^+ ions and Cl^- ions can generally hardly flow under these conditions and therefore have little influence on the resting membrane potential. Thus, not only the extracellular and intracellular concentrations of ions are crucial for the membrane potential of a cell, but also the permeability of the membrane for these ions. The ion for which the membrane is particularly permeable sets the tone, so to speak. It "pulls" the membrane potential close to its equilibrium potential. Ions with lower permeabilities have less influence on the membrane potential (Hodgkin 1964). The **Goldman-Hodgkin-Katz equation** takes into account not only the concentration difference of the ions but also the permeability and allows the membrane potential to be calculated:

$$E_M = \frac{R \cdot T}{z \cdot F} * \ln \frac{P_{Na} \cdot \left[Na^+\right]_a + P_K \cdot \left[K^+\right]_a + P_{Cl} \cdot \left[Cl^-\right]_i}{P_{Na} \cdot \left[Na^+\right]_i + P_K \cdot \left[K^+\right]_i + P_{Cl} \cdot \left[Cl^-\right]_a}$$

with E_M = membrane potential, R, T and F as in the Nernst equation, P_{Na}, P_K, P_{Cl} = permeability for K^+ ions, Na^+ ions and Cl^- ions.

As can be seen, the Goldman-Hodgkin-Katz equation is an extension of the Nernst equation. All ions that play a role in the membrane potential are included in the calculation. Under resting conditions—as already mentioned—the permeability for K^+ ions is high, that for Na^+ ions and Cl^- ions is low. Accordingly, the resting membrane potential is close to the equilibrium potential for K^+ (generally somewhat more positive, since the permeability for Na^+ ions and Cl^- ions is not zero).

If the permeability for K^+ ions increases as additional K^+-channels open, the membrane potential approaches the equilibrium potential for K^+, i.e. it becomes more negative. This is referred to as **hyperpolarization**. As we will see later, hyperpolarization causes the excitability of the neuron to decrease. The equilibrium potential for Na^+ is about +60 mV. This means that when the resting membrane potential is, say, −70 mV, Na^+ ions will strive to flow into the cell. This happens, for example, when a neuron is activated in such a way that the permeability of the membrane to Na^+ ions increases. In this case, the membrane potential approaches the equilibrium potential for Na^+. The membrane potential thus becomes less negative or even positive. This is referred to as **depolarization**.

4.4 Action Potential

Neurons are among the excitable cells of our body. This means that they are capable of firing an action potential (Hille 2001). An action potential is understood to be a very rapid change in the membrane potential, due to a depolarization of the membrane potential to approx. −50. At this so-called **threshold potential**, voltage-gated Na^+ channels (Na_V channels) open. Voltage-gated ion channels change their ion permeability depending on the membrane potential (Armstrong and Hille 1998). They thus differ from ligand-gated ion channels (= ionotropic receptor ion channel, ligand-gated), in which the binding of a transmitter causes the opening of the channel. Voltage-gated Na^+ channels are closed at normal resting membrane potential and open at a membrane potential of about −60 mV. As shown in ▶ Sect. 4.3, the opening of channels that are selectively permeable to Na^+ ions leads to depolarization (the so-called **rising phase** the action potential). The membrane potential becomes positive (**overshoot**) and approaches the equilibrium potential for Na^+ ions at about +60 mV. However, voltage-gated Na^+ channels close again very quickly. Voltage-gated K^+ channels open somewhat later than Na^+ channels. This leads to a **repolarization** of the membrane potential (◘ Fig. 4.4). The permeability of the membrane to K^+ ions is usually greater at this stage than before the action potential. This explains why the membrane potential is hyperpolarized after an action potential, i.e. closer to the equilibrium potential for K^+ ions than before the action potential. Action potentials follow an all-or-nothing law. This means that reaching the threshold potential always triggers an action potential, which then proceeds in a very stereotyped manner with a similar amplitude (approximately 80–100 mV) and temporal duration (approximately 1 ms). The increasing depolarization during the upstroke leads to the explosive opening of all voltage-gated Na^+ channels. Accordingly, after an action potential all voltage-gated Na^+ channels are also inactivated. The cell is then in the absolute **refractory period** during which no further action potential can be triggered. In the subsequent relative refractory period, voltage-gated Na^+ channels can be partially reactivated so that a strong depolarization

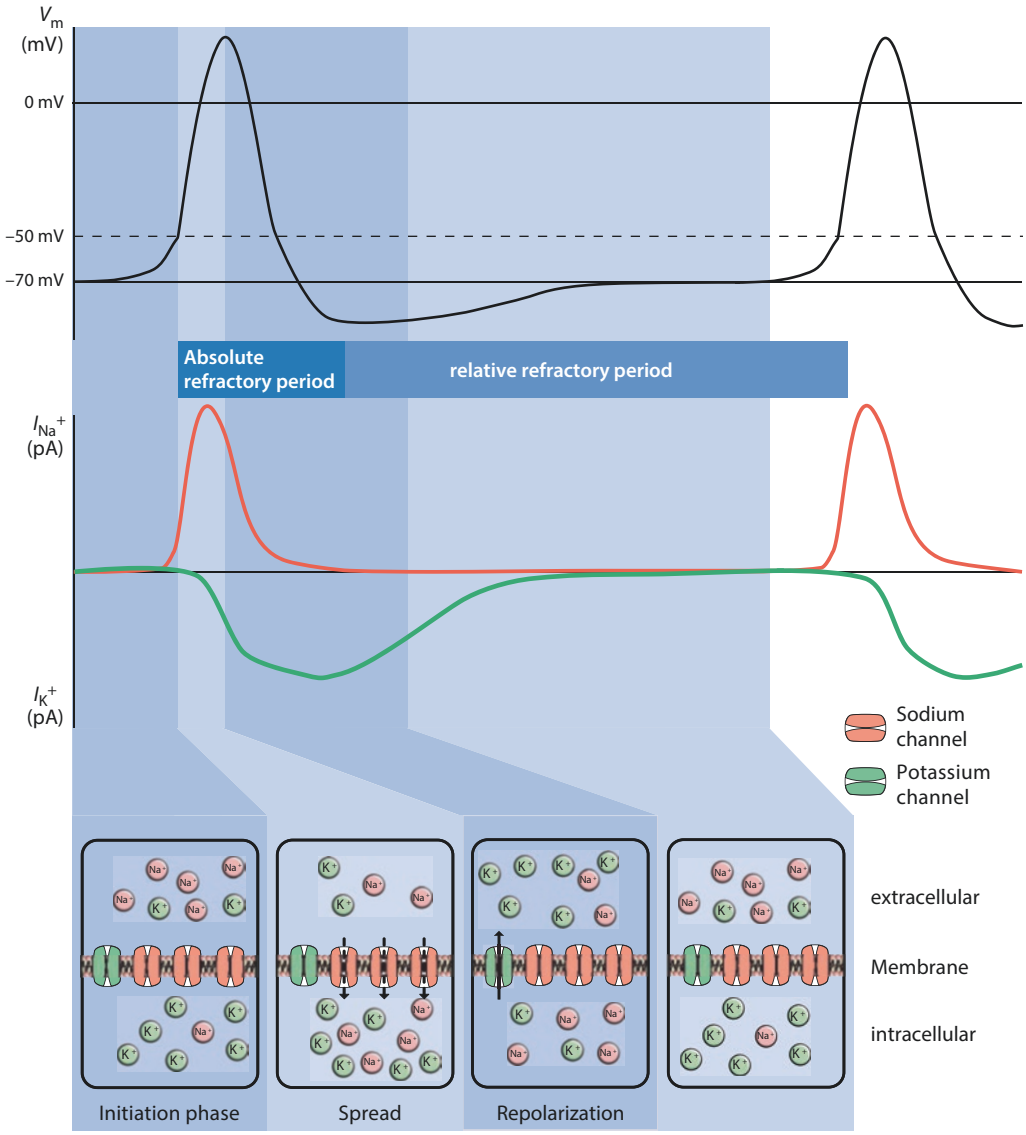

Fig. 4.4 The action potential. The upper part of the figure shows the course of the membrane voltage over time during two action potentials. Excitatory postsynaptic potentials depolarize the membrane potential during the initiation phase (▶ Sects. 4.14 and 4.16). When this then reaches the threshold potential (dashed line), there is a rapid, strong depolarisation with overshoot (positive membrane potential). During repolarization, the cell is briefly hyperpolarized. A further action potential can be formed at the earliest in the relative refractory period

can again trigger an action potential. The refractory period plays an important role in controlling the excitability of neurons. Mutations that shorten the duration of inactivation of voltage-gated Na^+ channels lead to hyperexcitability of neurons and are thus causative for certain forms of epilepsy (Catterall 2017).

4.5 Electrical Conduction

Changes in the membrane potential are passively and actively conducted in the membrane. To understand passive (*electrotonic*) conduction, it is helpful to think of the membrane as an electrical *capacitor-resistor element* (Fig. 4.5a). The lipid bilayer of the membrane is a capacitor because it can store electric charge as an insulating layer between two conducting media. However, depending on the number of open ion channels, the membrane also has some electrical conductivity (as the reciprocal of resistance). K^+ channels, for example, which are open even in resting neurons, reduce the membrane resistance and cause a leakage current. If the membrane potential changes at one site of a neuron, this membrane potential change will passively propagate across the membrane of the neuron. The capacitor property of the membrane explains why electrotonic conduction is quite slow and the amplitude of the propagated membrane potential change decreases with distance from the site of origin (Fig. 4.5). Accordingly, electrotonic conduction over long distances is not very effective. However, changes in membrane potential must sometimes be propagated over very long distances. The longest axons in humans, for example, can be over 2 m long (e.g., from neurons that conduct sensitive information from the big toe to the nucleus gracilis located in the brainstem). In the blue whale, axons can even be over 30 m long. Passive conduction would be much too slow here, and even with large changes in membrane potential at the axon hillock, there would be no measurable change in membrane potential at the synapses.

Electrical conduction over long distances must therefore be active. One speaks of active mechanisms when it comes to the opening of voltage-gated channels. Action potentials originate at the axon hillock. The density of voltage-gated Na^+ channels is particularly high in the membrane of this initial segment of the axon. From there, the action potential must be conducted to the synapses at the end of the axon. An axonal conduction of an action potential can be imagined as follows: An action potential arising at the axon hillock leads to depolarization of the neighboring axon membrane. This causes voltage-gated Na^+ channels to open. As in passive conduction, the action potential is thus continuously propagated. In contrast to passive conduction, however, the amplitude of the action potential does not reduce in active (*regenerative*) conduction (Fig. 4.5). An action potential is generally propagated only in the *orthodromic* direction, that is, from the axon hillock along the axon toward the synapses. Only in the orthodromic direction does the action potential encounter closed and activatable voltage-gated Na^+ channels. The inactivation of Na^+ channels that have just been activated and the opening of voltage-gated K^+ channels prevents *antidromic* conduction (▶ Sect. 4.4).

However, action potentials do not only play a role at the axon. So-called *back-propagating action potentials* can propagate from the axon hillock via the cell soma into the dendrites. The back-propagating conduction into the dendrites is based on passive (electrotonic) as well as active (activation of voltage-dependent Na^+ or Ca^{2+}-channels) mechanisms. The exact function of the retrograde action potentials has not yet been elucidated. However, it is likely that they remove the Mg^{2+} block of NMDA receptors (▶ Sect. 4.17) by depolarizing the dendritic membrane and thus influence synaptic signal transmission.

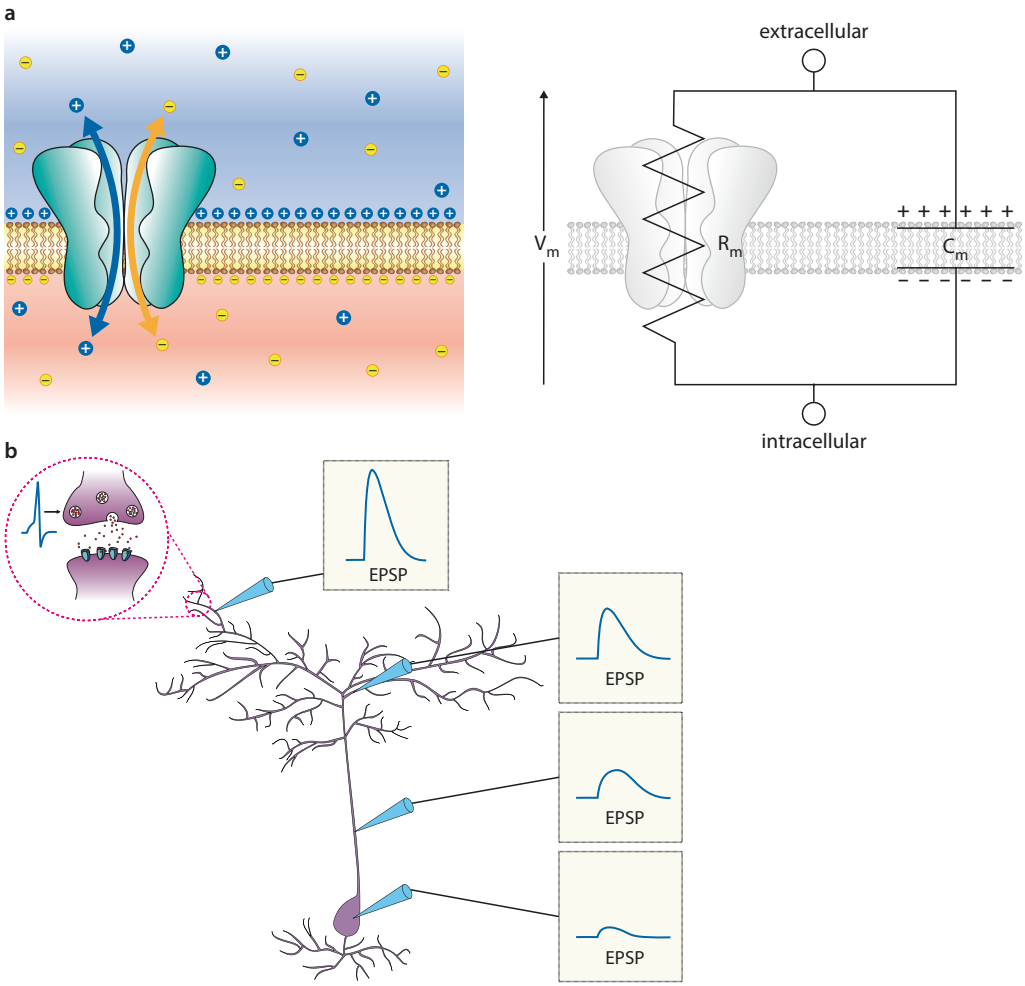

Fig. 4.5 Electrical conduction. **a** The membrane of neurons has properties of a capacitor-resistor element. Charges can be stored at the lipid bilayer, as in a capacitor. At the same time, a current can flow through ion channels. Depending on the number of open channels, the cell membrane has a resistance. **b** In passive (electrotonic) conduction, a current flow (e.g. by opening excitatory receptors) causes the membrane potential to change locally. As a result, the membrane potential of neighboring membrane sections is also changed, leading to passive conduction. Leakage currents flowing through open channels reduce the membrane potential change. In electrotonic conduction, the amplitude of the membrane potential change decreases with distance from the origin. At the same time, a high capacitance of the membrane slows electrical conduction. EPSP potential, excitatory postsynaptic: excitatory postsynaptic potential

4.6 Electrical Conduction Along Myelinated Axons

Although active/regenerative conduction prevents the reduction of the amplitude of the action potential, it is quite time-consuming. Very fast conduction is therefore not possible. In order for action potentials to be conducted not only over long distances but also very quickly, active mechanisms must be combined with passive mechanisms. This occurs in myelinated axons. **Myelination** is an electrically insulating layer that tightly surrounds the axon. It does not originate

from the neuron itself, but is formed by glial cells (oligodendrocytes in the central nervous system, Schwann cells in the peripheral nervous system), whose membrane protrusions grow in a spiral around the axon of the neuron (Fig. 4.6). As the degree of myelination increases, the number of lamellae enveloping the axon increases.

How does myelination affect conduction? In ▶ Sect. 4.5, we discussed that the membrane can be viewed as a capacitor-resistor element. Myelin, which has a high lipid content and a relatively low protein content, performs a similar function to the insulating plastic sheath around a power cable. It increases the membrane resistance and reduces the capacitance of the capacitor. The increased membrane resistance (reduction of leakage currents) and low electrical capacitance due to myelin reduce the amplitude drop of the electrotonically propagated action potentials and cause the membrane potential to change much more rapidly when current flows through it. As a result, myelinated axons conduct action potentials electrotonically much faster than unmyelinated axons. Since the thickness of the myelin layer increases the speed of conduction, long axons in particular, which have to conduct information very quickly, have a high degree of myelination, i.e. a thicker myelin sheath. This applies, for example, to axons of motor neurons in the cortex or spinal cord. Unmyelinated or poorly myelinated axons would not be able to initiate fast, coordinated movement patterns.

However, myelination cannot completely prevent the amplitude reduction of the action potentials. For this reason, there are regular interruptions of myelination in the course of the axon. These myelin-free axon regions are called **Nodes of Ranvier**. In the myelin-free regions are voltage-gated Na^+ channels in particularly high density. The opening of these channels leads to the regeneration of the action potential. While the electrotonic conduction velocity is very high in the myelinated region, there is a delay at the Nodes of Ranvier because the regeneration of the action potential by opening the Na^+ channels takes more time. The conduction is therefore not continuous, but the action potential appears to jump from one Node of Ranvier to the next Node of Ranvier. This is therefore referred to as saltatory conduction (Fig. 4.6). The maximum conduction velocity can reach 130 m s^{-1} in myelinated axons. In contrast, non-myelinated axons only conduct at a speed of 1–3 m s^{-1}.

Multiple Sclerosis and Guillain-Barré Syndrome

Multiple sclerosis (encephalomyelitis disseminata) is an inflammatory disease of the central nervous system that is associated with demyelination of axons. The resulting impaired conduction of excitation leads to deficits such as paralysis, sensory disturbances or visual disturbances. Since demyelination occurs in many different parts of the brain (hence dissiminata), the clinical picture is colourful. For example, patients may suffer from visual disturbance of the left eye, numbness of the right foot, and coordination problems and paralysis of both hands at the same time. As expected in a demyelinating disease, conduction is slowed, which can be documented by electroencephalogram (EEG). For example, a common examination in multiple sclerosis patients with visual disturbances is an EEG recording with visual stimulation. Shortly after the visual stimulus, a signal can be detected over the visual cortex, but because it is very small, it becomes visible only after averaging many EEG recordings. Signals that are independent of the stimulus are filtered out by averaging. The primary cortical visual evoked potential appears after about 100 ms in healthy individuals, and a latency prolongation is

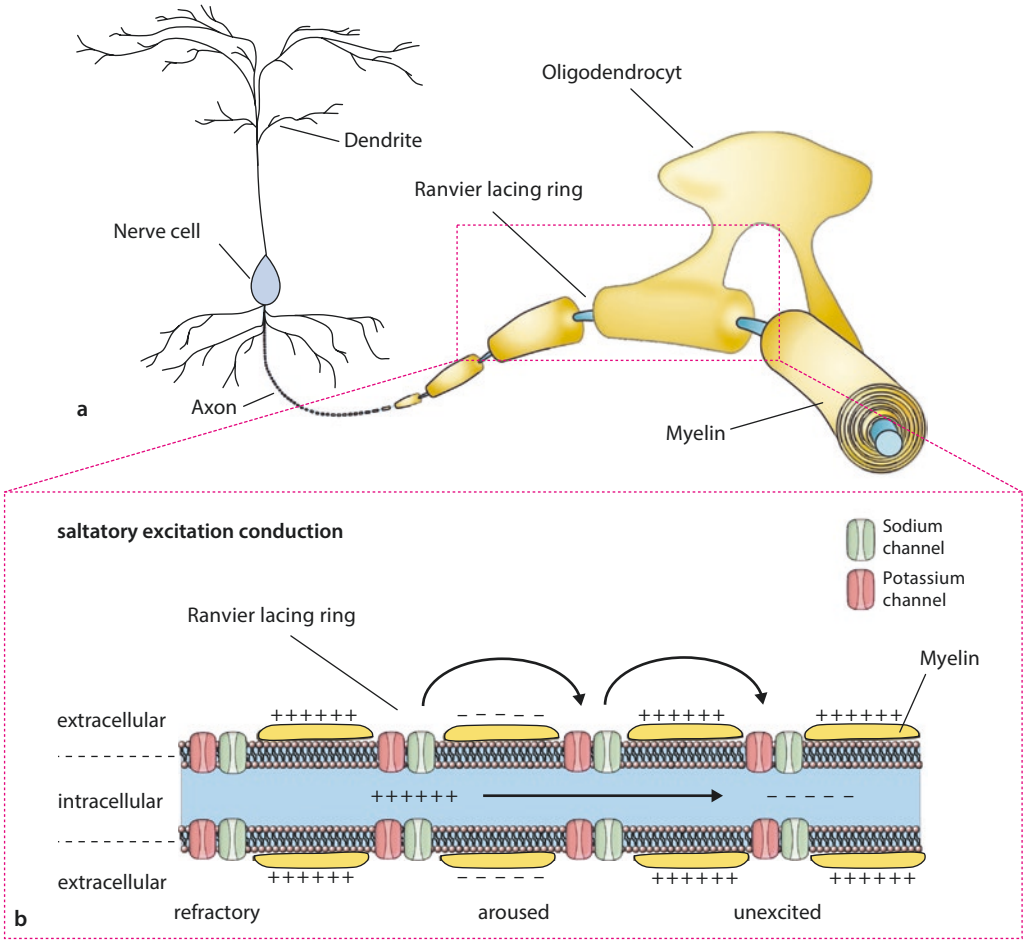

Fig. 4.6 Saltatory conduction. **a** The figure shows a neuron whose axon is covered by a myelin layer. The myelin layer, which is formed in the CNS by oligodendrocytes, is interrupted at regular intervals (Node of Ranvier). Myelination causes action potentials to be transmitted faster along the axon. **b** The high number of voltage gated sodium channels and the absence of myelin allow formation of action potentials at the Nodes of Ranvier. The depoplarisation is then passively transmitted from one Node of Ranvier to the next where a new regenerative action potential is formed. Condution of action potentials is usually unidirectional as the refractory period after sodium channel activation prevents backpropagation of the action potential

expected in patients with reduction in conduction. Demyelinating diseases are not unique to the central nervous system. Guillain-Barré syndrome, for example, is an inflammatory disease in which antibodies produced by the body (autoantibodies) are directed against the myelin sheath of the peripheral nerves. Here, too, the consequence of demyelination is slowed conduction, which leads to symptoms such as paralysis and sensory disturbances.

4.7 Synaptic Transmission

> So far as our present knowledge goes, we are led to think that the tip of a twig of the arborescence is not continuous with but merely in contact with the substance of the dendrite or cell body on which it impinges. Such a special connection of one nerve cell with another might be called a synapsis.
>
> Sir Charles Scott Sherrington 1897 (Foster 1897)

At the end of the nineteenth century, the Italian neuroanatomist Camillo Golgi developed a silver stain with which he could not stain the whole tissue, but only individual cells in the brain. This method, later further developed by the Spanish neuroanatomist Santiago Ramón y Cajal, made it possible for the first time to visualize the anatomy of neurons. Golgi and Ramón y Cajal thus laid the foundation for modern neuroscience and were awarded the Nobel Prize in 1906 for their achievements. However, both scientists drew very different conclusions from the observation of the stained cells. The different hypotheses were the cause of a bitter antagonism between the two scientists. Golgi's reticular theory stated that nerve tissue is a network, a "reticulum," of interconnected cells. Indeed, neurons can be directly connected to each other via *gap junctions*. However, such connections are the exception rather than the rule. Rather, neurons are separated from each other, as assumed by Ramón y Cajal, even if they are spatially in very close contact with each other. It was not until the 1950s that the dispute was settled in favor of the neuron theory by the development of electron microscopy. This microscopy is so high-resolution that it can be used to see the very small gap (30–50 nm) between the synapse **terminal** (**axon terminal** or *twig of the arborescence*) of one neuron and the dendrite or cell body of the second neuron. This point of contact between two neurons was named synapse by Charles Sherrington at the suggestion of his colleague Michael Foster and the philologist Arthur Verrall (Sherrington had initially preferred *syndesm as* a term, but was persuaded that synapse was the better choice, among other things because it allows a better adjective to be formed: synaptic versus syndesmic).

Today we know that neuronal "communication" between presynaptic and postsynaptic neuron takes place at the synapses. We speak of presynaptic and postsynaptic, since communication generally only goes in one direction: the presynaptic neuron "speaks", the postsynaptic neuron "listens". In this process, the electrical signal (the action potential) must be converted into a chemical signal. To do this, the presynaptic neuron releases transmitters that bind to receptors in the membrane of the postsynaptic neuron, causing it to be excited or inhibited. Presynaptic transmitter release is triggered by the action potential arriving at the axon terminal. In this process, voltage-gated Ca^{2+} channels are opened by depolarization of the cell membrane. Ca^{2+} entering the axon terminals binds to synaptotagmin—a protein that acts as a calcium sensor. The binding of Ca^{2+} to synaptotagmin in turn initiates the fusion of vesicles, i.e. small membrane-enveloped vesicles, with the presynaptic cell membrane via activation of proteins of the so-called SNARE complex (including synaptobrevin, syntaxin, SNAP25). The fusion results in the release (*exocytosis*) of transmitter molecules located in the vesicles into the synaptic cleft (Fig. 4.7) (Jahn and Fasshauer 2012). Presynaptic vesicles contain several thousand neurotransmitter molecules. Diffusion of the neurotransmitter across the synaptic cleft to the postsynaptic membrane occurs in a fraction of a millisecond. The membrane of the postsynaptic neuron contains receptors to which the transmitter molecules bind and thereby exert their effect.

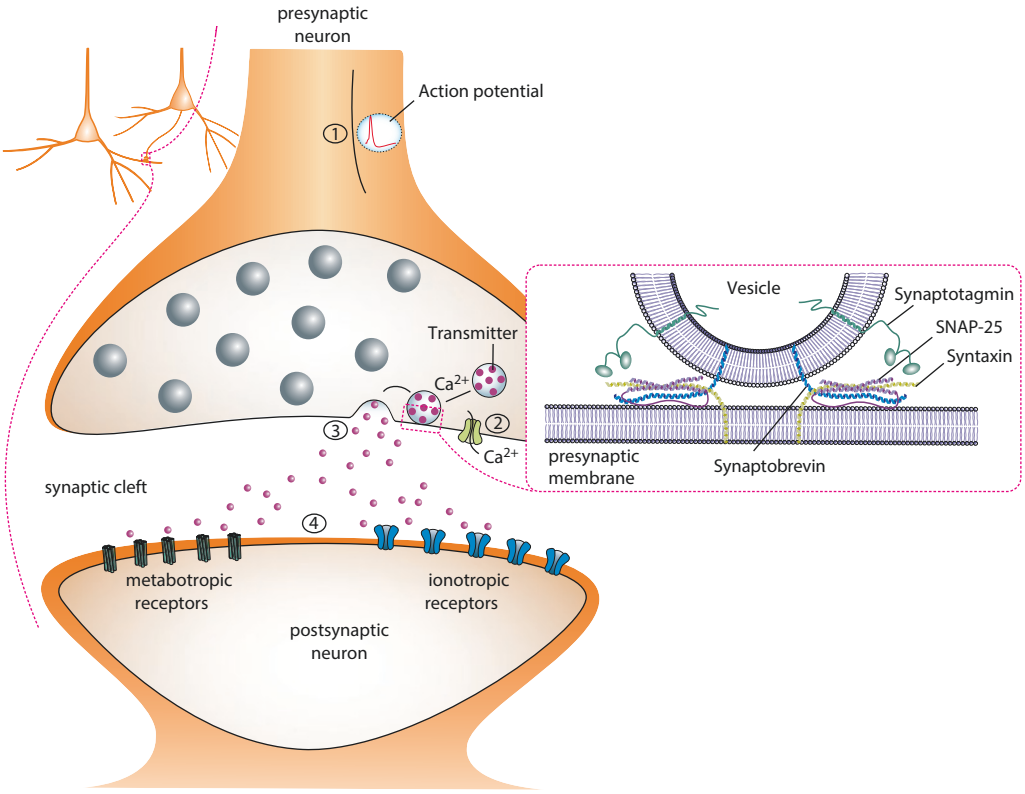

Fig. 4.7 Structure of a synapse. Top left: two nerve cells connected by a synapse. Middle: simplified structure of the synapse consisting of the axon terminal of the presynaptic nerve cell and the so-called *spine* of the postsynaptic nerve cell (Yuste 2015). An incoming action potential (1) leads to Ca^{2+} influx via presynaptic Ca^{2+} channels (2), which in turn initiates fusion of synaptic vesicles with the presynaptic membrane and subsequent transmitter release (3). The transmitter molecules then bind specifically to ionotropic or metabotropic receptors (4) localized in the postsynaptic membrane. Right: structure of synaptic proteins involved in calcium-dependent exocytosis. The inflowing Ca^{2+} binds to the vesicle protein synaptotagmin. As a consequence, synaptotagmin binds to the presynaptic membrane and to the SNARE complex, which is formed by the vesicle protein synaptobrevin and the two synaptic membrane proteins, syntaxin and SNAP-25. The SNARE complex contributes to the fusion of the vesicle membrane with the adjacent presynaptic membrane. (Jahn and Fasshauer 2012)

Tetanus and Botulism

The proteins of the SNARE complex play a crucial role in the symptoms of tetanus (lockjaw) and botulism. In both cases, bacteria (*Clostridium tetani* and *Clostridium botulinum*) produce toxins (tetanus toxin and botulinus toxin) that cleave proteins of the SNARE complex. Tetanus bacteria occur ubiquitously (especially in the soil). Infection occurs, for example, after an injury to a splinter of wood. If the injury is deep, the bacteria can multiply well, especially in an anaerobic (i.e. low-oxygen) environment, and produce tetanus toxin. This is transported retrogradely via axons of motor neurons into the spinal cord, where it exerts its effect in inhibitory neurons. Via cleavage of synaptobrevin, it inhibits synaptic transmission between inhibitory neurons and motoneurons. The reduced inhibition of motoneurons leads to increased activa-

tion of muscles and thus to the eponymous convulsions. Acute treatment consists of wound sanitation, antibiotic treatment, and administration of antitoxin (antibodies to tetanus toxin). Good protection is provided by active vaccination, in which the administration of tetanus toxoid (inactivated tetanus toxin) induces the production of protective antibodies.

Botulism is generally caused by food poisoning, usually meat or sausage, in which botulinum bacteria multiply due to improper food hygiene. Typically, these are home-cooked canned foods that have not been sterilized with overpressure and temperatures above 100 °C. In these, the also anaerobic bacteria can grow and build botulinus toxin. This toxin is then ingested and enters the motor neurons where, like tetanus toxin in inhibitory neurons, it cleaves synaptobrevin and thus inhibits the transmission from motor neuron to muscle. Paralysis is the result. In addition to symptomatic therapy (e.g. ventilation in the case of respiratory paralysis), the administration of antitoxin (antibodies against botulinus toxin) is indicated. In aesthetic medicine, the use of botulinus toxin as an anti-aging substance is widespread. There it is known as Botox and is mainly injected in the facial area, which leads to the slackening of muscles and thus to the temporary disappearance of wrinkles.

4.8 Motor End Plate

The motor endplate is a special form of synapse, as it does not allow communication between two neurons, but between neuron and skeletal muscle cell. However, the principles of chemical transmission are similar to synapses between two neurons. The neurotransmitter of the motor endplate is acetylcholine, which binds postsynaptically to **nicotinic acetylcholine receptors** of the muscle cell. These are ionotropic receptors (as distinct from *muscarinic* acetylcholine receptors; ▶ Sect. 4.10), through which ions can flow when the transmitter binds, depolarizing the muscle cell. The resulting action potential leads to a release of Ca^{2+} ions from the sarcoplasmic reticulum, which in turn causes the muscle cell to contract (electromechanical coupling). Acetylcholine is cleaved in the synaptic cleft within a very short time by the enzyme acetylcholinesterase to choline and acetate, which limits the duration of the chemical signal and thus also the muscle contraction.

4.9 Electrical Synapses

Camillo Golgi was not entirely wrong when he postulated that neurons form a syncytium in which neurons are directly connected. Although the spatial separation of neurons that communicate with each other via chemical synapses is the rule, neurons can also form direct connections via **gap junctions** (◘ Fig. 4.8; Bennett and Zukin 2004). These are channels formed from connexins. Six connexins form a connexon, and the interconnected cells each have a connexon that together form a gap junction. Gap junctions are found in many tissues (heart, liver, intestine, vessels), where they enable, among other things, a direct exchange of substances between neighbouring cells. However, the diameter of the gap junctions limits the exchange of substances to small molecules up to a size of approx. 500–1000 Da. This allows, for example, the exchange of second messengers such as cAMP or Ca^{2+}. In the brain, glial cells in

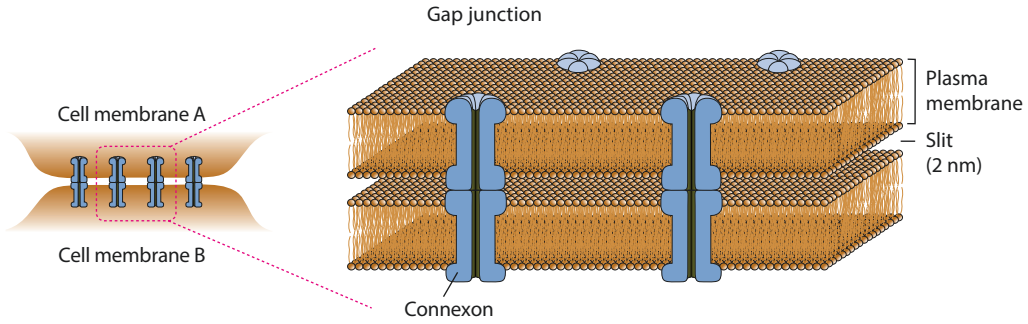

Fig. 4.8 Electrical synapses. Left: The plasma membranes of two cells are connected by gap junctions. Right: Schematic representation of a cross-section through the plasma membranes with connexons

particular express gap junctions. Direct connection between neurons via gap junctions is comparatively more common in invertebrates than in vertebrates. Gap junctions allow not only an exchange of substances, but also direct electrical communication between the two neurons. They are therefore also referred to as electrical synapses. In contrast to chemical synapses, the communication is bidirectional. Since the conversion of the electrical signal into a chemical signal is omitted, electrical synapses are also much faster. Thus, a depolarization in one neuron will cause a depolarization in a neuron connected by electrical synapses with minimal time delay. When many neurons are connected to each other via gap junctions, the activity of this neuron network can be synchronized by the electrical synapses. The synchronous activity of interneurons plays a role in the generation of oscillatory activity in the brain, for example.

4.10 Ionotropic Receptors

Ionotropic receptors are membrane proteins that not only bind neurotransmitters but also form an ion channel. The binding of the neurotransmitter on the extracellular membrane side leads to a structural change of the protein, which opens the ion channel. Depending on the membrane potential, ions flow in and out of the cell through the channel; thus, a current flows. However, ionotropic receptors are not equally permeable to all ions. Ionotropic **glutamate receptors** and **acetylcholine receptors** are examples of transmitter-gated cation channels. Binding of glutamate or acetylcholine opens channels that are permeable to Na^+ and K^+ ions. Depending on the membrane potential, there is an influx and efflux of Na^+ and K^+ ions (Fig. 4.9). At a resting membrane potential of −70 mV, the influx of Na^+ ions outweighs the efflux of K^+ ions resulting in a net inward current and thus a depolarization of the neuron. The membrane potential thus approaches the threshold potential for action potentials. Accordingly, glutamate and acetylcholine are excitatory neurotransmitters, and the neurons that release these transmitters are excitatory neurons. In contrast, the inhibitory neurotransmitters γ-aminobutyric acid (GABA) and glycine bind to transmitter-gated anion channels that are permeable to Cl^- ions. The influx of Cl^- ions into the cell through **$GABA_A$ receptors** or **glycine receptors** leads to hyperpolarization and thus to inhibition of the neuron.

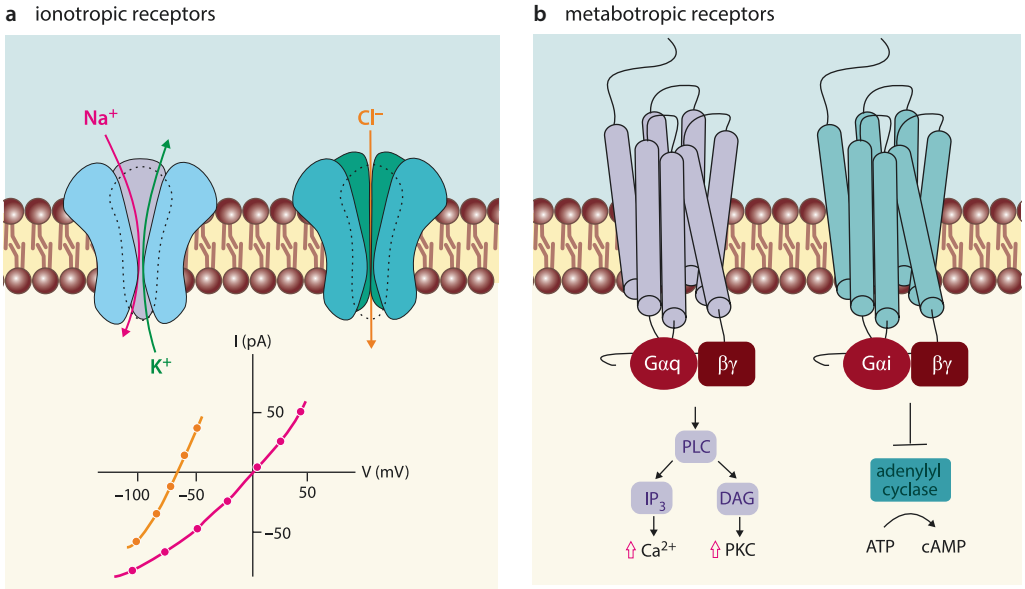

Fig. 4.9 Ionotropic and metabotropic receptors. **a** Structure and current voltage curves of ionotropic receptors. Ionotropic glutamate receptors and acetylcholine receptors (left) are (excitatory) cation channels that open after binding by a neurotransmitter and lead to membrane depolarization. The depolarization here is mainly carried by the inflowing sodium ions. As can be seen from the current-voltage curve, the reversal potentials of glutamate receptors are usually around 0 mV. At membrane voltages below 0 mV, the sodium influx predominates, i.e. the cell depolarizes; at membrane voltages above 0 mV, the potassium outflow predominates, and hyperpolarization occurs. However, physiologically important here is mainly the depolarizing effect, since membrane voltages above 0 mV are not seldom reached at the synapse—$GABA_A$ receptors conduct Cl^- ions, which means that opening the channel usually leads to a hyperpolarization of the cell. **b** Structure and intracellular signaling cascades of metabotropic receptors. Metabotropic receptors differ in their action primarily by the G protein coupled to the receptor. G_q-coupled receptors increase intracellular Ca^{2+} levels via activation of phospholipase C and stimulate protein kinase C activity. G_i-coupled receptors inhibit the adenylate cyclase and thereby increase the intracellular cAMP level

4.11 Metabotropic Receptors

The effect of binding of a neurotransmitter to metabotropic receptors is more indirect than that to ionotropic receptors. Metabotropic receptors are not channels, but exert their effect by activating intracellular signalling cascades and changing the concentration of intracellular messengers (second messengers). Primarily, they activate proteins on the intracellular side. Many neurotransmitters bind to G protein-coupled metabotropic receptors. Binding of the neurotransmitter to these receptors leads to activation of the guanosine triphosphate (GTP)-binding protein (G protein for short), which itself is composed of three subunits (α-, β- and γ-subunit). The consequence of this activation depends on which G protein is coupled to the receptor. For example, metabotropic glutamate receptors, of which there are eight subtypes ($mGluR_1$–$mGluR_8$), are either G_q-coupled or G_i-coupled. Activation of G_q-coupled receptors

($mGluR_1$ and $mGluR_5$) leads to activation of a signaling cascade that ultimately results in activation of protein kinase C and release of Ca^{2+} ions from the endoplasmic reticulum. In contrast, activation of G_i-coupled receptors ($mGluR_{2-4}$ and $mGluR_{6-8}$) inhibits adenylate cyclase, thereby reducing the intracellular concentration of the second messenger cAMP. The consequences of Ca^{2+} or cAMP concentration change and activation of protein kinase C are complex and dependent on neuron type.

Another example of a metabotropic receptor is the $GABA_B$ receptor. In contrast to the $GABA_A$ receptor, which is an ionotropic channel that inhibits the neuron via a Cl^- ion influx when opened, the $GABA_B$ receptor is a metabotropic receptor coupled to a G protein. Binding of GABA to the $GABA_B$ receptor leads to postsynaptic opening of K^+ channels via activation of the G protein. The efflux of K^+ ions from the cell hyperpolarises the cell (insofar as its membrane potential is less negative than the equilibrium potential for K^+), and an IPSP or IPSC (▶ Sects. 4.12, 4.13, and 4.14) can be measured. However, metabotropic receptors (the same is true to some extent for ionotropic receptors) are not only expressed postsynaptically. They are also found presynaptically, where, for example, activation of mGluRs or $GABA_B$ receptors reduces the likelihood that neurotransmitters will be released. Excessive transmitter release can thus be prevented via presynaptic receptors. The effect of metabotropic receptors lasts much longer than that of ionotropic receptors. For example, a $GABA_B$ receptor-mediated IPSP can last one second, whereas a $GABA_A$ receptor-mediated IPSP, in contrast, has kinetics that are approximately 50-fold faster. Metabotropic receptors are therefore also thought to have a signal-modulating effect.

4.12 Excitatory and Inhibitory Postsynaptic Potentials

The influence that a presynaptic neuron exerts on a postsynaptic neuron depends on which neurotransmitter has been released and which receptors the postsynaptic neuron carries on its membrane. Excitatory transmitters, such as glutamate and acetylcholine, generally lead to membrane depolarization by binding to their specific ionotropic receptors, while inhibitory transmitters lead to hyperpolarization. Excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) are thus potentials that either bring the membrane potential closer to or remove it from the threshold potential for triggering an action potential. The currents underlying an EPSP and IPSP are referred to as *excitatory postsynaptic current (EPSC)* and *inhibitory postsynaptic current (IPSC)*. EPSPs, IPSPs, EPSCs and IPSCs are short-lived (a few milliseconds) because the neurotransmitters very quickly unbinds from the receptors and diffuse out of the synapse (◘ Fig. 4.10). The duration of action of neurotransmitters is also limited by the fact that they are either degraded very rapidly (e.g. acetylcholine by acetylcholineesterase) or eliminated by uptake into neurons or glial cells.

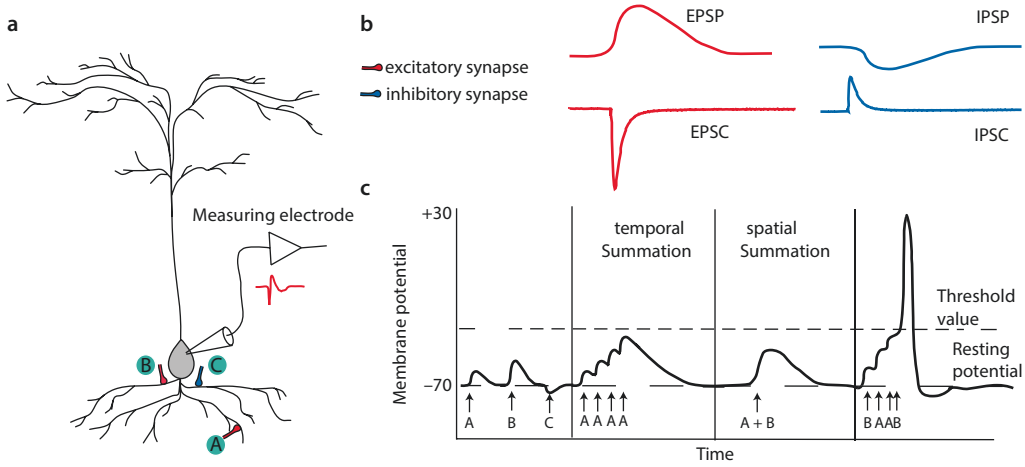

Fig. 4.10 Excitatory and inhibitory currents and potentials. **a** Excitatory and inhibitory synapses are located throughout the dendritic tree; inhibitory synapses are often located at the cell body. **b** Excitatory and inhibitory postsynaptic currents (EPSC and IPSC) and excitatory and inhibitory postsynaptic potentials (EPSP and IPSP) result from the activation of excitatory and inhibitory synapses. **c** Activation of individual excitatory and inhibitory synapses usually changes the membrane potential only slightly. When a synapse is activated repeatedly over a short period of time, temporal summation results in a greater change in membrane potential. When multiple synapses are activated, summation of the individual potentials occurs (spatial summation). If many synapses are active in a short period of time, the spatial and temporal summation can result in a potential change that is suprathreshold, so that an action potential is generated

4.13 mEPSPs and mIPSPs

The amplitude of EPSPs and IPSPs depends on the neurotransmitter concentration in the vesicles, the number of vesicles exocytosed in response to an action potential, and the number of postsynaptic receptors. So-called miniature EPSPs (mEPSPs) and miniature IPSPs (mIPSPs) occur when only one vesicle fuses with the presynaptic membrane. The quantum hypothesis developed in this context states that when multiple vesicles are released, the amplitudes of EPSPs and IPSPs are integer multiples of the amplitudes of mEPSPs and mIPSPs.[1] Here, the mEPSPs and mIPSPs represent the so-called "quanta" as the smallest and indivisible responses. The quantum hypothesis was postulated based on observations of the motor endplate by Bernard Katz. The motor endplate is the synapse between the motoneuron and muscle. The motoneuron releases acetylcholine as a transmitter, which binds to nicotinic acetylcholine receptors in the membrane of the muscle cell. The depolarization in response to exocytosis of an acetylcholine vesicle is referred to here as miniature endplate potential (mEPSP). Bernard Katz noted that the amplitudes of muscle cell depolarization are integer multiples of this mEPSPs.

mEPSPs and mIPSPs as well as their underlying currents (mEPSCs and mIPSCs) can be visualized by a methodical trick. This involves blocking voltage-gated Na^+ channels with tetrodotoxin (TTX) derived from puffer fish of the family Tetraodontidae. This prevents action potential generation and thus action potential-induced exocytosis of vesicles. In rare cases, however, fusion of a vesicle with the presynaptic membrane still occurs. Since this fusion is not triggered by an action potential, there is no temporally coordinated exocytosis of multiple vesicles.

1 Despite the similarity of terms, however, the quantum hypothesis has primarily nothing to do with quantum physical phenomena.

The response of the transmitter molecules of one vesicle can then be measured as mEPSP, mIPSP, mEPSC or mIPSC in the postsynaptic neuron. The analysis of these smallest responses is interesting for two main reasons: First, the frequency with which they occur correlates with the number of synapses of the postsynaptic neuron, and second, the amplitude of the responses correlates with the number of receptors per synapse.

4.14 Integration of EPSPs and IPSPs

A single EPSP is normally not able to depolarize a neuron to the extent that the threshold potential is reached at the axon hillock and the neuron generates an action potential. Rather, an integration of many EPSPs and IPSPs results in a constant fluctuation of the membrane potential. A presynaptic neuron often forms multiple synapses with a postsynaptic neuron. Nevertheless, the activity of a single presynaptic neuron is usually not sufficient to trigger an action potential in a postsynaptic neuron. The more or less coordinated activity of several excitatory neurons is necessary for the postsynaptic neuron to reach the threshold potential. This is partly because an action potential does not result in the release of vesicles in every synapse (the presynapse is not particularly reliable, ▶ Sect. 4.16), and partly because a single EPSP changes the membrane potential very little. Locally at the synapse, an EPSP often already has a small amplitude. Due to the electrotonic (i.e. purely passive) signal transmission from the dendritic synapses towards the cell body, the amplitude reduces even further (▶ Sect. 4.6) and the resulting depolarization at the axon hillock is very small (in fact, it is around 1 mV). Synapses located farther from the soma are thus at a disadvantage and have less weight in generating action potentials than synapses located closer to the axon hillock. Distally arising EPSPs or IPSPs may nevertheless play a relevant role because their amplitude at the site of origin is either relatively large or by activating dendritic voltage-gated Na^+ and Ca^{2+} channels.

Despite these amplification mechanisms, one can imagine that at a resting membrane potential of about −70 mV, a summation of many EPSPs is necessary to reach a threshold potential of about −50 mV (◘ Fig. 4.10). Here, the active synapses may be widely separated in different dendrites. The EPSPs are ultimately summed at the axon hillock. In addition to this **spatial summation**, there is also temporal summation (and combinations of spatial and temporal summation). **Temporal summation** of EPSPs occurs when they are not triggered by synaptic activity at the same time. For EPSPs to sum, they need only occur in a temporally overlapping time window; precisely coordinated synaptic activity is not required (◘ Fig. 4.10). An IPSP arriving in the same time window will again reduce the resulting depolarization and thus the probability that an action potential will be generated. Inhibitory synapses are found throughout the dendrites, but in particularly high density at the cell body, i.e., in strategic proximity to the axon hillock. There they are ideally positioned to effectively prevent the generation of action potentials.

Inhibitory transmitters such as GABA can inhibit a neuron even if the opening of their receptors does not lead to an IPSP. The reversal potential for Cl^- ions is quite close to the resting membrane potential. If the reversal potential is equal to the resting membrane potential, the opening of Cl^--permeable channels will lead to no IPSP. There are neurons whose resting membrane potential is even more negative than the reversal potential for Cl^- ions. In this case, the opening of Cl^--permeable channels even leads to a depolarization of the neuron (formally, one can then measure an EPSPs in electrophysiological studies). In general, the neuron is nevertheless inhibited by the opening of Cl^--permeable channels (e.g. $GABA_A$-receptors). This is partly because

the reversal potential for Cl^- ions is more negative than the threshold potential. Once the membrane potential through EPSPs approaches the threshold potential, and the membrane potential is then less negative than the reversal potential for Cl^- ions, the opening of Cl^--permeable channels will again trigger an IPSP. On the other hand, the opening of Cl^--permeable channels reduces the membrane resistance. In ▶ Sect. 4.6 we mentioned that the amplitude of membrane potential changes depends on the membrane resistance. The lower this is, e.g. if many K^+ channels are open causing leakage currents, the smaller the membrane potential change will be. In a very similar way, open Cl^--permeable channels reduce the amplitude of EPSP, even if the Cl^--permeable channels themselves do not trigger IPSP or even depolarize the neuron. This is referred to as ***shunting inhibition***.

Electroencephalography

Electroencephalography (EEG) is used to graphically record electrical brain activity via electrodes attached to the scalp. In the process, potential fluctuations are derived from many neurons, especially cortical neurons (primarily potentials that result from synaptic activity). Depending on the state of wakefulness or consciousness, the activity of populations of neurons synchronizes. This synchronous activity is reflected in oscillatory EEG signals. The frequency spectrum of the EEG can be analyzed quantitatively by Fourier analysis. Most often, however, the predominant frequency is read directly from the EEG by the experienced examiner. α-waves (alpha waves; 8–13 Hz) are found in the EEG as the dominant frequency in awake and relaxed people with eyes closed (especially over the occipital cortex). When the eyes are opened or mentally tense, desynchronization occurs and faster β-waves (beta-waves; 13–30 Hz) replace the α-waves (α-blocking; arousal response).

During sleep, the dominant EEG frequency slows down, ϑ-waves (theta-waves; 4–7 Hz) and with increasing sleep depth δ-waves (delta-waves; 0.5–3 Hz) are observed. The slow frequencies are explained by the fact that as the state of wakefulness or consciousness decreases, the thalamus determines the rhythm of cortical activity, leading to the synchronous activity of large populations of neurons. As the number of synchronously active neurons increases so does the amplitude of the recorded potential fluctuations. δ-waves therefore generally have a higher amplitude than β-waves. γ-waves (gamma-waves; 30–80 Hz) occur in the awake and alert state, but have such small amplitudes that intracortical EEG recordings are usually required for their analysis. The sensitivity of intracerebral EEG is so good that action potentials of individual neurons can also be recorded. This means, for example, that the single-cell activity of over a hundred neurons can be recorded simultaneously and correlated with specific behavioral patterns. Intracerebral EEGs are not only used in animal experiments as part of basic research, but are also performed on epilepsy patients as part of preoperative diagnostics. EEGs are of particular importance in epilepsy diagnostics. In addition to the frequency spectrum, the neurologist primarily analyses the forms of the potential fluctuations. During an epileptic seizure, the synchronous activity of large neuron populations can be seen in the high-amplitude potential fluctuations. In the seizure-free interval, characteristic potential fluctuations (epilepsy-typical potentials; e.g. *spike-waves*) indicate the presence of the disease. The EEG is also used in brain death diagnostics. Here, the absence of potential fluctuations (zero-line EEG) indicates irreversible brain damage or brain death.

4.15 Synaptic Short-Term Plasticity

The strength of communication between presynaptic and postsynaptic neuron is variable. This variability is the basis for adaptive processes, learning and memory formation. Different mechanisms cause the amplitude of postsynaptic responses to increase or decrease for short but also very long periods of time. If the change only lasts for a short time (a few seconds), we speak of short-term plasticity.

Short-term plasticity refers to the change in synaptic responses when a synapse is active several times in a short period of time, e.g. when the presynaptic neuron fires at a frequency of 10 Hz. This can increase or decrease the amplitude of the responses (Fig. 4.11). This is referred to as synaptic **facilitation** or **depression, respectively**. There are presynaptic and postsynaptic mechanisms that lead to a short-lasting change in synaptic strength. On the presynaptic side, high frequency activity generally leads to a change in the probability that a transmitter-filled vesicle will be released. In synapses where the probability of release is very high, each subsequent action potential reduces this probability. The first action potential and the resulting Ca^{2+} -influx have a high probability to trigger a release if there are vesicles that are very close to the cell membrane and are just "waiting" for the signal to exocytose. When the presynaptic neuron is active again shortly afterwards, these easily released vesicles no longer exist. Each subsequent action potential releases fewer vesicles. The postsynaptic responses therefore become smaller (depression). The situation is quite different in synapses where the release probability is low. In these synapses, the presynaptic Ca^{2+} influx is often insufficient for a vesicle to be exocytosed at all. The repetitive activity of such synapses leads to an accumulation of Ca^{2+} in the axon terminal so that the release probability increases with each action potential. This is referred to as synaptic facilitation.

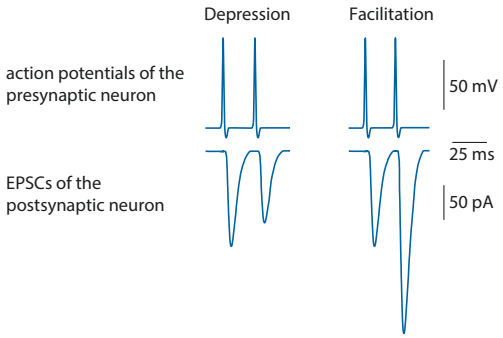

Fig. 4.11 Synaptic short-term plasticity. If a presynaptic neuron is excited several times within a short period of time, synaptic short-term plasticity may occur. Short-term plasticity can occur as facilitation or depression. Facilitation means that EPSC amplitudes increase. Depression causes the opposite, i.e., the EPSC amplitude decreases. Both pre- and postsynaptic mechanisms can be responsible for both mechanisms

In fact, the release probability in synapses of the central nervous system is mostly smaller than 1. There are for example synapses where the release probability is 0.05. This means that only every 20th action potential triggers the exocytosis of a vesicle. The effectiveness of communication between two neurons is increased by the fact that a presynaptic neuron often has more than one synapse with the postsynaptic neuron. Nevertheless, a single action potential does not always lead to a postsynaptic response. Synaptic facilitation then strengthens these less effective connections.

In some synapses with high release probability, postsynaptic mechanisms play an important role in short-term plasticity. The binding of the transmitter to its receptor not only causes it to open but also to desensitize, i.e. to become less sensitive. Some receptors are slow to recover from this desensitization. Thus, in synapses with a high release probability the prolonged desensitization of receptors can reduce the current amplitude and thereby contribute to synaptic depression.

4.16 Synaptic Long-Term Plasticity

In contrast to short-term plasticity, changes in synaptic strength in long-term plasticity can last for many hours or days. It is likely that long-term plasticity is a fundamental mechanism for the long-lasting storage of learned material in memory. Communication between neurons can be strengthened and weakened. This is referred to as long-term potentiation and long-term depression, respectively. The Canadian psychologist Donald Hebb postulated in 1949 that the synaptic communication of two neurons is strengthened if the activity of one neuron repeatedly causes activity in the other neuron (Hebb 1949) (*what fires together, wires together*). Long-term potentiation can explain, for example, the synaptic changes in **classical conditioning**: When an animal repeatedly hears a tone just before or during an electric shock *fear conditioning* occurs. After conditioning, the animal will respond with a fear response when it hears the tone. Before conditioning, the tone, as a **neutral stimulus**, will not elicit a fear response. At the cellular level, we can imagine that before conditioning the auditory neurons elicit an EPSP but no action potential in neurons of the amygdala, a brain region that is important for the fear response. The electrical shock will elicit an EPSP large enough to form an action potential in the same cells. Thus, as an **unconditioned stimulus** (UCS), the electrical shock will always elicit a fear response. If sound and shock occur repeatedly together the auditory neurons will be active more or less simultaneously with the neurons of the amygdala. Thus, as predicted by Hebb, synaptic communication is strengthened and in our example in such a way that, after conditioning, activity in the auditory neurons triggers a suprathreshold EPSP in the neurons of the amygdala. The tone thus becomes a **conditioned stimulus** (CS). If the tone is later repeatedly presented without electrical shock the fear response will decrease (**extinction**). The influence of auditory neurons on neurons of the amygdala will weaken again. Long-term synaptic depression, in which there is a prolonged weakening of synaptic communication, appears to play a role in extinction.

The timing of presynaptic and postsynaptic neuron activity is important in determining whether potentiation or depression occurs (Markram et al. 1997). This is referred to as ***spike-timing-dependent plasticity***. In general, long-term potentiation occurs when the presynaptic neuron is active just before the postsynaptic neuron (◘ Figs. 4.12 and 4.13). This temporal sequence suggests a causal link. In our fear conditioning example, the auditory neurons will be active before the neurons in the amygdala. With this apparent causal connection (tone triggers electrical shock) conditioning makes sense. The tone predicts the shock that is about to follow. If the tone is not followed by a shock, e.g., if the shock-tone sequence is reversed, then the tone has no predictive value for the occurrence of the tone. Synaptic connections in which the presynaptic neuron is repeatedly active after the postsynaptic neuron are generally weakened accordingly.

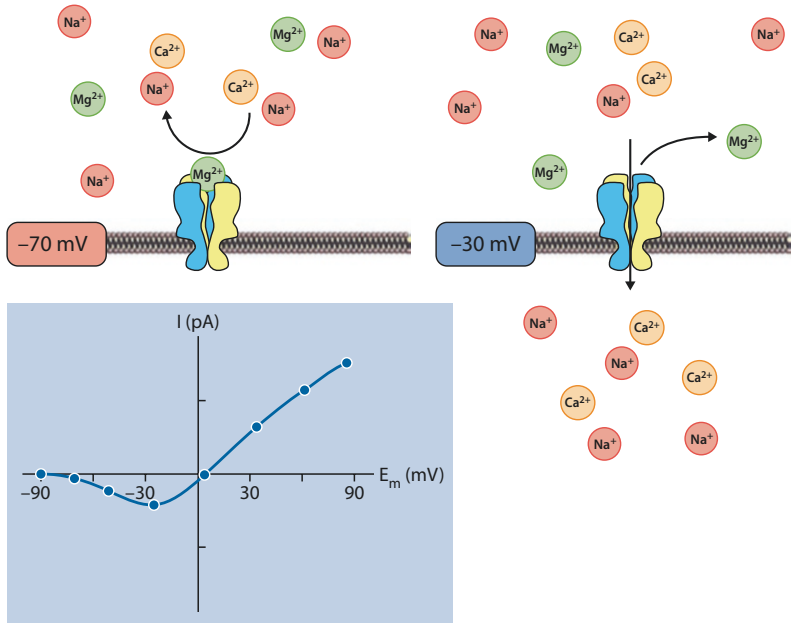

Fig. 4.12 Magnesium block of NMDA receptors. NMDA receptors have a voltage dependence mediated by Mg^{2+} ions. If the receptors open after binding glutamate at values around the resting membrane potential of a nerve cell (approx. −70 mV), the Mg^{2+} ions attracted by the negative cell interior block the receptor because they cannot pass through it. This so-called Mg^{2+}-block also prevents the other ions (especially Na^+ and Ca^{2+} ions) from passing through the channel. However, if the membrane potential shifts towards more positive values the Mg^{2+} block is reduced. As a result, more and more Na^+ and Ca^{2+} ions can pass the receptor and an EPSP is formed. The dependence of the ion flux on the membrane voltage can be shown particularly well in a so-called current-voltage curve in which the current flow through the receptor is plotted against the membrane voltage of the cell. In the curve shown here for an NMDA receptor it can be seen that no current flows through the receptor at values below −70 mV. With depolarization of the membrane potential, the Mg^{2+} block described above increasingly decreases and a current flow occurs

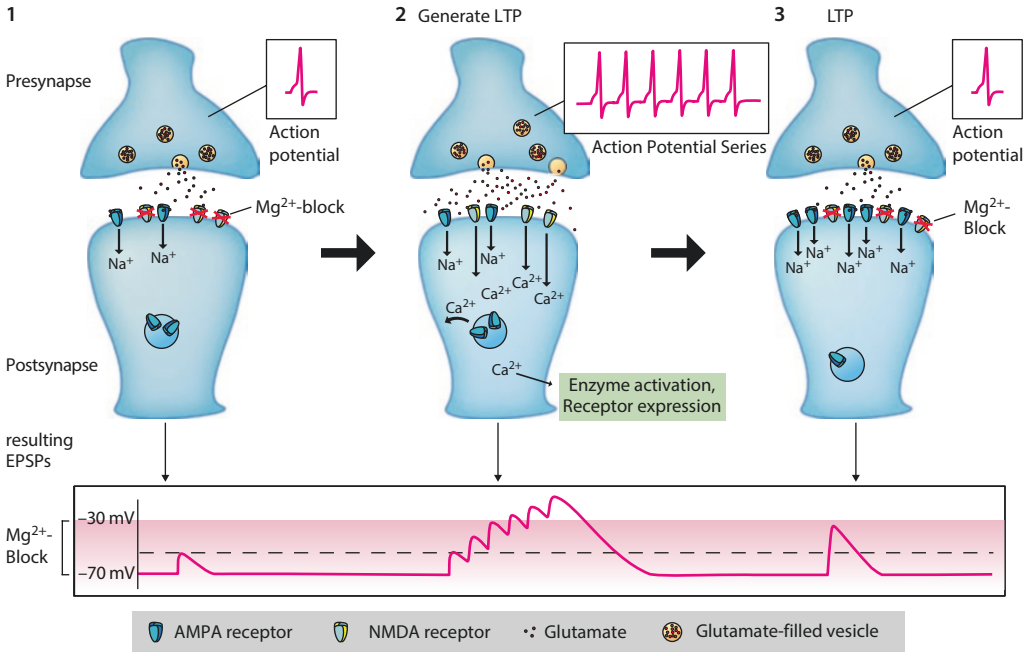

Fig. 4.13 Development of long-term potentiation. At excitatory synapses of the brain, action potentials arriving at the presynapse lead to Ca^{2+}-mediated release of glutamate-filled vesicles and binding of glutamate to postsynaptic glutamate receptors. (1) At negative resting membrane potential, only AMPA receptors open because NMDA receptors are blocked by Mg^{2+} ions. The depolarization resulting from opening of fewer AMPA receptors is too small to remove the Mg^{2+}-block. (2) Upon higher frequency activation of the synapse and temporal summation of EPSPs, the Mg^{2+} -block of NMDA receptors is removed. Ca^{2+} ions flowing into the cell through NMDA receptors lead to increased AMPA receptor incorporation into the synapse via various mechanisms. (3) Subsequently, when another action potential encounters a synapse enhanced by the incorporation of more AMPA receptors it generates an EPSP that is larger than the initial EPSP. Since the amplification of the EPSP can persist over long periods of time it is referred to as long-term potentiation. (Bredt and Nicoll 2003)

4.17 Cellular Mechanisms of Synaptic Long-Term Plasticity

What happens at the synaptic level during long-term potentiation and long-term depression? As with short-term synaptic plasticity, there are in principle presynaptic and postsynaptic mechanisms that can contribute to changes in synaptic communication strength. In contrast to short-term plasticity, however, postsynaptic mechanisms play a greater role in most synapses.

Changes in presynaptic function were analyzed in particular detail by the American neurophysiologist Erik Kandel and his colleagues in the marine snail *Aplysia*. They investigated the cellular mechanisms underlying associative and non-associative memory. **Associative learning** was studied using **classical conditioning** in which an electric shock was used as an unconditioned stimulus and touching the snail's siphon was used as a neutral stimulus. After repeated pairing of both stimuli, touching the siphon elicits a contraction of the snail's gill. This results in a long-lasting strengthening of communication between sensory neurons which are active when the siphon is touched and postsynaptic motor neurons which are responsi-

ble for the contraction of the gill. The **induction** of presynaptic long-term potentiation (LTP) requires the activity of interneurons that are active when the electric shock is applied. These interneurons secrete the transmitter serotonin, which binds to metabotropic receptors of sensory siphon touch neurons. In the axon terminals of sensory neurons, second messenger signaling cascades increase the release probability for glutamate vesicles. The resulting long-term potentiation of synaptic communication explains why touching the siphon triggers gill contraction even days after conditioning. Again, the order of stimulation plays a role. A long-term potentiation and conditioned response is observed when touching the siphon occurs just before the electrical shock. If the stimulation is reversed, long-term depression (LTD) occurs.

The mechanisms that lead to the strengthening of the synaptic connection in classical conditioning are in principle very similar to the mechanisms that play a role in **sensitization**. Here, a repeatedly applied strong electrical stimulus enhances the transmitter release of sensory neurons that are active upon electrical stimulation. These presynaptic changes are also triggered via the activity of serotonergic neurons. The resulting long-term potentiation between sensory and motor neurons causes gill contraction to increase in response to weak electrical stimuli. Because sensitization occurs on repeated application of an electrical stimulus, i.e., does not require pairing with another stimulus, it is a form of **nonassociative learning**. In contrast, with primarily rather weak electrical stimuli **habituation** occurs another form of nonassociative learning. The serotonergic interneurons are not activated to a sufficient extent so that the strength of synaptic communication between sensory and motor neurons weakens. Consequently, gill contraction decreases with repeated application of a weak electrical stimulus.

In more complex organisms, presynaptic and postsynaptic mechanisms lead to the sustained strengthening of neuronal communication. A prominent example of presynaptic long-term potentiation is the long-lasting strengthening of communication between granule cells and CA3 neurons in the hippocampus. Granule cells of the dentate gyrus project with their axons known as mossy fibers to CA3 neurons. High-frequency stimulation of mossy fibers induces long-term potentiation in granule cell-to-CA3 synapses. Activation of second messenger signaling cascades thereby increases the release probability of glutamate vesicles in axon terminals of granule cells (similar to sensory neurons of *Aplysia*). This potentiates EPSP amplitudes in postsynaptic CA3 neurons.

In the hippocampus, CA3 neurons project to CA1 neurons. Here, high-frequency stimulation or even low-frequency stimulation in which a presynaptic action potential is paired with depolarization of the postsynaptic neuron induces a postsynaptic form of long-term potentiation. Crucial for the induction of potentiation are the activation of NMDA-type glutamate receptors and an influx of Ca^{2+} ions into the postsynaptic neuron. NMDA receptors have two properties that make them central players in the induction of synaptic plasticity (◘ Figs. 4.12 and 4.13). First, at a resting membrane potential of, say, −70 mV, NMDA receptors are blocked by Mg^{2+} ions which sit on the extracellular side in the channel of NMDA receptors and prevent other ions from flowing through the channel (◘ Fig. 4.12). Thus, binding of glutamate to NMDA receptors does not result in current flow in nondepolarized neurons. When the neuron is depolarized, Mg^{2+} ions leave the channel of NMDA receptors. At membrane potentials more positive than −30 mV, Mg^{2+} ions do not block NMDA receptors so that binding of glutamate can induce a current. Thus, NMDA receptors are **coincidence detectors** that open only in the

presence of simultaneous activity of the presynaptic (release of glutamate) and postsynaptic neuron (depolarization). Let us recall Donald Hebb. He had postulated that neurons strengthen their connection when they are active together. Thus, the molecular basis for "Hebbian learning" is the NMDA receptor, since it can detect joint activity. This also explains why synaptic plasticity is generally restricted to the synapses where this simultaneous activity occurs (**input specificity**). High-frequency presynaptic activity, for example, can lead to postsynaptic depolarization sufficient to open NMDA receptors. Less active synapses, where depolarization is insufficient, are not potentiated. However, if they are active at the same time as a synapse that leads to strong depolarization they too can be potentiated (**associativity**). Potentiation of "weak" synapses upon synchronous activation of "strong" synapses is a form of synaptic plasticity that likely underlies associative learning. In our example above, before conditioning, auditory stimuli activate weak synapses. Simultaneous application of electrical stimuli stimulates strong synapses such that depolarization is sufficient to open NMDA receptors in the weak synapses. These are thereby potentiated, the primarily neutral stimulus becomes a suprathreshold conditioned stimulus.

The second property of NMDA receptors that is crucial for the induction of synaptic plasticity is their high permeability for Ca^{2+} ions. Ca^{2+} ions flowing into the postsynaptic neuron activate intracellular signaling cascades as second messengers, which ultimately lead to the expression of long-term potentiation. Kinases such as Ca^{2+}/Calmodulin kinase II (CaMKII), protein kinase A and C play important roles in this process (Raymond 2007). The subsequent phosphorylation of AMPA receptors increases their conductance. In addition, new AMPA receptors are incorporated into the synaptic membrane. Both mechanisms lead to a rapid potentiation of synaptic transmission (Fig. 4.13).

AMPA receptors are not expressed in every excitatory synapse. These synapses are also referred to as *silent synapses* since they do not form EPSPs at a resting potential of, for example, −70 mV. However, silent synapses may express NMDA receptors the activation of which leads to the incorporation of AMPA receptors during the induction of synaptic plasticity by the mechanisms mentioned above. This form of long-term potentiation is also referred to as *unsilencing* (Bredt and Nicoll 2003).

For a long-lasting potentiation, an increased protein synthesis is also necessary. A crucial step in this process is the transcription of DNA into RNA, which is induced by transcription activators such as CREB (*cAMP response element-binding protein*). This leads to increased synthesis of AMPA receptors and structural proteins. The result is not only an increased incorporation of AMPA receptors into the synapse, but also a restructuring of the synapse. However, the potentiation results not only from the strengthening of the existing synapses, which become larger and contain more AMPA receptors, but also from a new formation of additional synaptic connections.

However, the activation of NMDA receptors can induce not only long-term potentiation but also long-term depression. In this case, the activation of NMDA receptors is generally weaker than during the induction of long-term potentiation and consequently the increase in the intracellular Ca^{2+} ion concentration is also lower. The signalling cascades activated after induction of long-term depression thus also differ from those after induction of long-term potentiation. For example, whereas kinases play a special role in long-term potentiation, phosphatases, i.e. enzymes that dephosphorylate phosphorylated proteins, play a more important role in long-term depression. The depression of synaptic strength results in particular from a reduction in the number of synaptic AMPA receptors.

Summary

How does consciousness arise? How does our memory work? What causes diseases that influence our behavior? These are only three examples of current scientific questions, the answers to which are indispensably linked to knowledge of the basic neurophysiological processes in our brain, as we have described them in the present chapter. Although great progress has been made in the field of basic neurophysiological research due to the enormous technical progress since the establishment of the membrane theory by Julius Bernstein in the nineteenth century, we are still far from understanding the complexity of the human brain in all its facets. Above all, experiments on rodents, usually mice or rats, provided important insights into the understanding of physiological as well as pathophysiological processes in the brain. For example, optogenetic stimulation of individual nerve cells in the brains of living mice has been able to manipulate their memory leading to changes in their behaviour. The findings of basic neurophysiological research also play a major role in medical research. The autism spectrum disorders, for example, are associated with a pathological change in the number of synapses. Here, too, the basic principles of the disease were studied in rodents and the findings were transferred to humans. The investigation of pathophysiological changes in brain function is essential for the understanding of brain diseases such as stroke, migraine, Parkinson's disease, multiple sclerosis, epilepsies, Alzheimer's disease, schizophrenia, autism, depression, anxiety disorders and thus an important building block for the development of new therapeutic approaches.

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