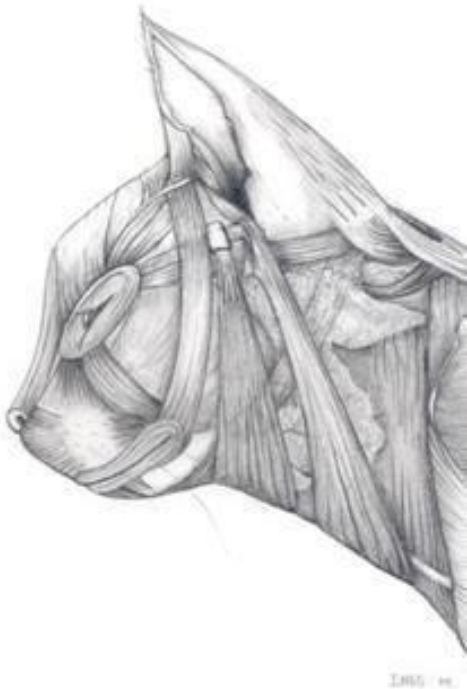
FIZIOLOGIJA ŽIVALI

Laboratorijske vaje

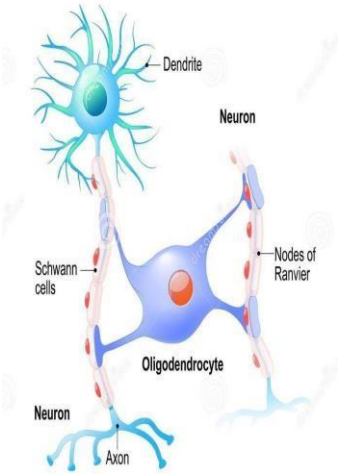
FIZIOLOGIJA ŽIVČNIH IMPULZOV



dr. Katja Adam

UP FAMNIT

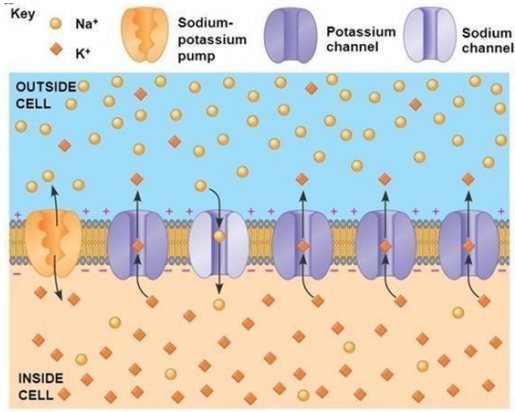
MEMBRANSKI POTENCIAL



*celice živčnega sistema: NEVRONI + NEUROGLIA CELICE

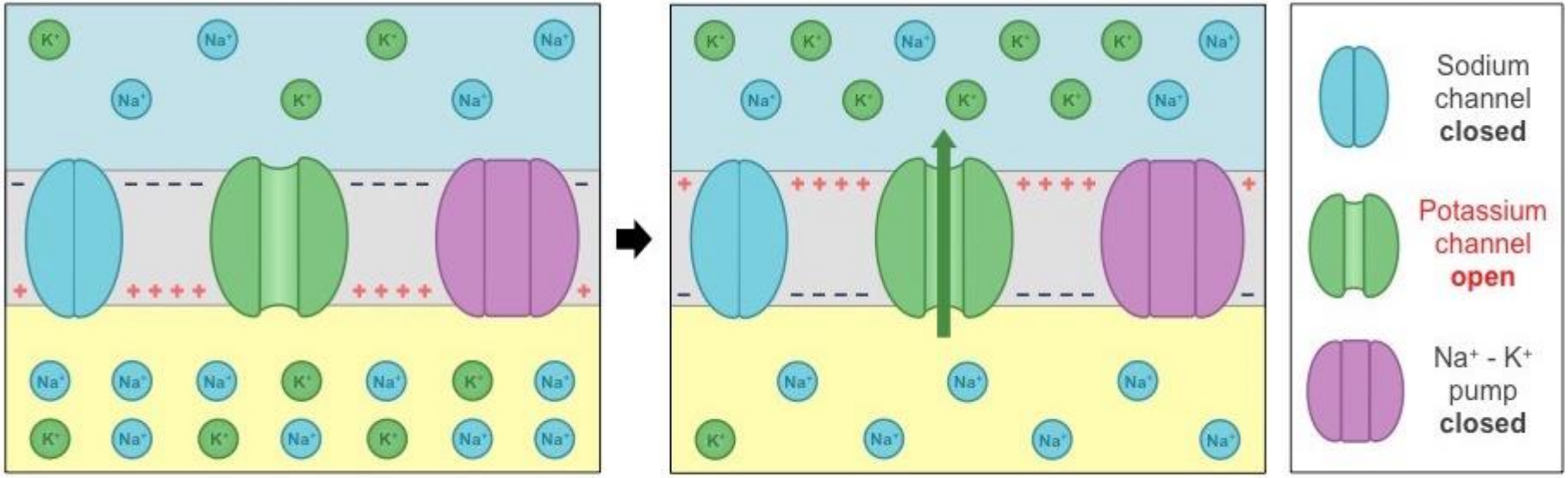
MEMBRANSKI POTENCIAL = razlika v potencialu (napetosti) čez membrano med notranjostjo in zunanjostjo celice

- merimo v mV
- membrana je **polarizirana** – ima **+** in **–** nabito stran
- s pretokom ionov čez kanalčke se membranski potencial spreminja
- LOČIMO (spremembe v MP glede na stanje v mirovanju):
  - 1. receptorski potencial
  - 2. AP
  - 3. sinaptični potencial



MIROVNI MEMBRANSKI POTENCIAL (resting membrane potential) - MMP

- STANJE MEMBRANE – vsi 3 (receptorski, AP, sinaptični) ga imajo
- odvisen od
  - permeabilnosti membrane za ione v mirovanju
  - koncentracij ionov zunaj in znotraj membrane, za katere je permeabilna
  - -70mV
- za večino nevronov sta Na^+ in K^+ najpomembnejša, prenos z Na^+ - K^+ črpalko
  - not malo Na^+ (~5mM), veliko K^+ (~150mM) – zunaj obratno
- v MMP gre K^+ ven iz celice z difuzijo skozi pasivne K^+ kanalčke, kar pusti za sabo negativni naboj **-70mV** v mirovanju

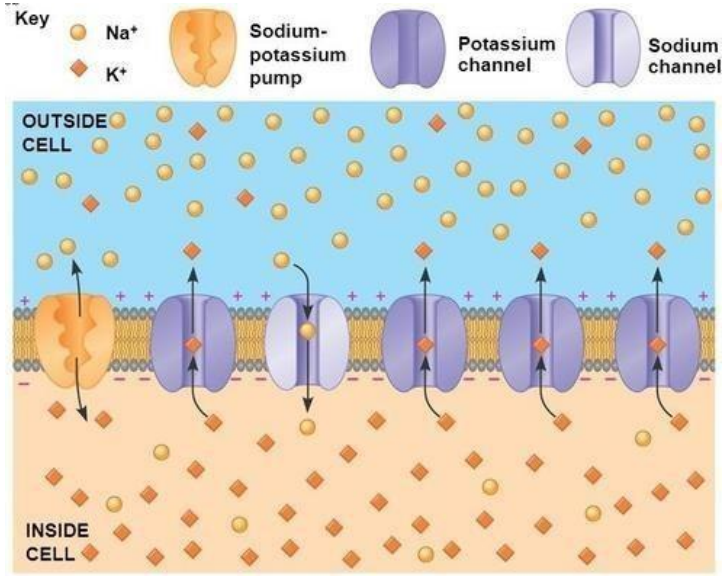


Before: Na^+ in ; K^+ in (*inside: +30 mV*)

After: K^+ efflux (*inside: –80 mV*)

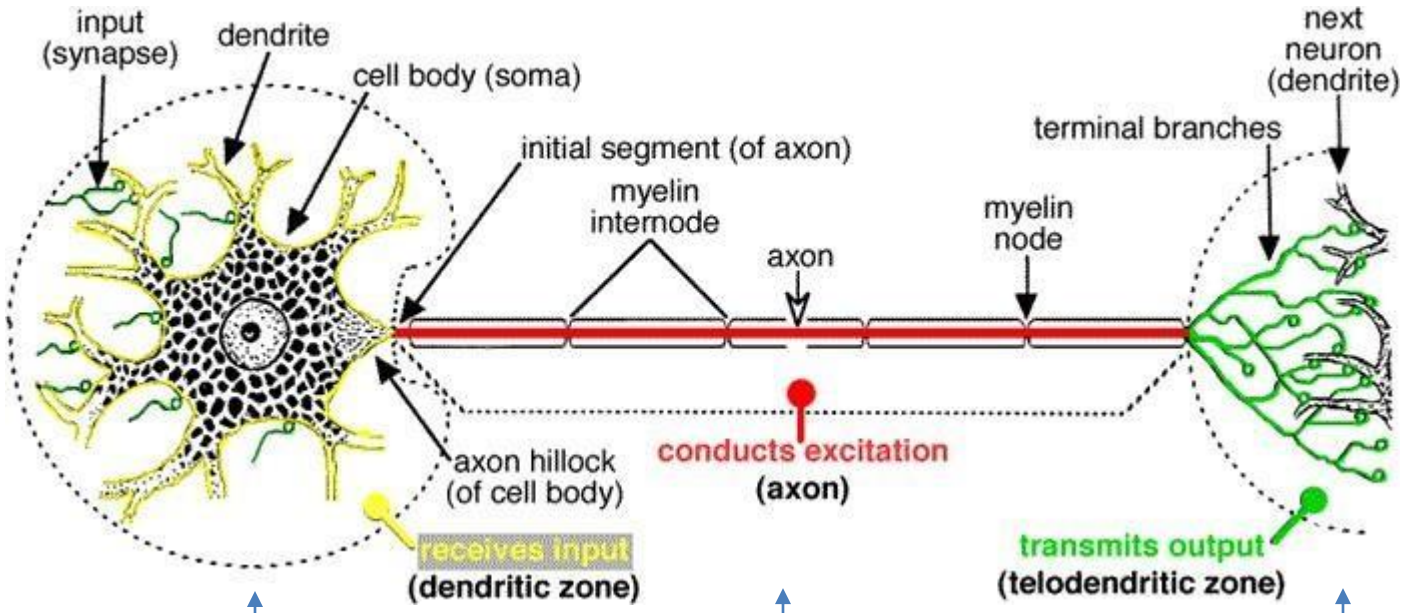
NEVROFIZIOLOGIJA ŽIVČNIH IMPULZOV

- signali, ki jih generirajo in prenašajo nevroni, so električni → nastanejo pod vplivom + ali – nabitih ionov
- ioni običajno prehajajo skozi membrano s **prenašalnimi proteini - kanalčki** (olajšana difuzija ali aktivni transport)
- kanalčki: lahko odprti, lahko regulirano odprti, lahko selektivni
- smer ionov: koncentracijski gradient!



NEVROFIZIOLOGIJA ŽIVČNIH IMPULZOV

- vsak nevron ima **3 funkcionalne regije**:
- sprejemna - dendritsko območje in telo nevrona
- **regija za prenos** (akson)
- **izhodna regija** (terminalni končiči)

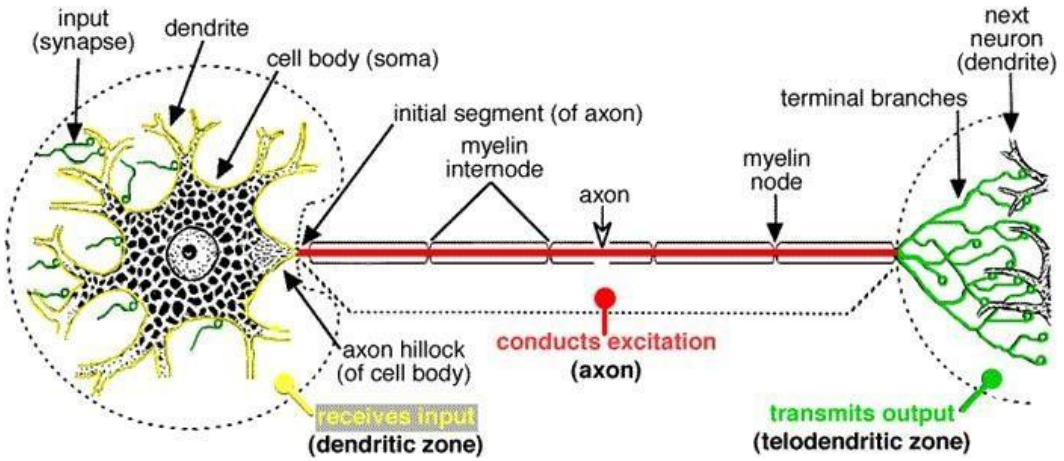


receptorski potencial — AP — sinaptični potencial

NEVROFIZIOLOGIJA ŽIVČNIH IMPULZOV

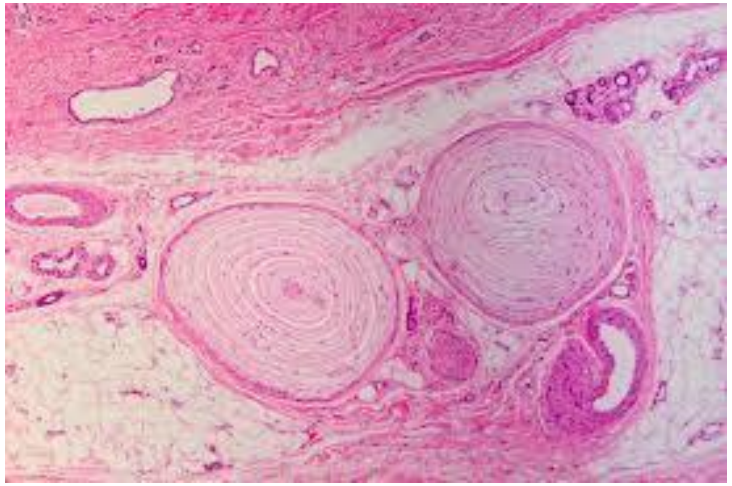
- membrana nevrona ima v vsaki regiji specifične **proteine**, ki sodelujejo pri specifičnih nalogah te regije
  - **sprejemni del**: receptroski proteini in proteini, ki generirajo receptorski potencial
  - **prenašalska regija**: proteini, ki generirajo in prenašajo AP
  - **izhodna regija**: proteini, ki sodelujejo pri izločanju nevrotransmiterjev in ustvarjajo sinaptični potencial

  - poleg naštetih so pa zelo pomembni tudi proteini vzdolž membrane, ki prenašajo določene ione



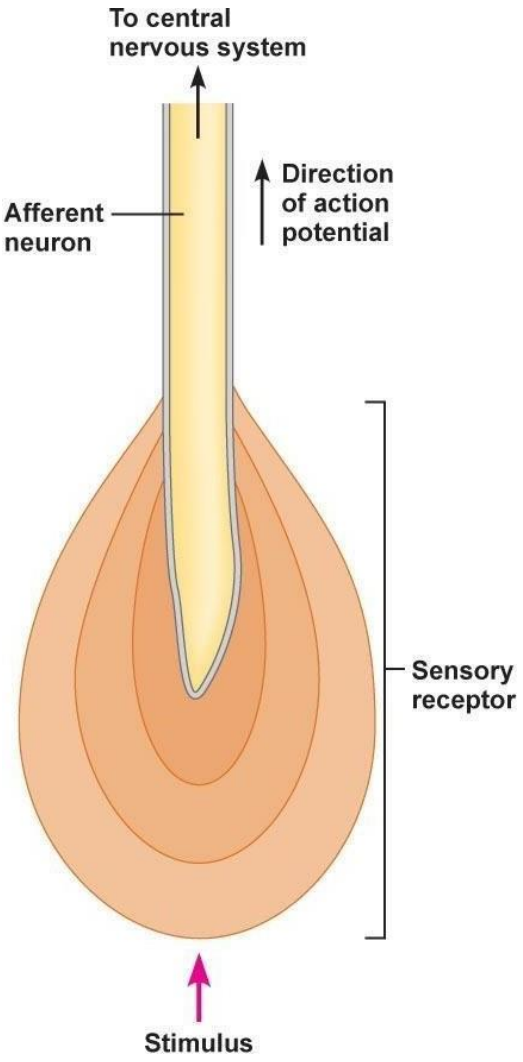
RECEPTORSKI POTENCIAL

- nevroni se odzovejo na okolje z generiranjem električnega signala
- Npr. čutilni nevroni v nosu ustvarijo signal - RECEPTORSKI POTENCIAL, ko se molekule vonja vežejo na njihovo membrano
  - **senzorični nevroni** (čutilni) reagirajo direktno na dražljaj z generiranjem receptorskega potenciala
- čutilni nevroni imajo pogosto sprejemno regijo specializirano za zaznavanje specifičnih dražljajev (vonj, svetloba, zvok, tip)
  - npr. Vater-Paccinijevo telesce za pritisk, receptorji v nosu za vonj, gustoreceptorji za okus itd.

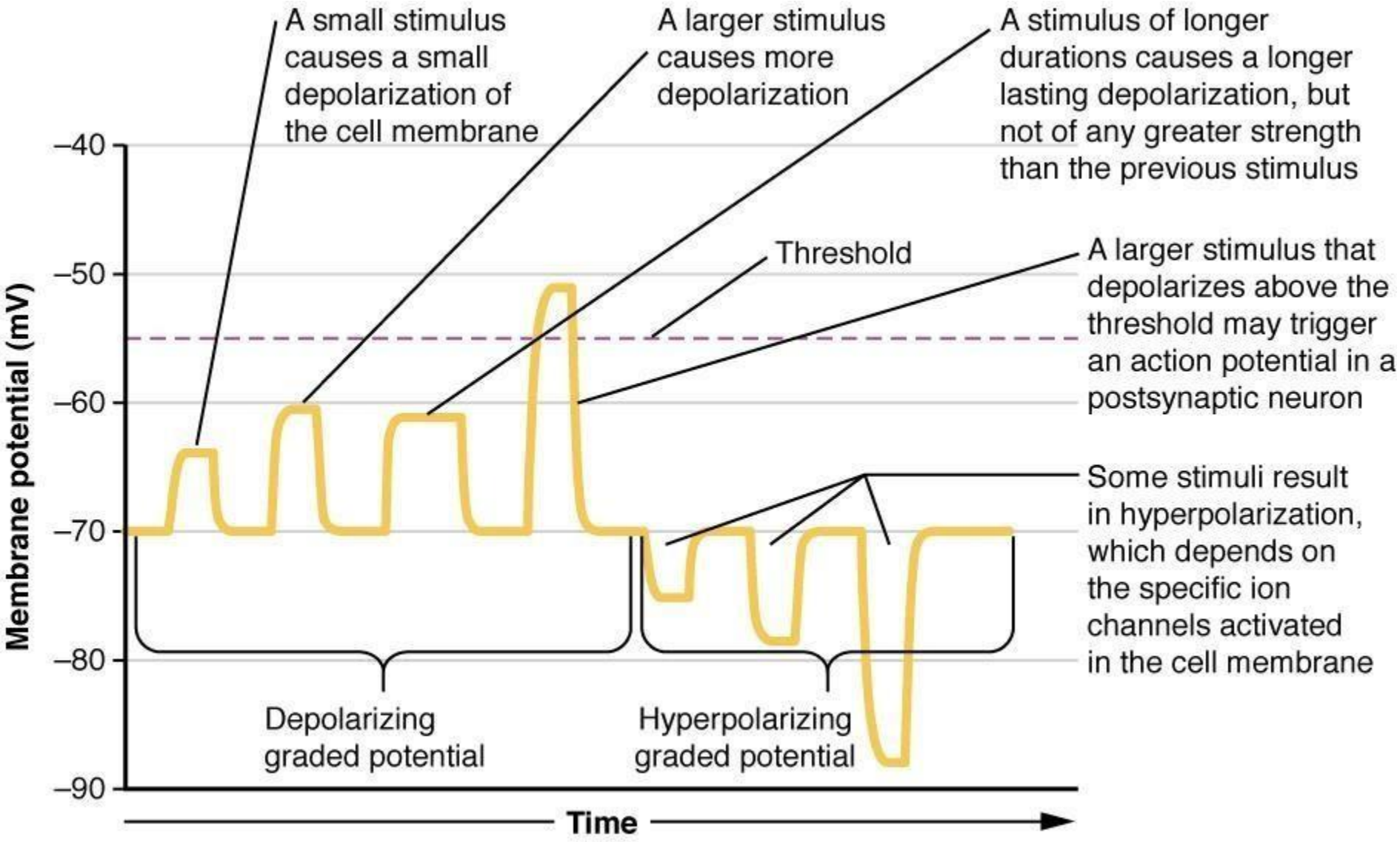


RECEPTORSKI POTENCIAL

- čutilni nevron ima v sprejemnem delu proteine, ki generirajo **receptorski potencial,** ko je nevron stimuliran z utreznim dražljajem
- energija dražljaja (toplota, kemijska, fizična) se spremeni v električni odgovor, kar ima za posledico odprtje ali zaprtje ionskih kanalčkov- temu pravimo **senzorična transdukcija**
  - do nje pride na koncu receptorskega predela čutilnega nevrona
- z intenziteto stimulusa se amplituda receptorskega potenciala poveča, čemur pravimo **stopnjevalni potencial (graded)**
- če pride do spremembe v MP iz negativne v manj negativni MMP –> **DEPOLARIZACIJA**
- **ČE JE RECEPTORSKI POT. DOVOLJ VELIK → sproži AP na aksonu….**
- **stopnjevalni potencial ni isti kot AP!**

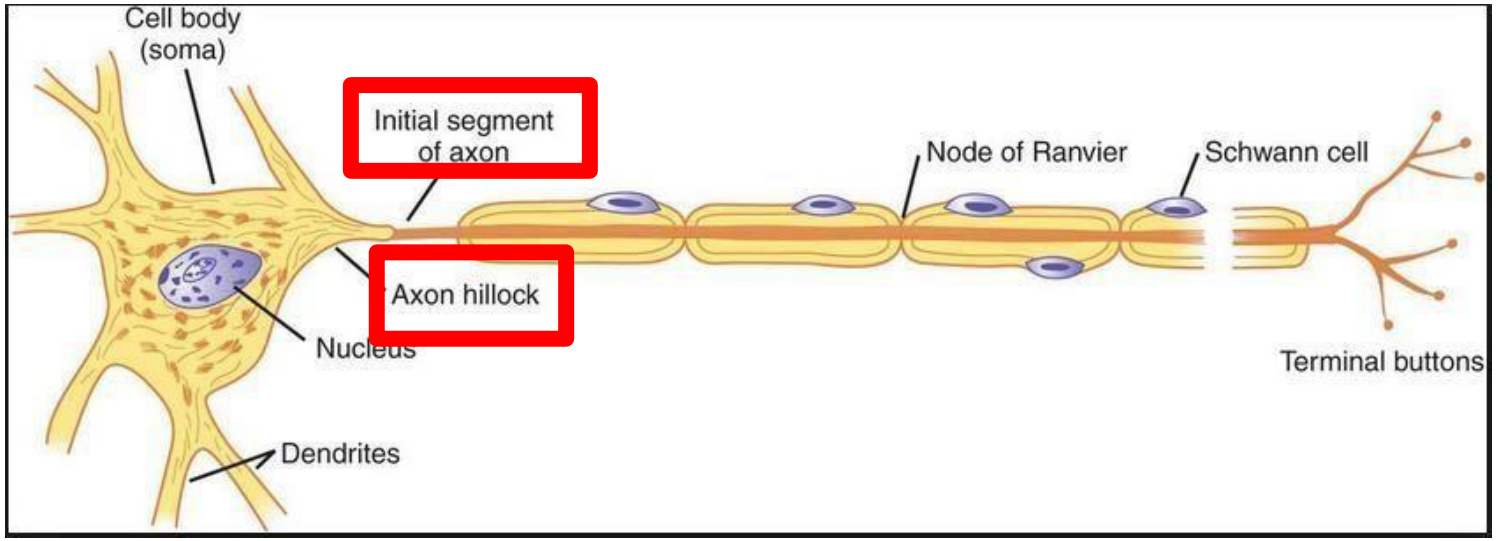


STOPNJEVALNI POTENCIAL



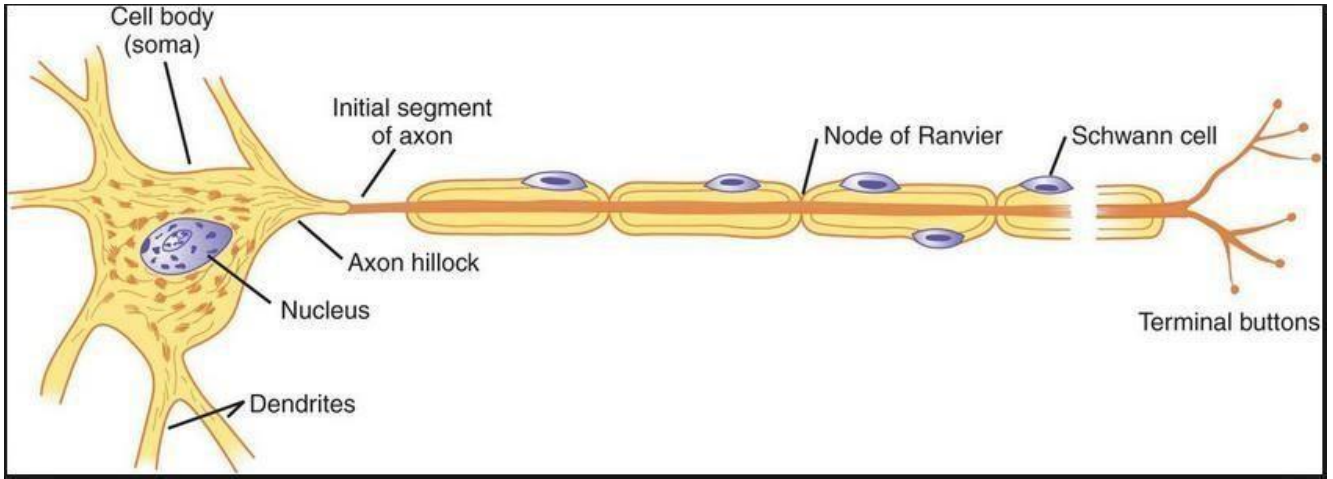
AKCIJSKI POTENCIAL & VZDRAŽNI PRAG

- akcijski potencial = spremembe v MP na aksonu
- multipolarni nevron: del telesa celice, iz katere izhaja akson, imenujemo **aksonski hrib** (axon hillock)
- prvo regijo aksona (se pravi tisto, ki izhaja iz aksonskega hriba) pri mieliniziranih aksonih imenujemo **začetni segment**
- **sprožitvena regija (trigger zone)**: na meji med aksonskim hribom in začetnim segmentom aksona; je mesto, kjer se sproži AP (pod vplivom dovolj velikega receptorskega potenciala)



AKCIJSKI POTENCIAL & VZDRAŽNI PRAG

- V ČUTILNEM NEVRONU: depolarizirajoči receptorski potencial se pasivno prenese do aksonskega hriba in prenese depolarizacijo, potrebno za generiranje AP
- če je receptorski potencial dovolj velik, se AP sproži (pravimo, da ta potencial doseže vzdražni prag) in se **regenerira (propagira/prevaja)** vzdolž membrane aksona
    - prevaja se lahko linearno (če aksoni niso mielinizirani) ali skokovito (mielinizirani aksoni)



AP ŽIVČNE CELICE: MMP, DEPOLARIZACIJA, REPOLARIZACIJA, HIPERPOLARIZACIJA

Za lažje razumevanje:
https://www.youtube.com/watch?v=iBDXOt_uHTQ

AP ŽIVČNE CELICE: MMP, DEPOLARIZACIJA, REPOLARIZACIJA, HIPERPOLARIZACIJA

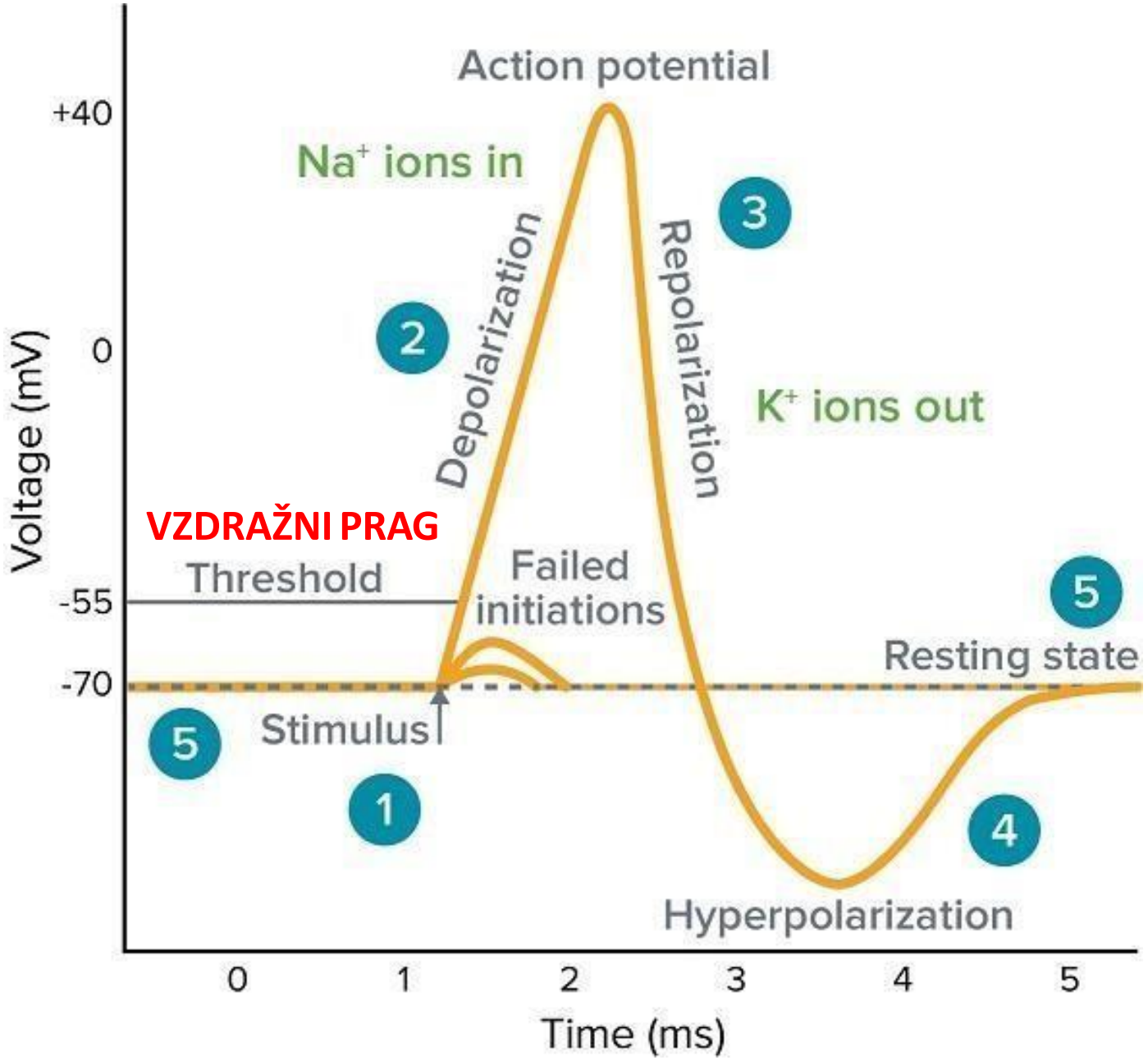
- v **DEPOLARIZACIJI MEMBRANE** živčne celice se odprejo napetostno odvisni (VOLTAGE-GATED) **Na^+ kanalčki** (tako kot pri (srčni) mišici!)
- ko je dovolj Na^+ kanalčkov odprtih, da gre dovolj Na^+ v celico in je večji vnos Na^+ ionov kot izhod K^+ ionov iz celice (ti izhajajo pasivno in vzržujejo negativni MMP), pride do sprožitve akcijskega potenciala
- po 1-2 msec se tej kanalčki inaktivirajo → Na ne gre več v celico
  - ne morejo biti odprti vsaj še nekaj msec (refraktarnost, zato se AP prevaja samo v eno smer!)
- odprejo in zaprejo se hitreje od K^+ kanalčkov, hitra depolarizacija

AP ŽIVČNE CELICE: MMP, DEPOLARIZACIJA, REPOLARIZACIJA, HIPERPOLARIZACIJA

- napetostno odvisni **K^+ kanalčki**
  - odprejo se med depolarizacijo, vendar počasnejši od Na kanalčkov
  - zaradi izhoda K^+ iz celice sledi **REPOLARIZACIJA MEMBRANE** nazaj na negativne vrednosti (več K^+ gre ven kot skozi pasivne K^+ kanalčke, ki so vedno odprti)

- ker gre veliko K^+ ven, membranski potencial postane nekoliko manj negativen od MMP – HIPERPOLARIZACIJA
- Na-K črpalka vrne ione nazaj ven/not -> MMP

- DOMAČA NALOGA: poglejte še enkrat teorijo akcijskega potenciala srčne mišice, primerjajte, kako sta si podobna oz. različna AP srčne mišice in nevrona!


Action potential
+40
Na+ ions in
3
Depolarization
Repolarization
2
0
K+ ions out
Voltage (mV)
VZDRAŽNI PRAG
Threshold
Failed
initiations
5
-55
Resting state
-70
5
Stimulus
1
4
Hyperpolarization
0
1
2
3
4
5
Time (ms)

STOPNJEVALNI POTENCIAL vs. AP

STOPNJEVALNI POTENCIAL: močnejši dražljaj dražljaj da večji odgovor (npr. več molekul neke vonjave sproži večji odgovor čutilnih nevronov, kot manj molekul vonjave)

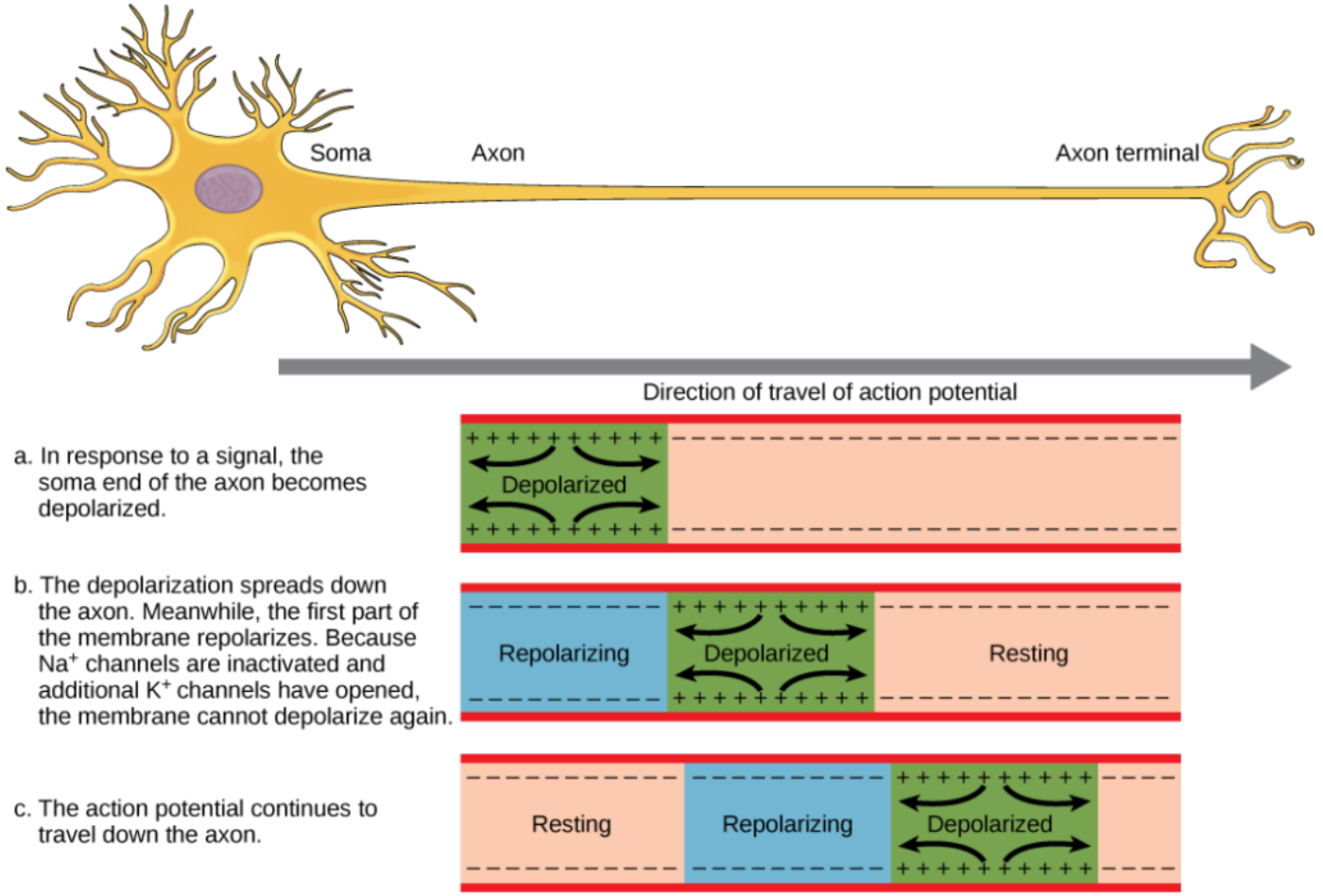
- če je receptorski potecial dovolj močan – sproži AP v aksonu
- če je dražljaj daljši, je daljša tudi depolarizacija

AP ima vedno isto amplitudo – ne glede na moč dražljaja

- deluje po principu **vse ali nič** – ko je dosežen vzdražni prag, pride do AP
- po prvem AP se lahko generira nov, če je še vedno prisoten prvoten dražljaj (AMPAK! Refraktarnost!)

PREVAJANJE AP VZDOLŽ AKSONA

- ko je AP generiran, se prevaja (generirajo se novi AP) vzdolž celega aksona – gre za enosmerno prevajanje



PREVAJANJE AP VZDOLŽ AKSONA

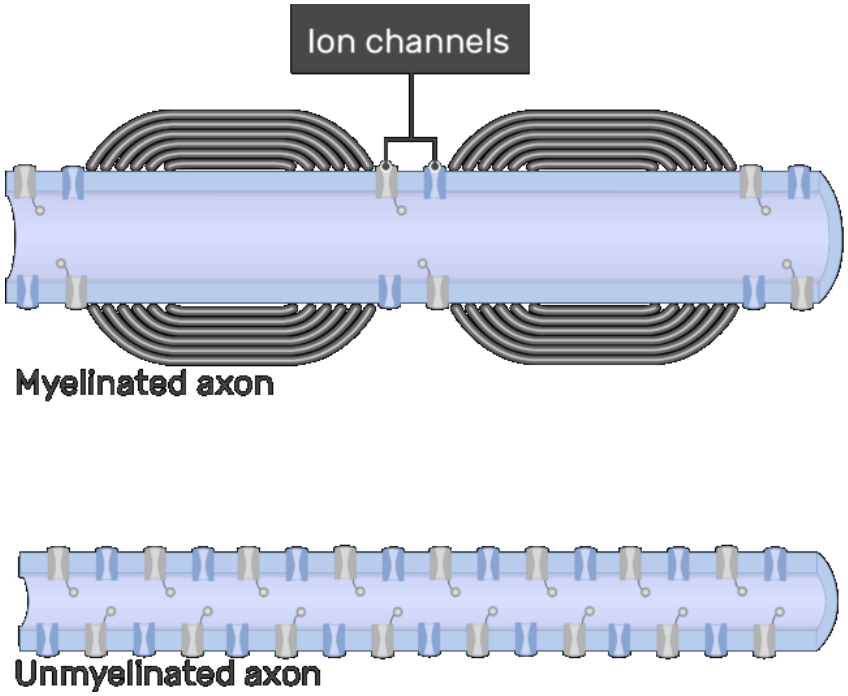
- amplituda AP se ne zmanjša vdolž aksona

- propagacijo/prevajanje AP omogočajo:
  - napetostno odvisni Na^+ in K^+ kanalčki, ki jih najdemo vzdolž aksona
  - močna depolarizacija AP z lahkoto sproži AP v sosednji regiji oz. iz enega zažemka v drugega pri mieliniziranih aksonih (jo privede do praga)

- **HITROST PREVAJANJA AP** izračunamo iz razdalje, ki jo mora AP opraviti, in časa, ki je za to potreben (izraženo v m/s)
  - odv. od **debeline aksona** in **mieliniziranosti**

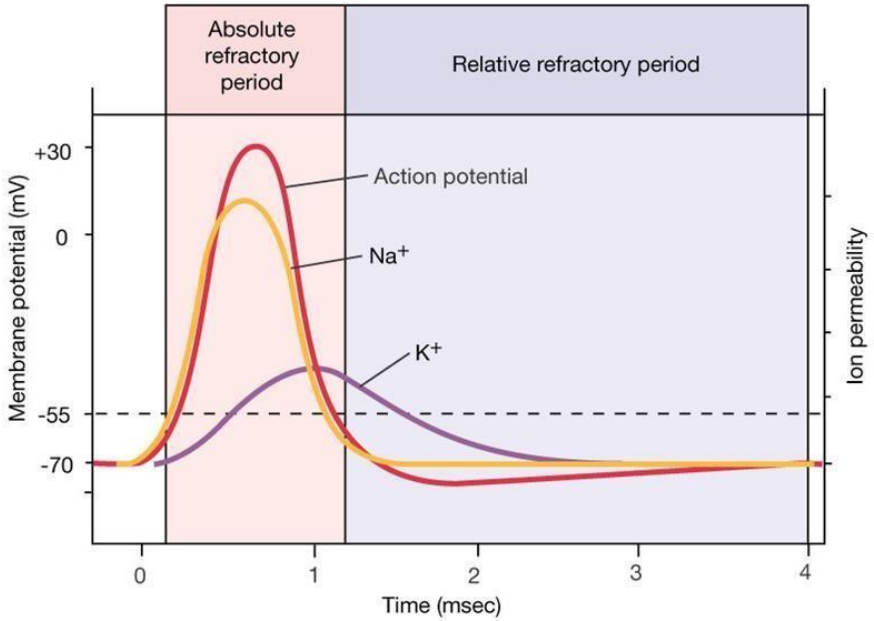
PREVAJANJE AP VZDOLŽ AKSONA

- MIELINIZACIJA = ovoj aksona, sestavljen iz neuroglia celic
  - CŽS – oligodendrocite
  - periferni ŽS – Schwannove celice
- Ranvierjevi zažetki – mesta brez mielinske ovojnice

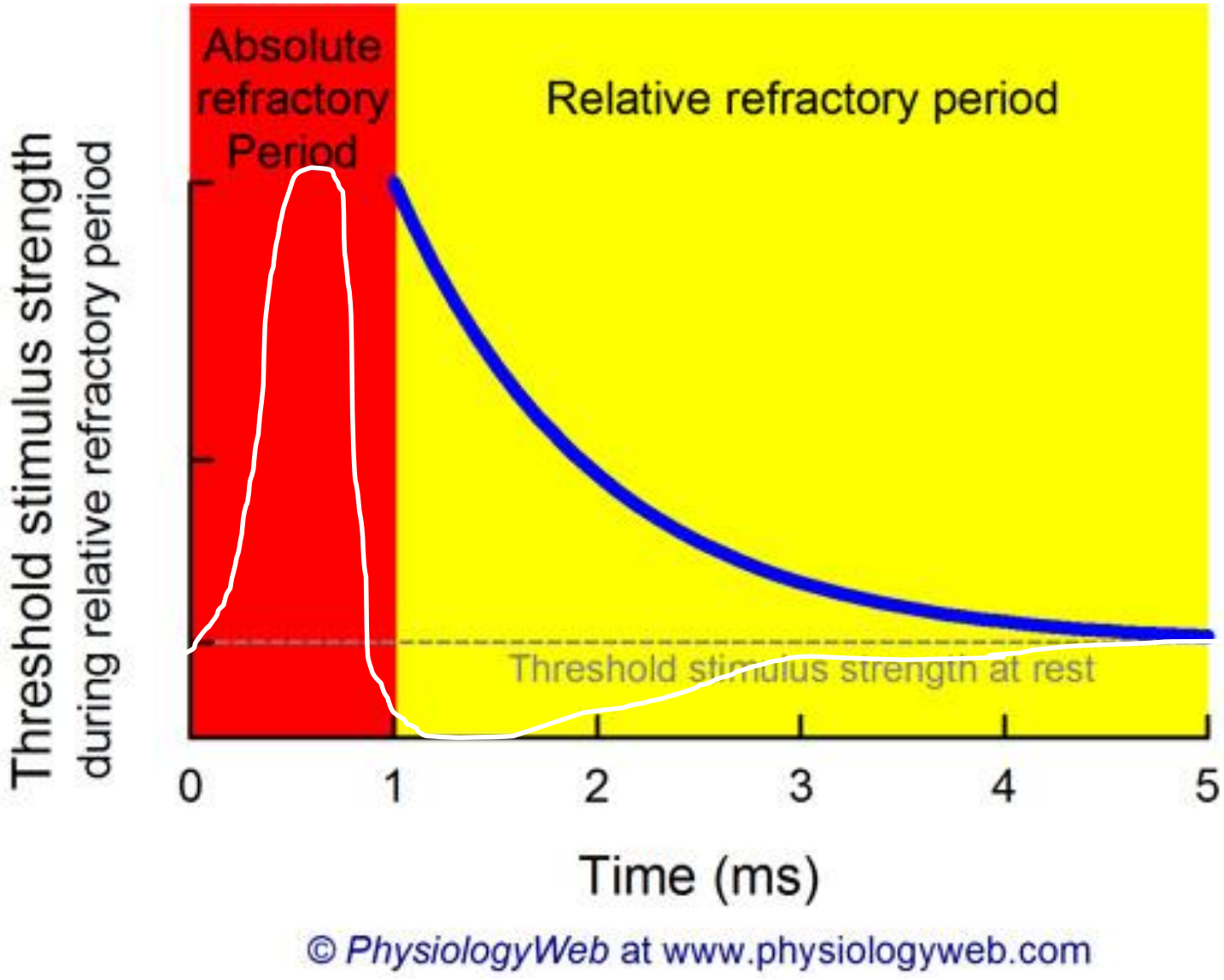


OBDOBJE REFRAKTARNOSTI AKSONA Ž. C.

- **Absolutna refraktarna doba** – čas po akcijskem potencialu, v katerem nevron ne more generirati novega AP (ne glede na moč dražljaja)
- **Relativna refraktarna doba** – čas po prvem AP, ko lahko sprožimo nov AP, vendar mora biti moč dražljaja večja od pražnega!



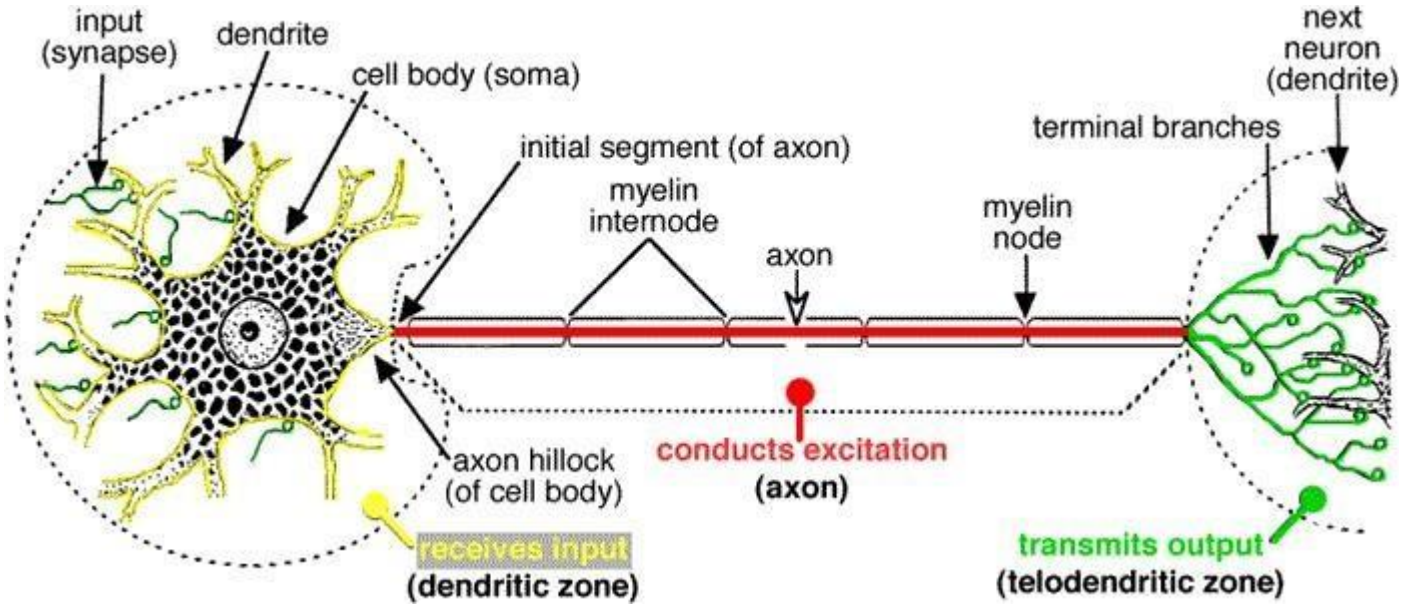
KOLIKO VEČJA MOČ???



Moč dražljaja, ki je potreben, da generiramo nov AP tekom relativne refr. dobe – na začetku relativne refr dobe mora biti dražljaj zelo močan za izzvanje novega AP, potem pa potrebna moč pada do pražne vrednosti (konec relativne refr dobe)

AKSONI –> PREVAJANJE AP NA DRUGE CELICE (SINAPTIČNI POTENCIAL)

- akson prevaja AP na drugo preko razvejitev – **terminalnih končičev** nevrona, ponavadi na več celic v istem času
- terminalni končiči nevrona specializirani za prenos skupkov nevrotransmiterjev iz majhnih znotrajceličnih mešičkov – **sinaptičnih mešičkov**

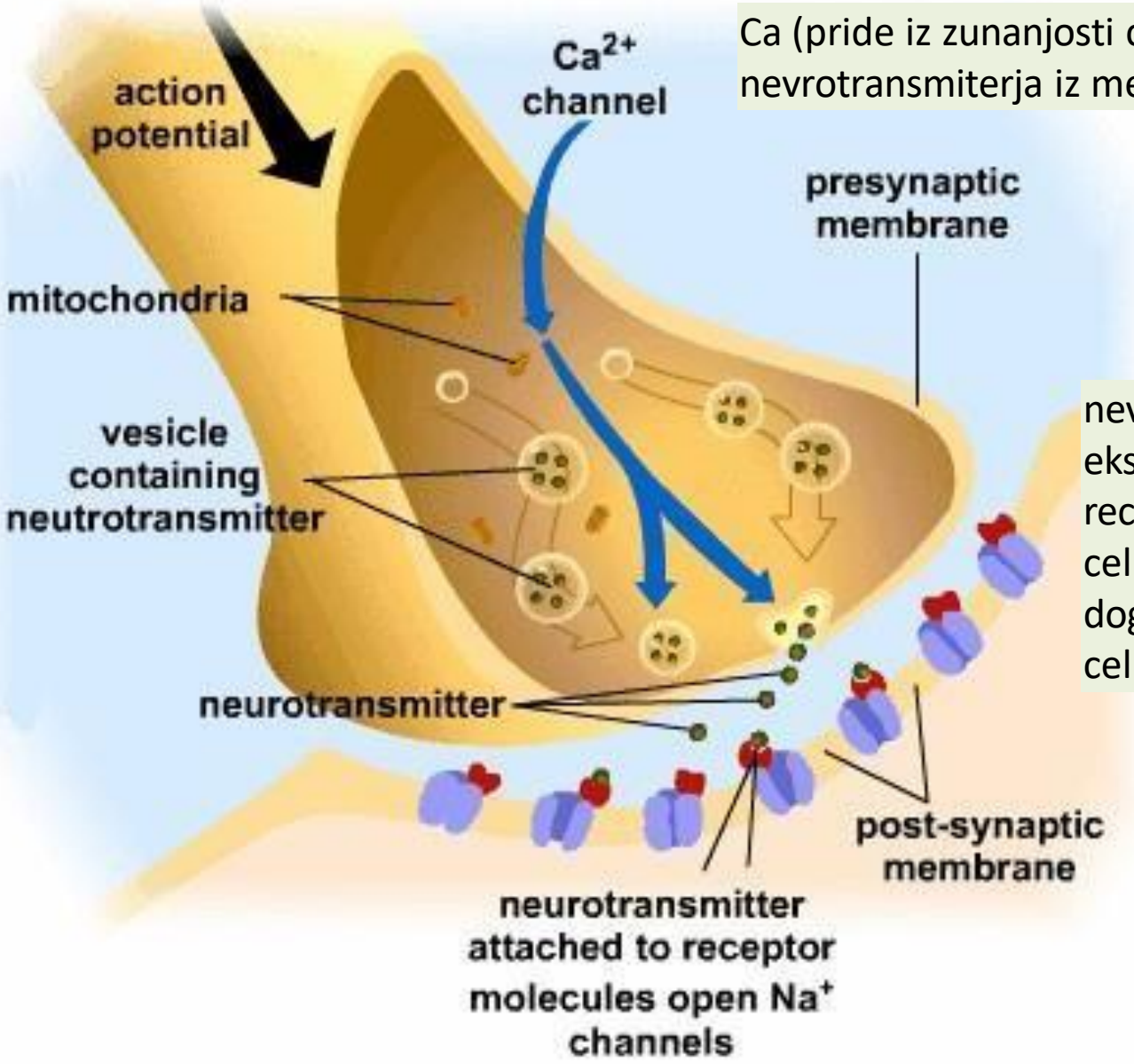


AKSONI –> PREVAJANJE AP NA DRUGE CELICE (SINAPTIČNI POTENCIAL)

- NEVROTRANSMITERJI
- zunajcelične molekule, ki jih celica izloči z **eksocitozo** (**ponavadi pod vplivom Ca! – ta pride v celice iz zunanjosti**)
- → z difuzijo čez majhen zunajcelični proctor (**sinaptično špranjo**) do druge celice (nevron/mišica/žleza)
- nevrotransmiterji se pogosto vežejo na proteine v membrani post-sinaptične celice → val dogodkov, ki odpirajo/zapirajo membranske ionske kanalčke in povzročijo, da se MMP pri njih spremeni (**postsinaptični potencial**)

slika na naslednji strani

Ca (pride iz zunanjosti celice) sproži izločanje nevrotransmiterja iz mešičov v sinaptično špranjo



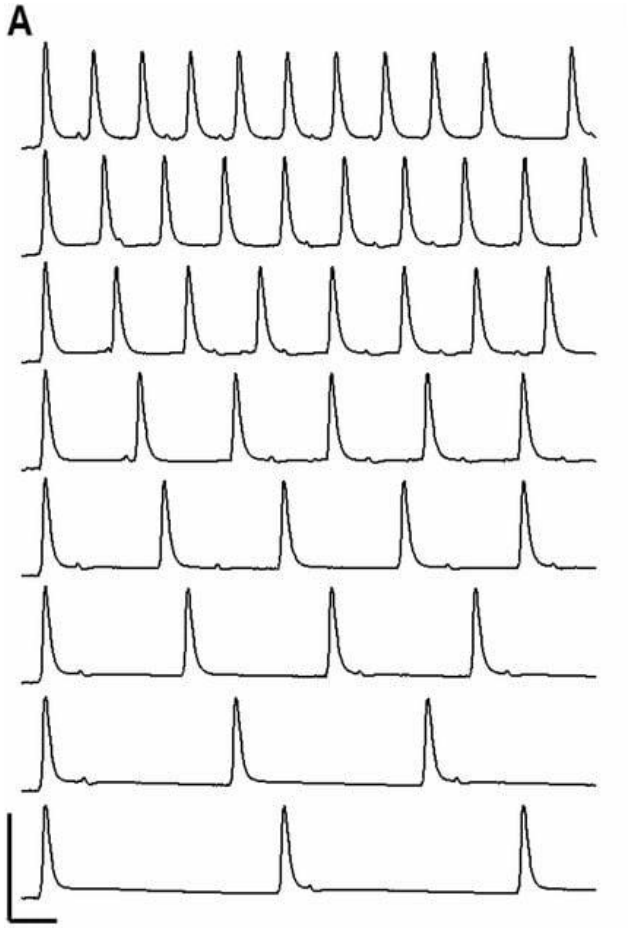
nevrotransmiter se z eksocitozo izloči in veže na receptorje v membrani druge celice – to sproži serijo dogodkov na postsinaptični celici

Močnejši dražljaj → več Ca pride v celico, iz več mešičkov se sprosti nevrotransmiter

FREKVENCA POJAVLJANJA AP

- izračunamo iz podatkov o času med enim in drugim AP

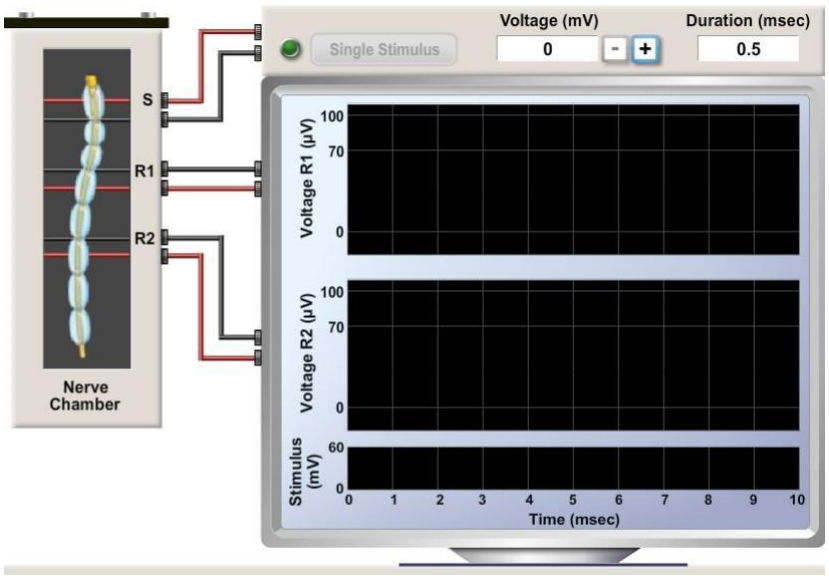
frekvenca =1/”čas”

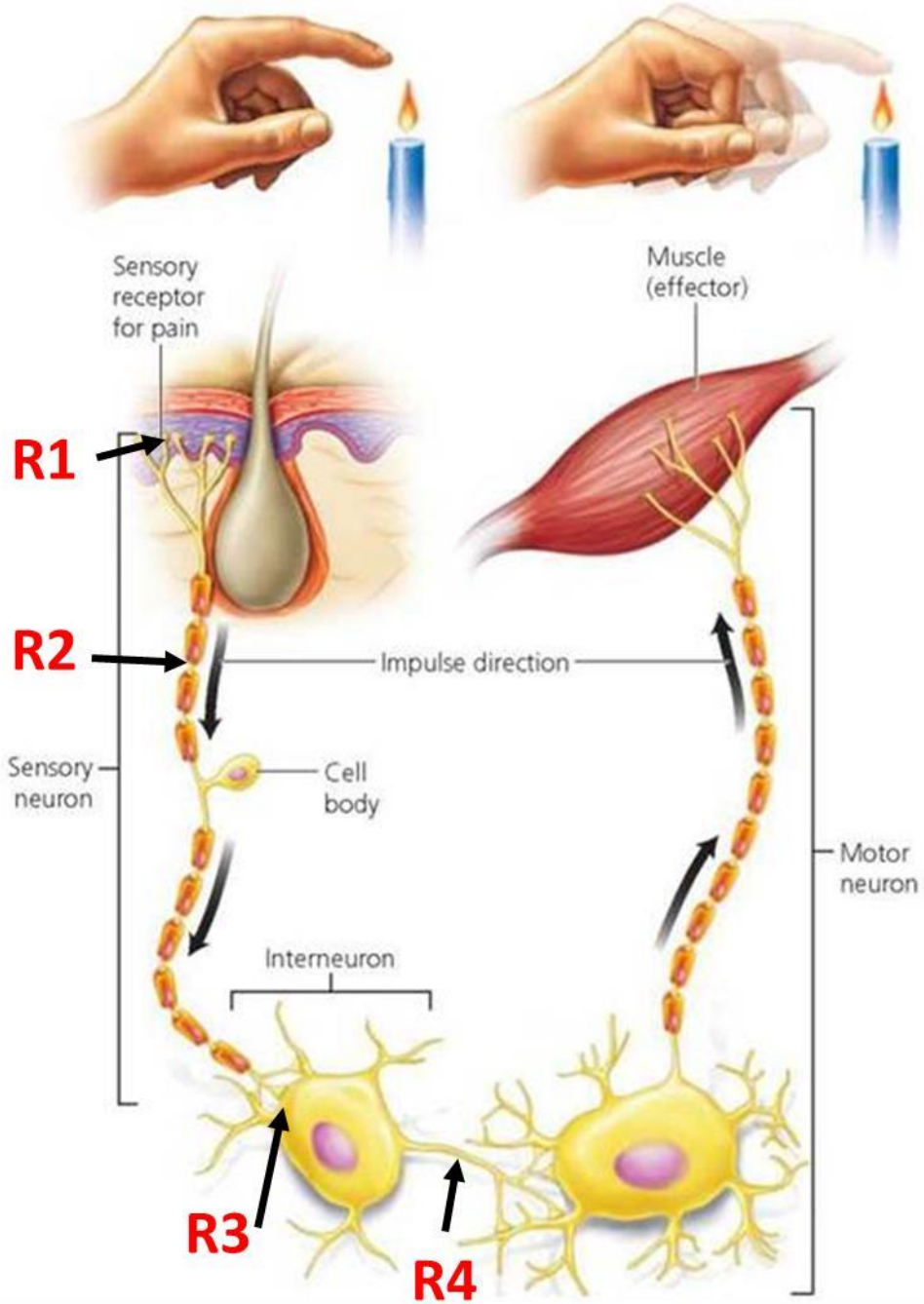


* VAJE ZA MERJENJE AKTIVNOSTI NEVRONOV

- v eksperimentih imamo **samo aksone**, brez telesa celice in dendritov
- do aksonov so speljane žičke, ki beležijo električno aktivnost na membrani aksona
- el. tok gre iz stimulatorja v eno žičko – po aksonu – nazaj po drugi žički v osciloskop
- ta električni tok depolarizira akson
- merimo razmere ekstracelulrano, saj je vstavljanje žičk v akson zelo težko

- pražna napetost – kjer opazite prvič AP




Sensory receptor for pain
Muscle (effector)
R1
R2
Impulse direction
Sensory neuron
Cell body
Motor neuron
Interneuron
R3
R4

DANAŠNJE VAJE

REŠUJETE NALOGE:
3_5 – refraktarnost
3_6 - intenziteta dražljaja
3_8 – nevrotransmiterji
3_9 – povzetek

Vaj 3-01, 3-02, 3-03, 3-04 in 3-07 ni potrebno opraviti, vendar pa morate poznati teorijo (zajeta v tem pptju)

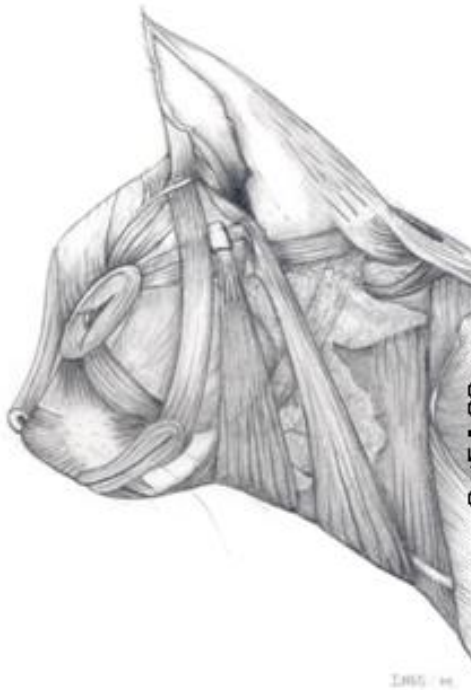
- MMP
- vzdražni prag
- receptorski potencial
- Na kanalčki
- hitrost prevajanja

FIZIOLOGIJA ŽIVALI

Laboratorijske vaje

REFLEKSI

dr. Katja Adam
UP FAMNIT



REFLEKSI

- hitri, nehoteni, nezavedni odzivi mišic ali žlez na okoljske dražljaje, ki jih zaznajo čutilni receptorji
- omogočajo jih nevrološke poti, ki jih imenujemo **refleksni lok**

- Delitev glede na efektor:
  - **somatski refleks**: odgovor je skrčenje prečno progaste skeletne mišice
  - **avtonomni (visceralni) refleks**: vključuje prečno porgasto srčno mišičje, gladko mišičje ali žleze

Somatic versus Autonomic Pathways

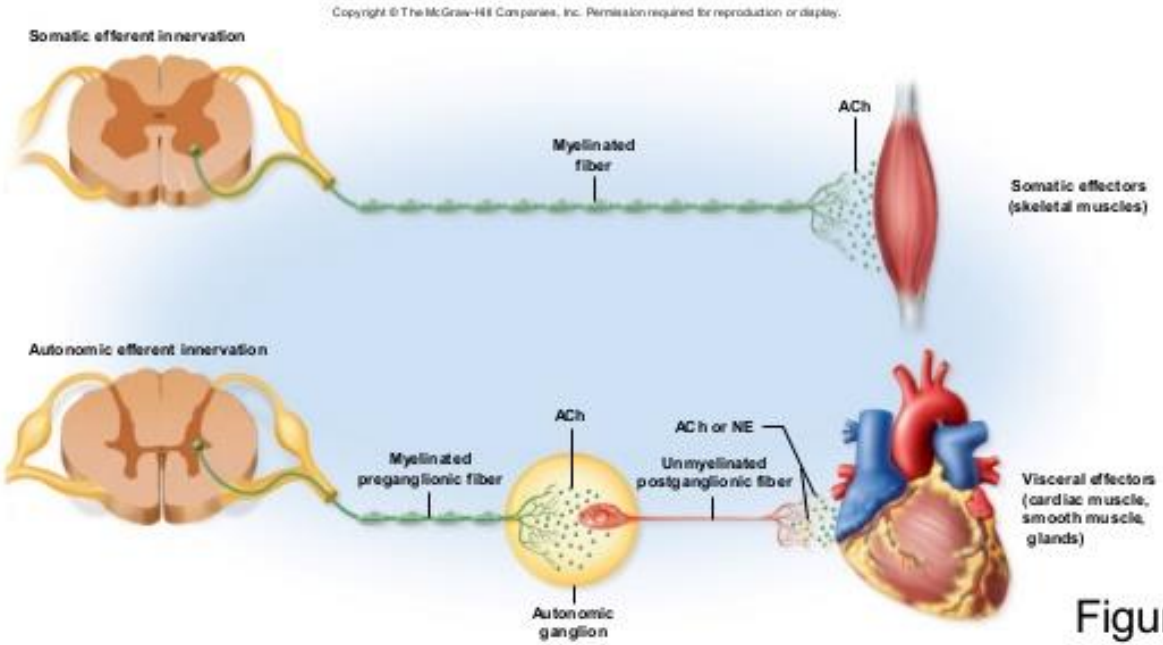


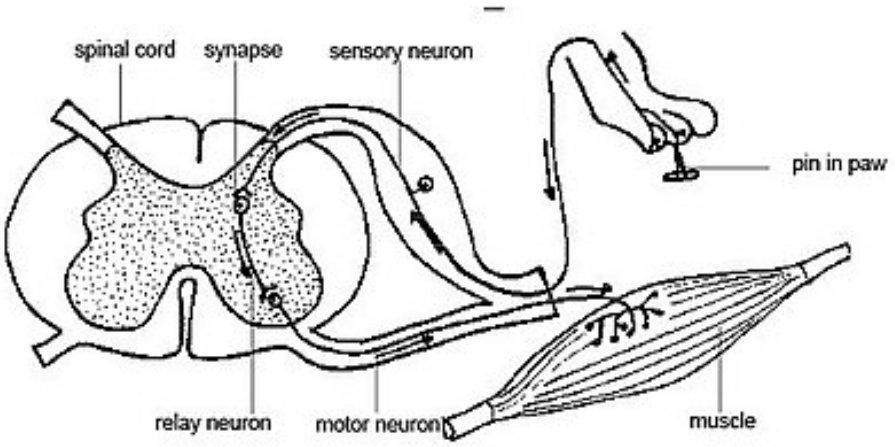
Figure 15.2

Delitev glede na pot refleksnega loka (živce):

spinalni refleksi: preko hrbtenjačnih živcev

kranialni refleksi: preko možganskih živcev

KOMPONENTE REFLEKSNEGA LOKA



<u>5 komponent:</u>

1. čutilni receptor
2. čutilni (senzorični) nevron
3. center integracije
   - monosinaptični R.L. (direktna povezava senzorični-motorični nevron)
   - polisinaptični R.L. (internevroni, 1 ali več)
4. motorični nevron
5. efektor (mišica/žleza)

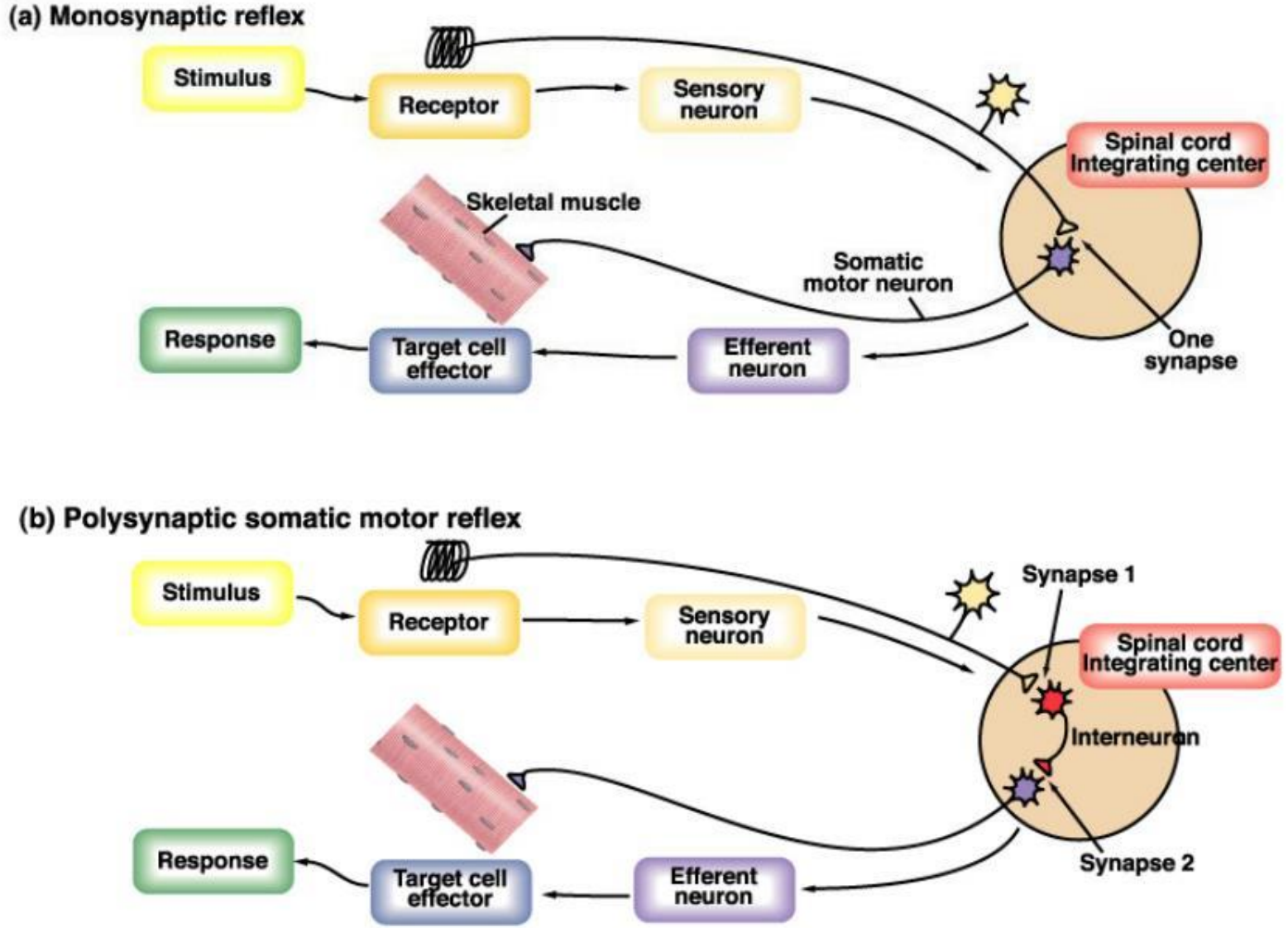


Fig 13.1 – Monosynaptic and polysynaptic reflexes

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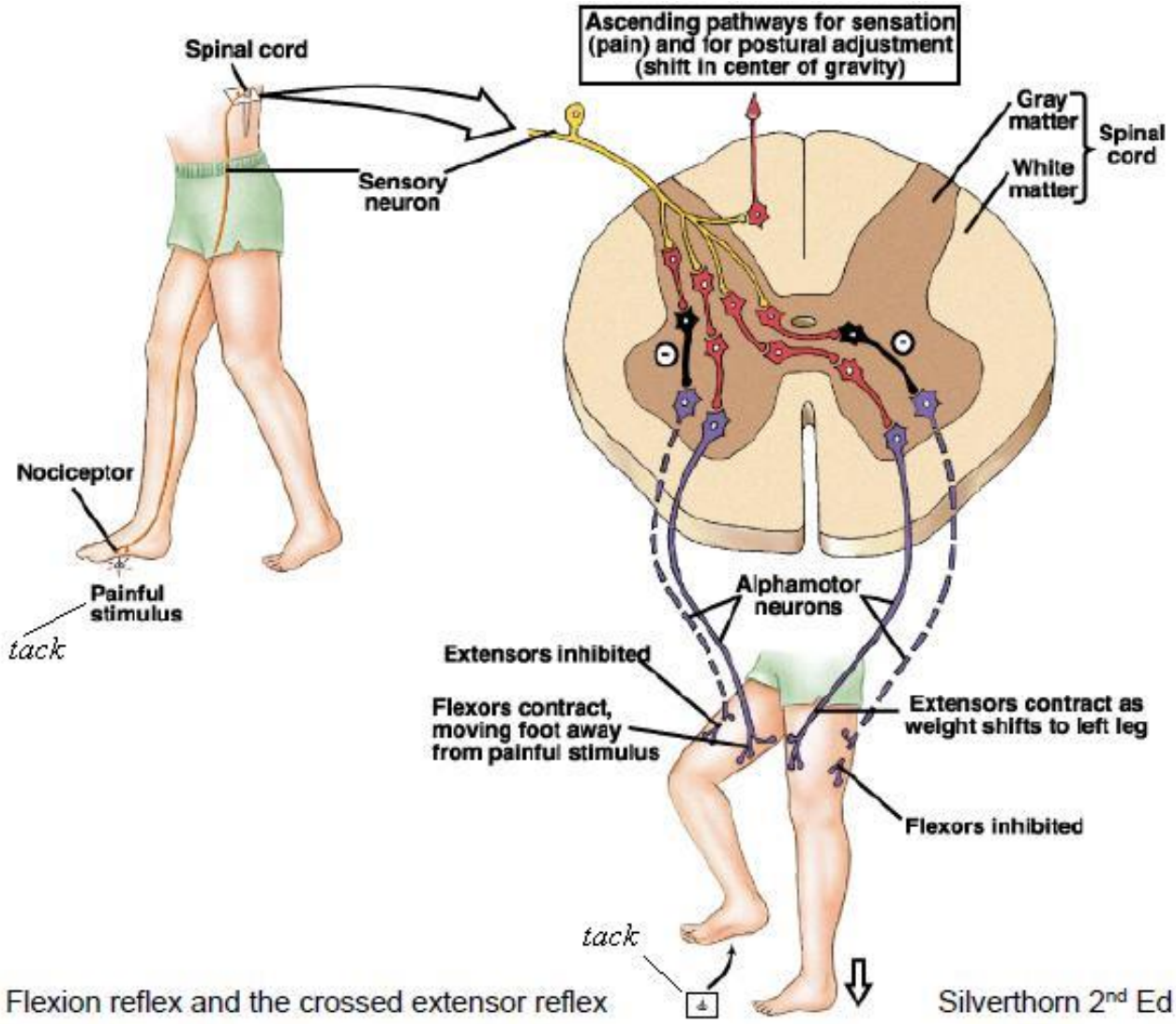
IPSILATERALNI/ KONTRALATERALNI R. L



Flexion reflex and the crossed extensor reflex

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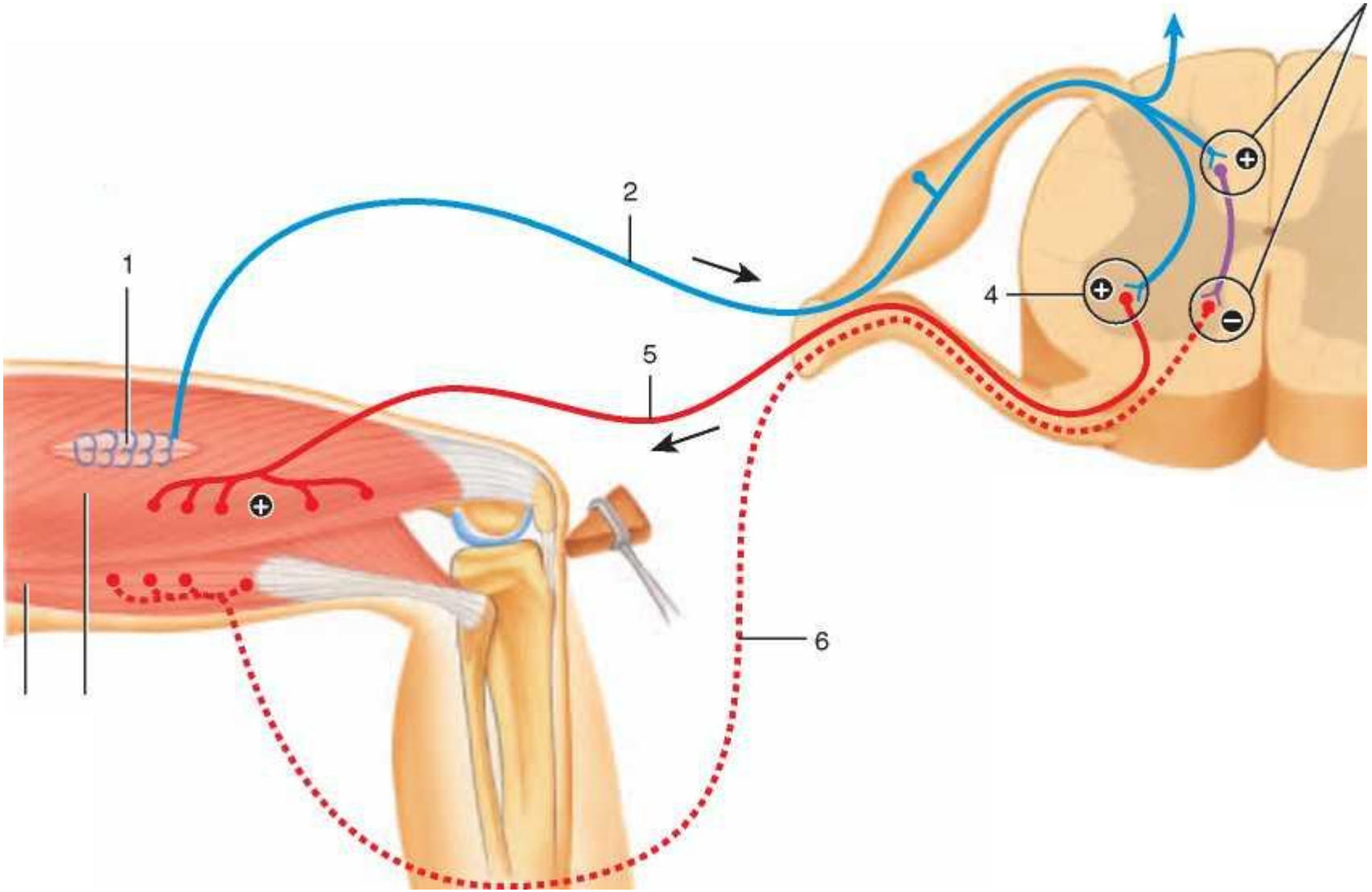
PATELARNI REFLEKS



PATELARNI REFLEKS

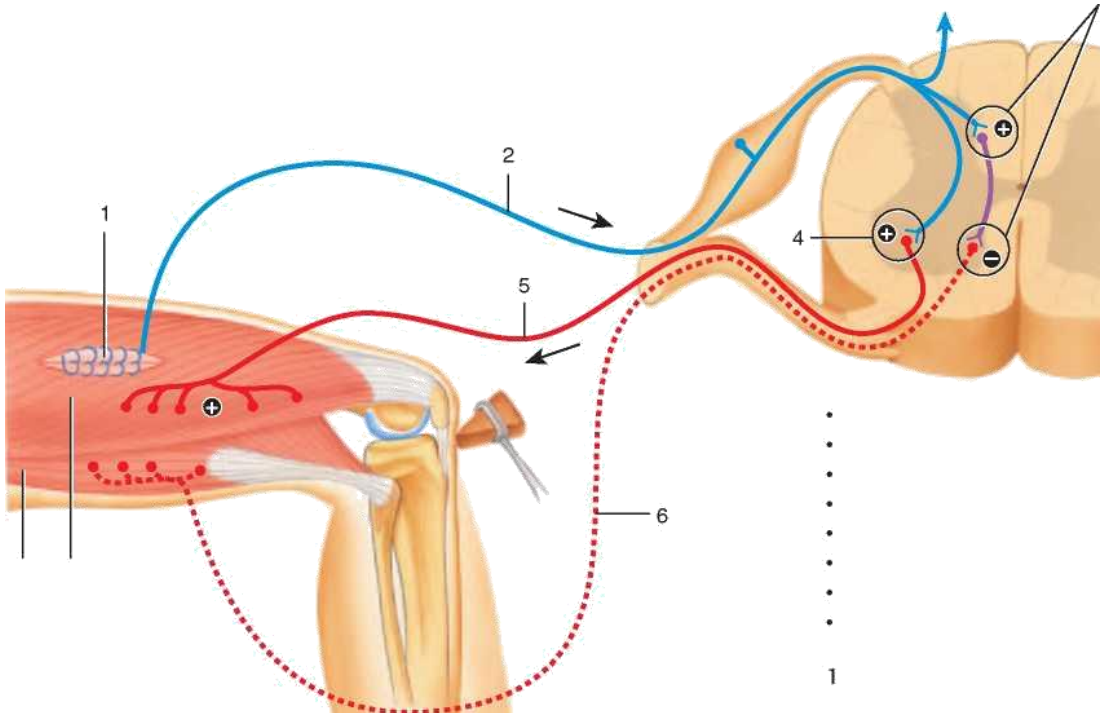
- ekstenzija kolena ob skrčenju kite mišice quadriceps femoris (štiriglava stegenska mišica) QF
- skrčenje kite – kontrakcija mišice

- **KOMPONENTE PATELARNEGA REFLEKSNEGA LOKA**:
- **mišična vretena (spindle)** v QF
    - z dotikom kite – mišice v QF se skrčijo – stimulacija vreten – inicicacija živčnih impulzov v aksonih senzoričnih nevronov
- **senzorični nevroni**: senzorični aksoni, ki prenašajo impulze v integracijski center (sivina) v hrbtenjači
- **integracijski center**: v anteriornem rogu hrbtenjače; sonzorični nevroni vzdražijo motorične, ki inervirajo QF
- **motorični nevron** – aksoni gredo do QF
- **Efektor**: QF se skrči in podaljša nogo, ko je stimulirana

PATELARNI REFLEKS

- senzorični nevron je del tudi **polisinaptičnega refleksnega loka** (3 nevroni, 2 sinapsi) – zavira delovanje motoričnega nevrona v antagonističnih mišicah – te ostanejo relaksirane

-> RECIPROČNA INERVACIJA – stimulacija kontrakcije agonistične mišice s simultano inhibicijo antagonistične mišice



BICEPSNI REFLEKS

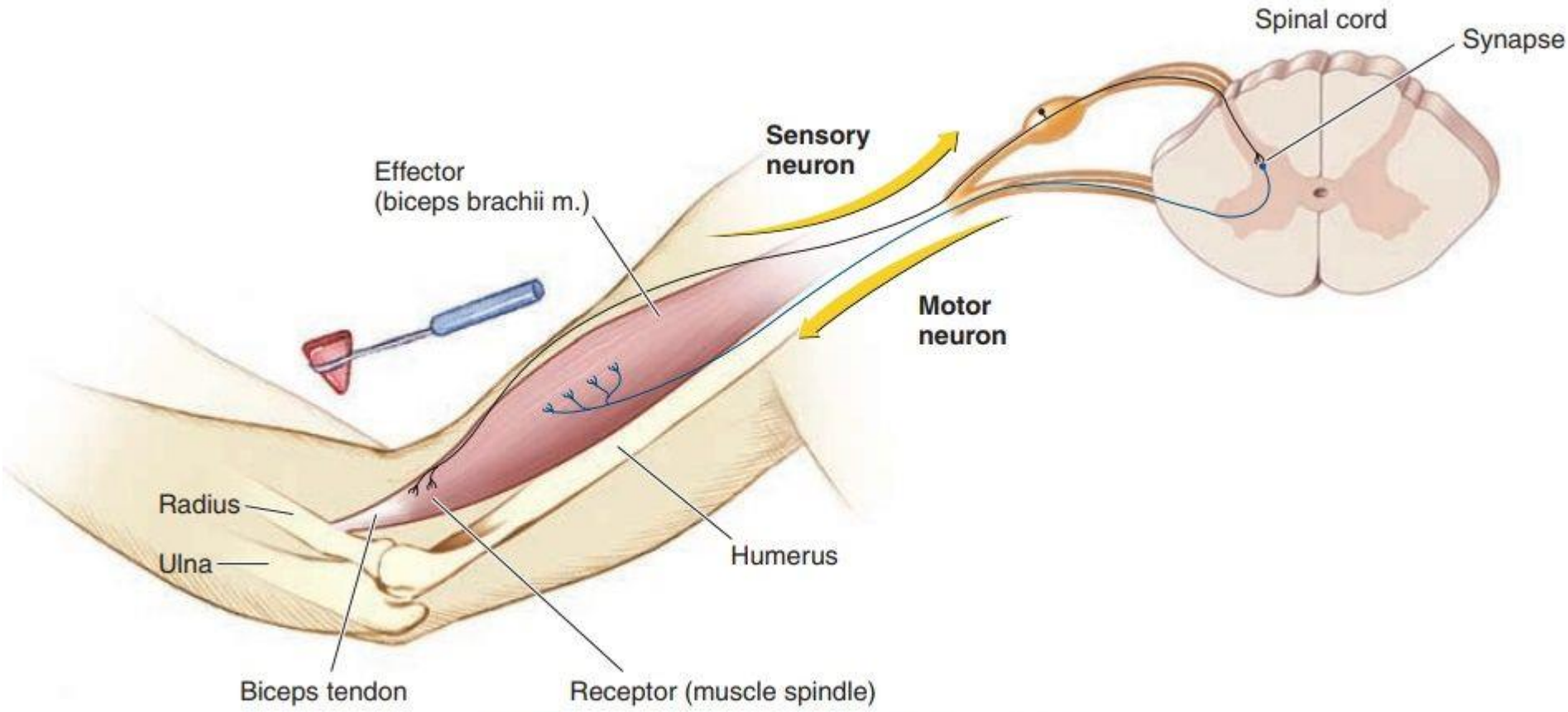
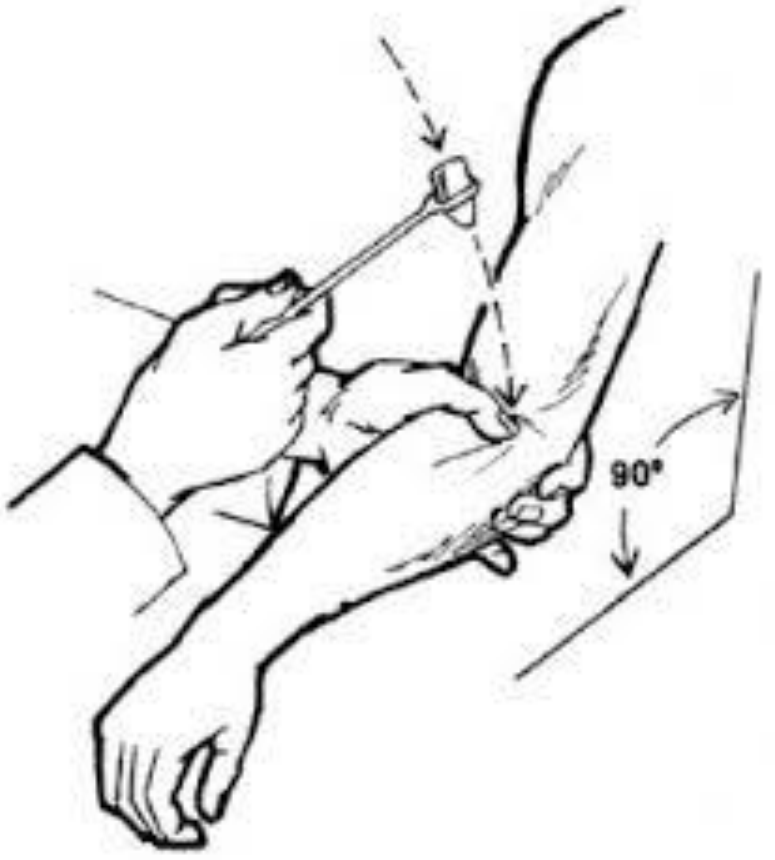
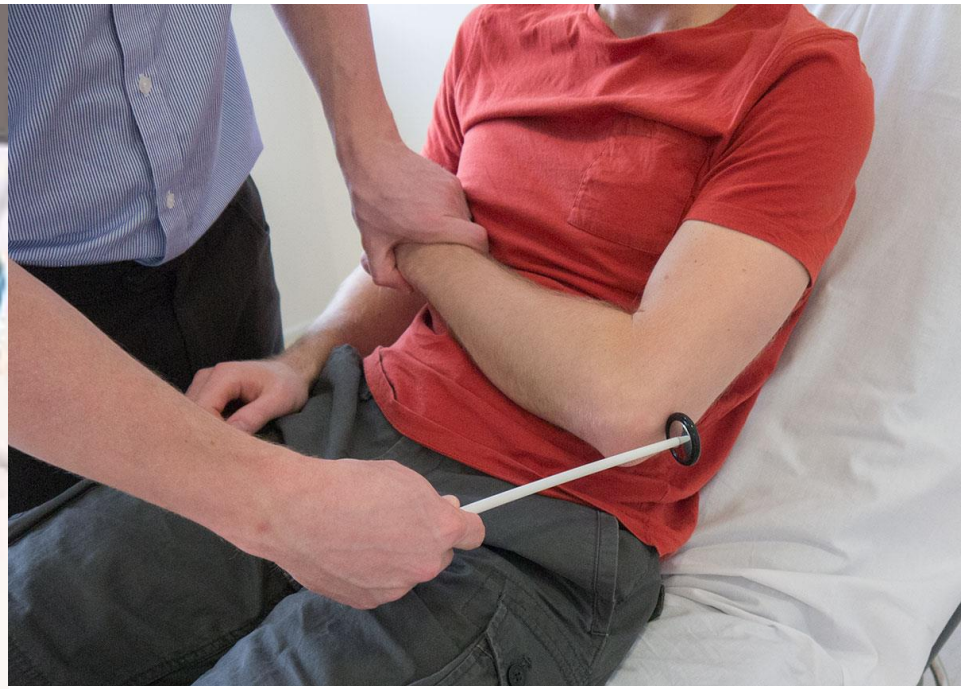


FIGURE . A Stretch Reflex—The Biceps Jerk

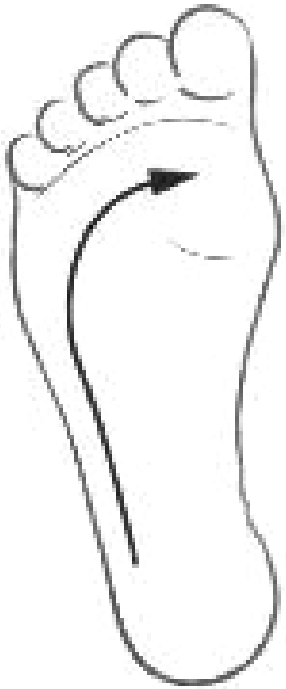
BICEPSNI REFLEKS

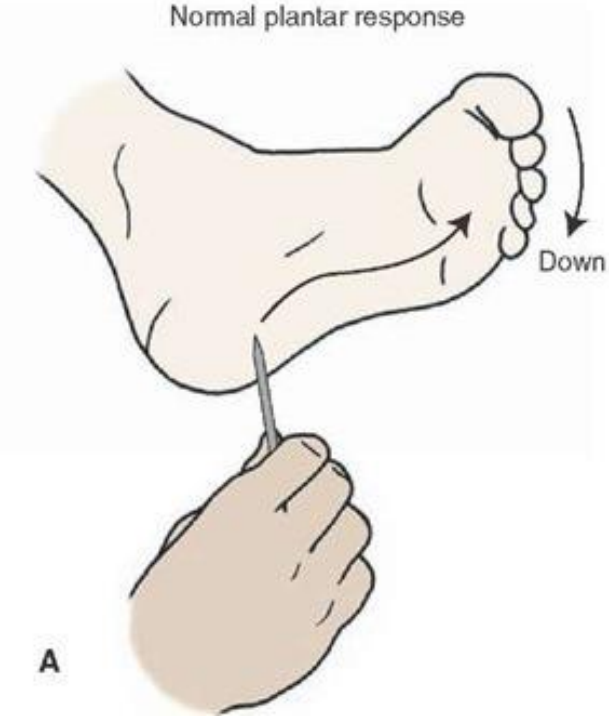


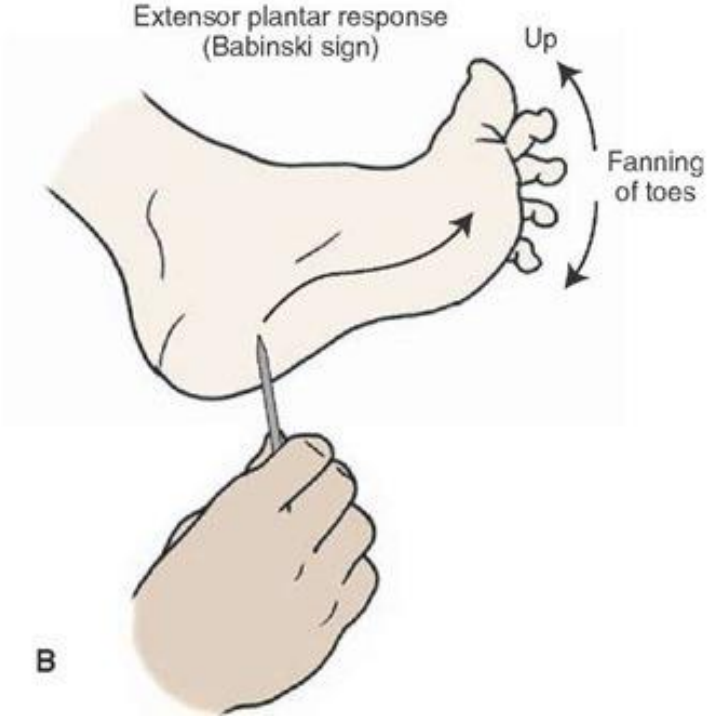
TRICEPSNI REFLEKS



STOPALNI ODZIV







Overview

Exercise Overview

Neurophysiology of Nerve Impulses

The nervous system contains two general types of cells: **neurons** and neuroglia (or glial cells). This exercise focuses on neurons. Neurons respond to their local environment by generating an electrical signal. For example, sensory neurons in the nose generate a signal (called a **receptor potential**) when odor molecules interact with receptor proteins on the membrane of these olfactory sensory neurons. Thus, sensory neurons can respond directly to sensory stimuli. The receptor potential can trigger another electrical signal (called an **action potential**), which travels along the membrane of the sensory neuron's axon to the brain—you could say that the action potential is conducted to the brain.

The action potential causes the release of **chemical neurotransmitters** onto neurons in olfactory regions of the brain. These chemical neurotransmitters bind to receptor proteins on the membrane of these brain **interneurons**. In general, interneurons respond to chemical neurotransmitters released by other neurons. In the nose the odor molecules are sensed by sensory neurons. In the brain the odor is perceived by the activity of interneurons responding to neurotransmitters. Any resulting action or behavior is caused by the subsequent activity of **motor neurons**, which can stimulate muscles to contract (see Exercise 2).

In general each neuron has three functional regions for signal transmission: a receiving

Exercise Overview

region, a conducting region, and an output region, or secretory region. Sensory neurons often have a receptive ending specialized to detect a specific sensory stimulus, such as odor, light, sound, or touch. The **cell body** and **dendrites** of interneurons receive stimulation by neurotransmitters at structures called **chemical synapses** and produce **synaptic potentials**. The conducting region is usually an **axon**, which ends in an output region (the axon terminal) where neurotransmitter is released (view Figure 3.1). Although the neuron is a single cell surrounded by a continuous plasma membrane, each region contains distinct membrane proteins that provide the basis for the functional differences. Thus, the receiving end has receptor proteins and proteins that generate the receptor potential, the conducting region has proteins that generate and conduct action potentials, and the output region has proteins to package and release neurotransmitters. Membrane proteins are found throughout the neuronal membrane—many of these proteins transport ions (see Exercise 1).

The signals generated and conducted by neurons are electrical. In ordinary household devices, electric current is carried by electrons. In biological systems, currents are carried by positively or negatively charged **ions**. Like charges repel each other and opposite charges attract. In general, ions cannot easily pass through the lipid bilayer of the plasma membrane and must pass through **ion channels** formed by integral membrane proteins. Some channels are usually open (leak channels) and others are gated, meaning that the channel can be in an open or closed configuration. Channels can also be selective for which ions are allowed to pass. For

Exercise Overview

example, sodium channels are mostly permeable to sodium ions when open, and potassium channels are mostly permeable to potassium ions when open. The term **conductance** is often is used to describe **permeability**. In general, ions will flow through an open channel from a region of higher concentration to a region of lower concentration (see Exercise 1). In this exercise you will explore some of these characteristics applied to neurons.

Although it is possible to measure the ionic currents through the membrane (even the currents passing through single ion channels), it is more common to measure the potential difference, or voltage, across the membrane. This membrane voltage is usually called the **membrane potential**, and the units are **millivolts** (**mV**). One can think of the membrane as a battery, a device which separates and stores charge. A typical household battery has a positive and negative pole so that when it is connected, for example, through a light bulb in a flashlight, current flows through the bulb. Similarly, the plasma membrane can store charge and has a relatively positive side and a relatively negative side. Thus, the membrane is said to be **polarized**. When these two sides (intracellular and extracellular) are connected through open ion channels, current in the form of ions can flow in or out across the membrane and thus change the membrane voltage.

Introduction

The receptor potential, synaptic potentials, and action potentials are important signals in the nervous system. These potentials refer to changes in the membrane potential from its resting level. In this activity you will explore the nature of the resting potential. The **resting membrane potential** is really a potential difference between the inside of the cell (intracellular) and the outside of the cell (extracellular) across the membrane. It is a steady-state condition that depends on the resting permeability of the membrane to ions and on the intracellular and extracellular concentrations of those ions to which the membrane is permeable.

For many neurons, Na^+ and K^+ are the most important ions, and the concentrations of these ions are established by transport proteins, such as the Na^+-K^+ pump, so that the intracellular Na^+ concentration is low and the intracellular K^+ concentration is high. Inside a typical cell, the concentration of K^+ is ~150 m*M* and the concentration of Na^+ is ~5 m*M*. Outside a typical cell, the concentration of K^+ is ~5 m*M* and the concentration of Na^+ is ~150 m*M*. If the membrane is permeable to a particular ion, that ion will diffuse down its concentration gradient from a region of higher concentration to a region of lower concentration. In the generation of the resting membrane potential, K^+ ions diffuse out across the membrane leaving behind a net negative charge—large anions that cannot cross the membrane.

The membrane potential can be measured with an amplifier. In the experiment the extracellular solution is connected to a ground (literally, the earth) which is defined as 0 mV. To record the voltage across the membrane, a microelectrode is inserted through the membrane without

Introduction

significantly damaging it. Typically, the microelectrode is made by pulling a thin glass pipette to a fine hollow point and filling the pulled pipette with a salt solution. The salt solution conducts electricity like a wire, and the glass insulates it. Only the tip of the microelectrode is inserted through the membrane, and the filled tip of the microelectrode makes electrical contact with the intracellular solution. A wire connects the microelectrode to the input of the amplifier so that the amplifier records the membrane potential, the voltage across the membrane between the intracellular and grounded extracellular solutions.

The membrane potential and the various signals can be observed on an oscilloscope. An electron beam is pulled up or down according to the voltage as it sweeps across a phosphorescent screen. Voltages below 0 mV are negative and voltages above 0 mV are positive. For this first activity, the time of the sweep is set for 1 second per division, and the sensitivity is set to 10 mV per division; a division is the distance between gridlines on the oscilloscope.

Equipment Used

- Neuron (in vitro)—a large, dissociated (or cultured) neuron
- Three extracellular solutions—control, high potassium, and low sodium
- Microelectrode—a probe with a very small tip that can impale a single neuron (In an actual wet lab, a microelectrode manipulator is used to position the microelectrode. For simplicity,

Introduction

the microelectrode manipulator will not be depicted in this activity.)

- Microelectrode manipulator controller—controls movement of the manipulator
- Microelectrode amplifier—used to measure the voltage between the microelectrode and a reference
- Oscilloscope—used to observe voltage changes

Introduction

The receiving end of a sensory neuron, the **sensory receptor**, has receptor proteins (as well as other membrane proteins) that can generate a signal called the **receptor potential** when the sensory neuron is stimulated by an appropriate, adequate stimulus. In this activity you will use the same recording instruments and microelectrode that you used in Activity 1. However, in this activity, you will record from the sensory receptor of three different sensory neurons and examine how these neurons respond to sensory stimuli of different modalities.

The sensory region will be shown disconnected from the rest of the neuron so that you can record the receptor potential in isolation. Similar results can sometimes be obtained by treating a whole neuron with chemicals that block the responses generated by the axon. The molecules localized to the sensory receptor ending are able to generate a receptor potential when an adequate stimulus is applied. The energy in the stimulus (for example, chemical, physical, or heat) is changed into an electrical response that involves the opening or closing of membrane ion channels. The general process that produces this change is called **sensory transduction**, which occurs at the receptor ending of the sensory neuron. Sensory transduction can be thought of as a type of signal transduction where the signal is the sensory stimulus.

You will observe that, with an appropriate stimulus, the amplitude of the receptor potential increases with stimulus intensity. Such a response is an example of a potential that is graded with stimulus intensity. These responses are sometimes referred to as *graded potentials*, or *local potentials*. Thus, the receptor potential is a graded, or local, potential. If the response (receptor

Introduction

potential) is a change in membrane potential from the negative resting potential to a less negative level, the membrane becomes less polarized and, thus, the change is called **depolarization**.

Equipment Used

- Three sensory receptors—Pacinian (lamellar) corpuscle, olfactory receptor, and free nerve ending
- Microelectrode—a probe with a very small tip that can impale a single neuron (In an actual wet lab, a microelectrode manipulator is used to position the microelectrode. For simplicity, the microelectrode manipulator will not be depicted in this activity.)
- Microelectrode amplifier—used to measure the voltage between the microelectrode and a reference
- Stimulator—used to select the stimulus modality (pressure, chemical, heat, or light) and intensity (low, moderate, or high)
- Oscilloscope—used to observe voltage changes

Introduction

In this activity you will explore changes in potential that occur in the axon. Axons are long, thin structures that conduct a signal called the **action potential**. A **nerve** is a bundle of axons.

Axons are typically studied in a nerve chamber. In this activity the axon will be draped over wires that make electrical contact with the axon and can therefore record the electrical activity in the axon. Because the axon is so thin, it is very difficult to insert an electrode across the membrane into the axon. However, some of the charge (ions) that cross the membrane to generate the action potential can be recorded from outside the membrane (extracellular recording) as you will do in this activity. The molecular mechanisms underlying the action potential were explored more that 50 years ago with intracellular recording using the giant axons of the squid, which are about 1 millimeter in diameter.

In this activity the axon will be artificially disconnected from the cell body and dendrites. In a typical multipolar neuron (view Figure 3.1), the axon extends from the cell body at a region called the **axon hillock**. In a myelinated axon, this first region is called the initial segment. An action potential is usually initiated at the junction of the axon hillock and the initial segment; therefore, this region is also referred to as the **trigger zone**.

You will use an electrical stimulator to explore the properties of the action potential. Current passes from the stimulator to one of the stimulation wires, then across the axon, and then back to the stimulator through a second wire. This current will depolarize the axon. Normally, in a

Introduction

sensory neuron, the depolarizing receptor potential spreads passively to the axon hillock and produces the depolarization needed to evoke the action potential. Once an action potential is generated, it is regenerated down the membrane of the axon. In other words, the action potential is **propagated**, or *conducted*, down the axon (see Activity 6).

You will now generate an action potential at one end of the axon by stimulating it electrically and record the action potential that is propagated down the axon. The extracellular action potential that you record is similar to one that would be recorded across the membrane with an intracellular microelectrode, but much smaller. For simplicity, only one axon is depicted in this activity.

Equipment Used

- Nerve chamber
- Axon
- Oscilloscope—used to observe timing of stimuli and voltage changes in the axon
- Stimulator—used to set the stimulus voltage and to deliver pulses that depolarize the axon
- Stimulation wires (S)
- Recording electrodes (wires R1 and R2)—used to record voltage changes in the axon (The first set of recording electrodes, R1, is 2 centimeters from the stimulation wires, and the second set of recording electrodes, R2, is 2 centimeters from R1.)

Introduction

The action potential (as seen in Activity 3) is generated when voltage-gated sodium channels open in sufficient numbers. **Voltage-gated sodium channels** open when the membrane depolarizes. Each sodium channel that opens allows Na^+ ions to diffuse into the cell down their electrochemical gradient. When enough sodium channels open so that the amount of sodium ions that enters via these voltage-gated channels overcomes the leak of potassium ions (recall that the potassium leak via passive channels establishes and maintains the negative resting membrane potential), threshold for the action potential is reached, and an action potential is generated.

In this activity you will observe what happens when these voltage-gated sodium channels are blocked with chemicals. One such chemical is tetrodotoxin (TTX), a toxin found in pufferfish, which is extremely poisonous. Another such chemical is lidocaine, which is typically used to block pain in dentistry and minor surgery.

Equipment Used

- Nerve chamber
- Axon
- Oscilloscope—used to observe timing of stimuli and voltage changes in the axon
- Stimulator—used to set the stimulus voltage and the interval between stimuli and to deliver pulses that depolarize the axon

Introduction

- Stimulation wires (S)
- Recording electrodes (wires R1 and R2)—used to record voltage changes in the axon (The first set of recording electrodes, R1, is 2 centimeters from the stimulation wires, and the second set of recording electrodes, R2, is 2 centimeters from R1.)
- Tetrodotoxin (TTX)
- Lidocaine

Introduction

Voltage-gated sodium channels in the plasma membrane of an excitable cell open when the membrane depolarizes. About 1-2 milliseconds later, these same channels inactivate, meaning they no longer allow sodium to go through the channel. These inactivated channels cannot be reopened by depolarization for an additional period of time (at least a few milliseconds). Thus, during this time, fewer sodium channels can be opened. There are also voltage-gated potassium channels that open during the action potential. These potassium channels open more slowly. They contribute to the repolarization of the action potential from its peak, as more potassium flows out through this second type of potassium channel (recall there are also passive potassium channels that let potassium leak out, and these leakage channels are always open). The flux through extra voltage-gated potassium channels opposes the depolarization of the membrane to threshold, and it also causes the membrane potential to become transiently more negative than the resting potential at the end of an action potential. This phase is called after-hyperpolarization, or the undershoot.

In this activity you will explore the consequences the conformation states of the voltage-gated channels have for the generation of subsequent action potentials.

Equipment Used

- Nerve chamber
- Axon

Introduction

- Oscilloscope—used to observe timing of stimuli and voltage changes in the axon
- Stimulator—used to set the stimulus voltage and the interval between stimuli and to deliver pulses that depolarize the axon
- Stimulation wires (S)
- Recording electrode (wires R1)—used to record voltage changes in the axon (The recording electrode is 2 centimeters from the stimulation wires.)

Introduction

As seen in Activity 3, the action potential has a constant amplitude, regardless of the stimulus intensity—it is an "all-or-none" event. As seen in Activity 5, the absolute refractory period is the time after an action potential when the neuron cannot fire a second action potential, no matter how intense the stimulus, and the relative refractory period is the time after an action potential when a second action potential can be generated if the stimulus intensity is increased.

In this activity you will use these concepts to begin to explore how the axon codes the stimulus intensity as *frequency*, the number of events (in this case, action potentials) per unit time. To demonstrate this phenomenon you will use longer periods of stimulation that are more representative of real-life stimuli. For example, when you encounter an odor, the odor is normally present for seconds (or longer), unlike the very brief stimuli used in Activities 3-5. These longer stimuli allow the axon of the neuron to generate additional action potentials as soon as it has recovered from the first. As seen in Activity 5, the length of this recovery period changes depending on the stimulus intensity. For example, at threshold, a second action potential can occur only after the axon has recovered from the absolute refractory period and the entire relative refractory period.

We will not consider the phenomenon of adaptation, which is a decrease in the response amplitude that often occurs with prolonged stimuli. For example, with most odors, after many seconds, you no longer smell the odor, even though it is still present. This decrease in response is due to adaptation.

Introduction

Equipment Used

- Nerve chamber
- Axon
- Oscilloscope—used to observe timing of stimuli and voltage changes in the axon
- Stimulator—used to set the voltage and duration of stimuli and to deliver pulses that depolarize the axon
- Stimulation wires (S)
- Recording electrode (wires R1)—used to record voltage changes in the axon (The recording electrode is 2 centimeters from the stimulation wires.)

Introduction

Once generated, the action potential is propagated, or conducted, down the axon. In other words, all-or-none action potentials are regenerated along the entire length of the axon. This propagation ensures that the amplitude of the action potential does not diminish as it is conducted along the axon. In some cases, such as the sensory neuron traveling from your toe to the spinal cord, the axon can be quite long (in this case, up to 1 meter). Propagation/conduction occurs because there are voltage-gated sodium and potassium channels located along the axon and because the large depolarization that constitutes the action potential (once generated at the trigger zone) easily brings the next region of the axon to threshold. The **conduction velocity** can be easily calculated by knowing both the distance the action potential travels and the amount of time it takes. Velocity has the units of distance per time, typically meters/second. An experimental stimulus artifact (see Activity 3) provides a convenient marker of the stimulus time because it travels very quickly (for our purposes, instantaneously) along the axon.

Several parameters influence the conduction velocity in an axon, including the axon diameter and the amount of myelination. **Myelination** refers to a special wrapping of the membrane from glial cells (or neuroglia) around the axon. In the central nervous system, oligodendrocytes are the glia that wrap around the axon. In the peripheral nervous system, the Schwann cells are the glia that wrap around the axon. Many glial cells along the axon contribute a myelin sheath, and the myelin sheaths are separated by gaps called nodes of Ranvier.

Introduction

In this activity you will compare the conduction velocities of three axons: (1) a large-diameter, heavily myelinated axon, often called an A fiber (the terms axon and fiber are synonymous), (2) a medium-diameter, lightly myelinated axon (called the B fiber), and (3) a thin, unmyelinated fiber (called the C fiber). Examples of these axon types in the body include the axon of the sensory Pacinian corpuscle (an A fiber), a visceral sensory fiber (a B fiber), and the axon of both the olfactory sensory neuron and a free nerve ending (C fibers).

Equipment Used

- Nerve chamber
- Three axons—A fiber, B fiber, and C fiber
- Oscilloscope—used to observe timing of stimuli and voltage changes in the axon
- Stimulator—used to set the stimulus voltage and to deliver pulses that depolarize the axon
- Stimulation wires (S)
- Recording electrodes (wires R1 and R2)—used to record voltage changes in the axon (The first set of recording electrodes, R1, is 2 centimeters from the stimulation wires, and the second set of recording electrodes, R2, is 2 centimeters from R1.)

Introduction

A major function of the nervous system is communication. The axon conducts the action potential from one place to another. Often, the axon has branches so that the action potential is conducted to several places at about the same time. At the end of each branch, there is a region called the axon terminal that is specialized to release packets of chemical neurotransmitters from small (~30 nm diameter) intracellular membrane-bound vesicles, called **synaptic vesicles**. **Neurotransmitters** are extracellular signal molecules that act on local targets as paracrine agents, on the neuron releasing the chemical as autocrine agents, and sometimes as hormones (endocrine agents) that reach their target(s) via the circulation. These chemicals are released by exocytosis and diffuse across a small extracellular space (called the synaptic gap, or synaptic cleft) to the target (most often the receiving end of another neuron or a muscle or gland). The neurotransmitter molecules often bind to membrane receptor proteins on the target, setting in motion a sequence of molecular events that can open or close membrane ion channels and cause the membrane potential in the target cell to change. This region where the neurotransmitter is released from one neuron and binds to a receptor on a target cell is called a **chemical synapse**, and the change in membrane potential of the target is called a synaptic potential, or **postsynaptic potential**.

In this activity you will explore some of the steps in neurotransmitter release from the axon terminal. Exocytosis of synaptic vesicles is normally triggered by an increase in calcium ions in the axon terminal. The calcium enters from outside the cell through membrane calcium channels that are opened by the depolarization of the action potential. The axon terminal has

Introduction

been greatly magnified in this activity so that you can visualize the release of neurotransmitter. Different from the other activities in this exercise, however, this procedure of directly seeing neurotransmitter release is not easily done in the lab; rather, neurotransmitter is usually detected by the postsynaptic potentials it triggers or by collecting and analyzing chemicals at the synapse after robust stimulation of the neurons.

Equipment Used

- Neuron (in vitro)—a large, dissociated (or cultured) neuron with magnified axon terminal
- Four extracellular solutions—control Ca^{2+}, no Ca^{2+}, low Ca^{2+}, and Mg^{2+}

Introduction

In the nervous system, sensory neurons respond to adequate sensory stimuli, generating action potentials in the axon if the stimulus is strong enough to reach threshold (the action potential is an "all-or-nothing" event). Via chemical synapses, these sensory neurons communicate with interneurons that process the information. Interneurons also communicate with motor neurons that stimulate muscles and glands, again, usually via chemical synapses.

After performing Activities 1-8 you should have a better understanding of how neurons function by generating changes from their resting membrane potential. If threshold is reached, an action potential is generated and propagated. If the stimulus is more intense, then action potentials are generated at a higher frequency, causing the release of more neurotransmitter at the next synapse. At an excitatory synapse the chemical neurotransmitter binds to receptors at the receiving end of the next cell (usually the cell body or dendrites of an interneuron), causing ion channels to open, resulting in a depolarization toward threshold for an action potential in the interneuron's axon. This depolarizing synaptic potential (called an excitatory postsynaptic potential) is graded in amplitude, depending on the amount of neurotransmitter and the number of channels that open. In the axon, the amplitude of this synaptic potential is coded as the frequency of action potentials. Neurotransmitters can also cause inhibition, which will not be covered in this activity.

In this activity you will stimulate a sensory neuron, predict the response of that cell and its target, and then test those predictions.

Introduction

Equipment Used

- Sensory neuron (in vitro)—a large, dissociated (or cultured) neuron
- Interneuron (in vitro)—a large, dissociated (or cultured) interneuron
- Microelectrodes—small probes with very small tips that can impale a single neuron (In an actual wet lab, a microelectrode manipulator is used to position the microelectrodes. For simplicity, the microelectrode manipulator will not be depicted in this activity.)
- Hook electrodes—used to record extracellular voltage changes in the axon.
- Oscilloscope – used to observe the changes in voltage across the membrane of the neuron and interneuron
- Stimulator—used to set the stimulus intensity (low or high) and to deliver pulses to the neuron