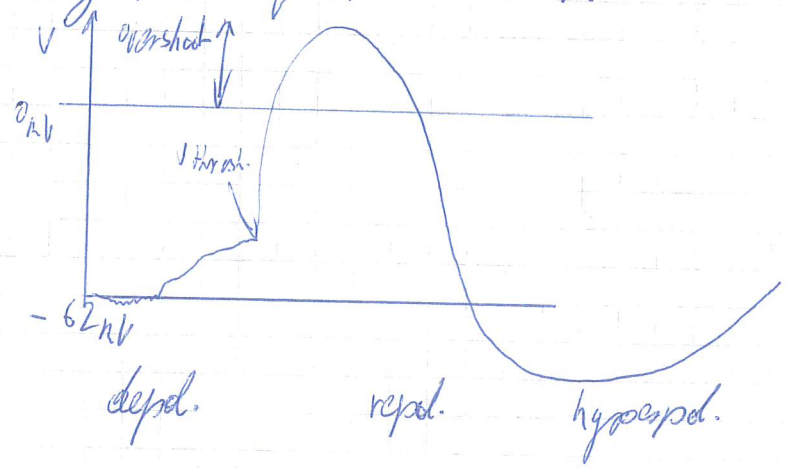
biophysics - Zeeh 1

action potential

always looks the same, neuron signals are frequency coded

resting memb. pot. ~ -62 mV



too little input - no AP - back to resting pot.

motivation

- understanding single cell neural activity
- techniques:
  - patch clamp electrophysiology
  - computational modeling of neuronal activity
  - (immunohistochemistry (staining) of tissue)

on retina ON and OFF cells, always comparative

light on ON cells fire more frequently
OFF cells stop (hyperpol.)

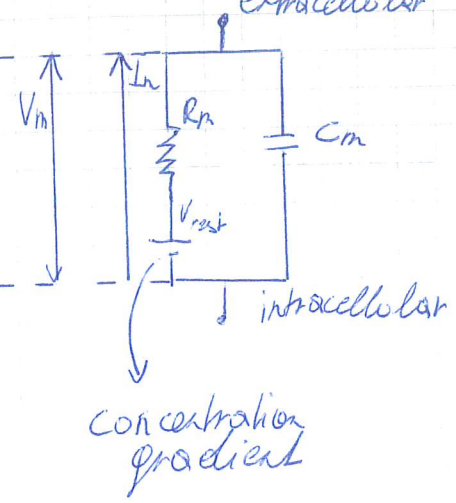
nervous sys. always noise (few AP)

equivalent circuit model

pyramidal cell (brain)

assume only 1 point, no 3D structure

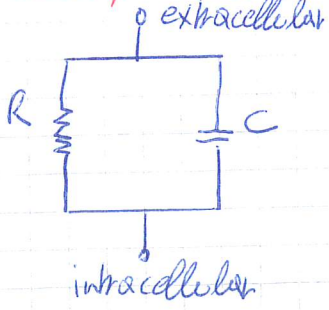
model as RC circuits



- across a wire, the pot. is the same
- charge inflow into one element must equal the outflow
- Kirchhoff's law: at a junction of wires, the total current is 0, $\Sigma I = 0$
- pot. changes by a fixed amount across a battery
- pot. changes by a variable amount across a resistor
  - Ohm's law: $U = V = R \cdot I$
  - alt. formulation $I = G \cdot U$
    conductance $G = \frac{1}{R}$

holes in membrane - resistors
bilayer - capacitor

the passive membrane



Kirchhoff: $I_R + I_C + I_{ext} = 0$

Resistor: $V = I_R \cdot R$ - charge

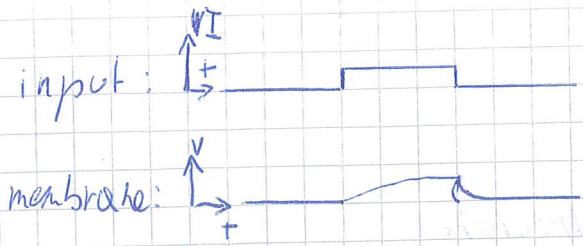
Capacitor: $C = \frac{Q}{V}$

$Q = CV$

$I_C = \frac{dQ}{dt} = C\frac{dV}{dt}$

ion channel open → more ion flow → R decrease

$$C\frac{dV}{dt} = -\frac{V}{R} - I_{ext}$$



time const. $\tau = RC$

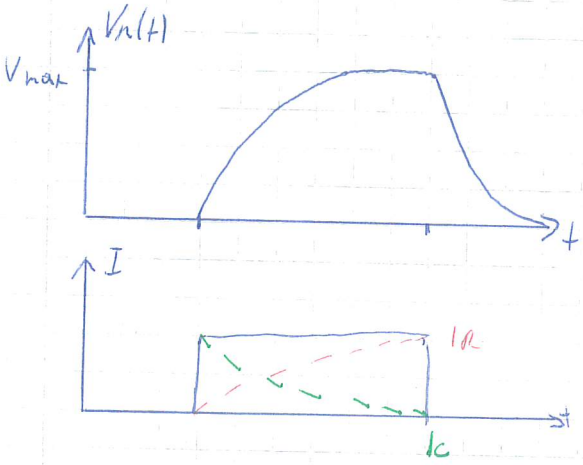
time it takes to reach steady state response after change in voltage across membrane

multiply R ×2: changes time parameter τ, V reaches higher plateau if V_{max} was not reached before

multiply C ×2: time const. changes, but not peak size (τ V_{max}) steeper curve

shape of AP depends on initial condition a little (resting memb. pot.)

$V_m(t) = V_{max}\ (1 - e^{-\frac{t}{\tau}})$ — $\tau = R\,C$



as C gets charged, Ic feels more resistance

and R gets more current, in correlation less resistance

if battery is added, same relationship, but battery (resting potential) influences V_{max}

Hodgkin – Huxley experiment

physics of neural signals, propagate as membrane voltage along axon; nonmyelinated squid axon, stimulus electrode = long uninsulated wire → every part of membrane has to react same way (propagation of signal prevented)

- why -62mV? because of diff. ions, Na^+, K^+ in/out
- effect of certain ions? remove from solution, see what happens
- concentration gradient → diffusion (ions are charges)
- how do they flow? ion channels in favor of K^+
- Nernst in model like batteries

each ion type has its own battery

- diff. ion channels have associated equilibrium (reversal) Nernst potentials
- a given conductance moves the membrane voltage to the equilibrium potential for that specific ion

$E_{NA} \sim 50mV$

$E_K \sim -90mV$

higher Na^+, Cl^-, Ca^{2+}

Nernst equation $V_{eq.} = \frac{RT}{zF} \ln\left(\frac{[X]_{out}}{[X]_{in}}\right)$

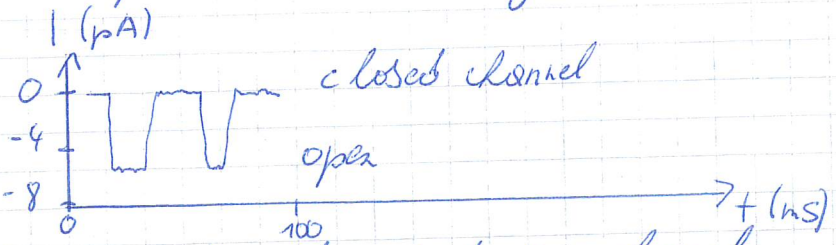
z = charge in mol of ions transferred

Na^+ depolarize membrane → sodium inflow

K^+ hyperpolarize membrane → potassium outflow

voltage dependent ion channels

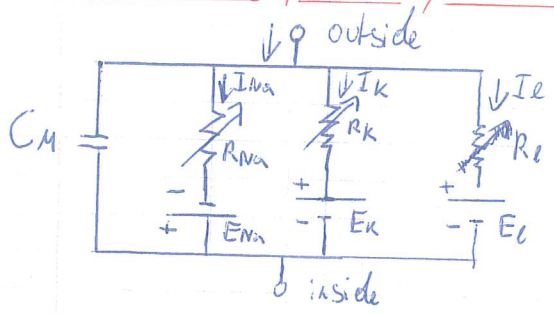
- single ion channels open stochastically (zufällig)
- all ions have their own equilibrium
- ion channels can be modeled as a conductance coupled with a battery



current through a single ion channel

- ions flow through volt. gated channels based on state of membrane (have flaps)
- billions of channels

parallel paths for ions across the membrane



for every ion

$I_i = g_i \cdot (V - E_i)$

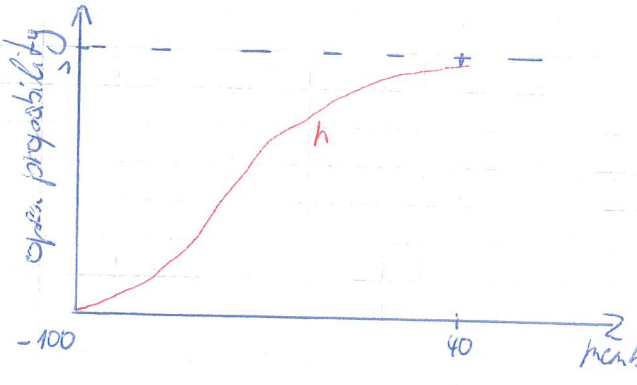
g_i ... conductance of channel

R_{Na}, R_K are variable because channels can open and close

l ... leakage (constant)

ion channel dynamics

- ion channel conductance is variable
- Hodgkin-Huxley model:
  - sodium ion channel
  - potassion ion channel
  - leakage conductance (constant)
- ion channels have gates
- sensor senses potential between in- and outside of the membrane (membrane voltage)
- propability of the gate being open or closed depends on the membrane potential



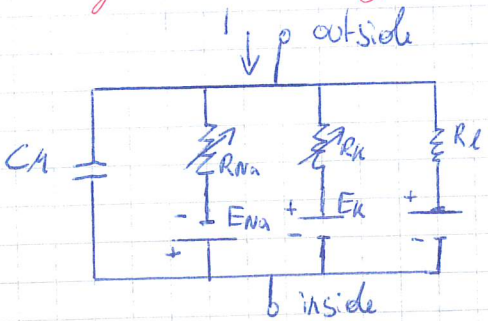
$i_K = g_{K\,max} \cdot n^4 \cdot (V - E_K)$

example: inject positive current into cell → K^+ channels open → K^+ flows out → counterbalances injected current

$$i_{Na} = g_{Na,max} \cdot m^3 \cdot h \cdot (V - E_{Na})$$

- Sodium channels have activation (m, opening) and inactivation (h, closing)
- the product of m^3 and h give the total opening probability
- the inactivation stops the sodium influx during an action potential
- if m or h is 0 → all is 0
- Na^+ ions are for upshoot (much faster), K^+ ions to calm down (open late)

Hodgkin-Huxley model



apply Ohm's law and Kirchhoff's law

$$C_m \frac{dV}{dt} = -\sum_i g_i (V - E_i) - I_{ext}$$

$C_m \frac{dV}{dt}$: capacitive membrane; $\sum_i g_i (V - E_i)$: ionic; I_{ext}: stimulus

$$-C_m \frac{dV}{dt} = g_L (V - E_L) + \bar{g}_K n^4 (V - E_K) + \bar{g}_{Na} m^3 h (V - E_{Na}) + I_{ext}$$

→ no analytical solution

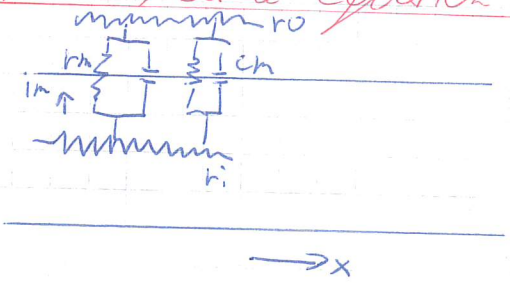
geometry

until here, we approximated the neuron to be a point model (soma, dendrites, axons)
but the geometry is also important, how the dendrites act, input and output are different

dendrites process information

smaller and wider axes
current leaks in membrane
AP was initiated in soma, at dendrite AP delayed and with smaller amplitude
EPSP arrives at dendrite delayed, smaller amplitude and slower decay at soma

(neural) cable equation



voltage is both a function of t and x → partial differential equation

r_m... membrane resistance
r_i... axial specific resistance
r_o... assumed to be 0
outside 0mV, inside -62mV

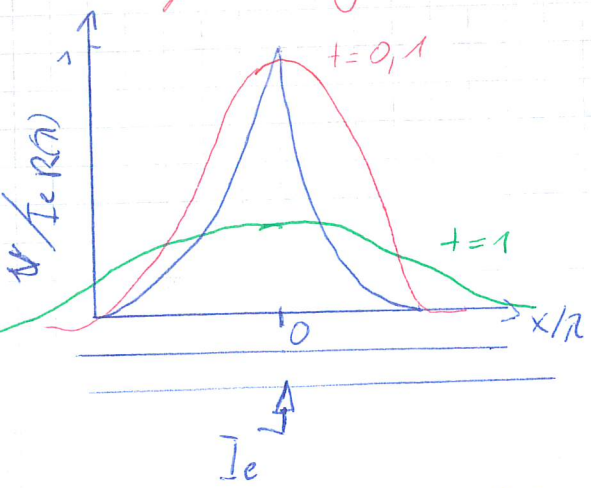
for point approximation:

$$i_m = i_c + i_{ionic} = c_m \frac{dV_m}{dt} + \frac{V_m}{r_m}$$

now also consider axial current i_i, which is proportional to the changes in x

voltage decay in space/time

$\lambda = \sqrt{\frac{r_m}{r_i}}$ ... time, constant and space



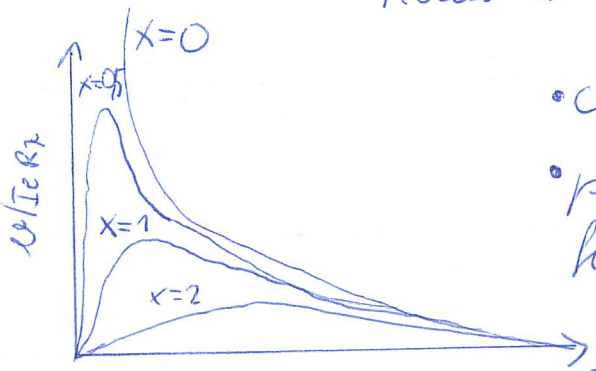
potential decays exponentially from $x=0$ (point of current injection)

$$v(x) \propto e^{-\frac{|x|}{\lambda}}$$

falls off in space and time

model in multiple Hodgkin-Huxley

- current pulse at $x=0$, $t=0$
- potential peaks later and lower for points further away



diffuse spread and exponential decay

compartment models

real neurons have ion channels and complex geometries

analytical solution complicated

→ compartments and numerical solutions

for each compartment:

- V_m is calculated by a set of differential equations (usually independent of x)
- spatial dependence is modeled by coupling the compartments

How complex must the geometry be?

if the diameters of the subbranches d1 and d2 of a branch d1 are in the following relationship, they can be approximated with a simple compartment of equal surface area

→ 1 soma compartment 1 dendrite

$d_{11}^{3/2} + d_{12}^{3/2} = d_1^{3/2}$

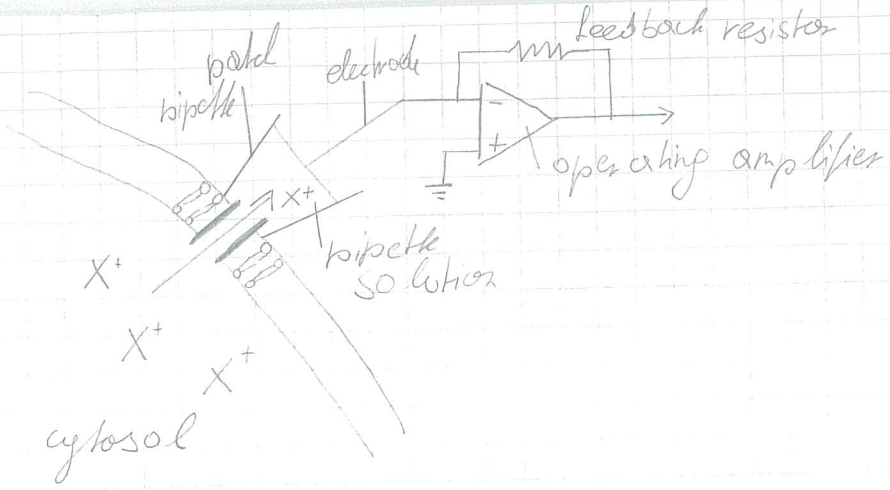
only true for passive membranes

full compartment models

- each compartment consists of part of the neuron with similar ion channel densities and properties
- compartments are modeled similar to the Hodgkin-Huxley model before, but are coupled to their neighboring compartments with a conductance term

patch clamp electrophysiology

- used to record membrane voltage (V_m) or currents through (single) ion channels
- fine-tipped (diameter 1-2 µm) glass micropipette attached to the neuron. low electrode resistance (2-8 MΩ)
- pros:
  - highly detailed information about membrane currents and membrane voltage
  - dissociated neurons and neurons in slice can be cultured and recorded for many weeks
  - neurons in an ex-vivo retina - measured up to one week
- cons:
  - time consuming - one cell per recording
  - sophisticated equipment required
  - once you patched the neuron with an electrode, the recording time is limited to ~1 h



patch clamp setup

- camera
- peristaltic pump
- micromanipulator control
- amplifier
- oscilloscope

- micromanipulator
- retina tissue + perfusion
- headstage / pre-amp

patch clamp - configurations

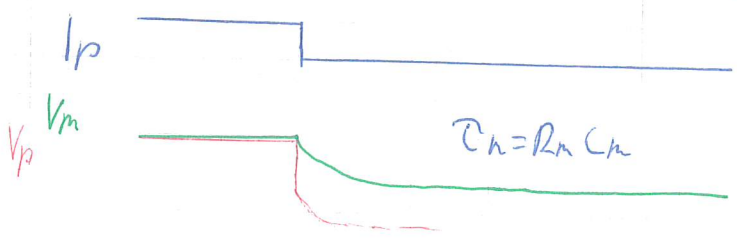
- loose patch
  - approach cell and apply little suction
  - $R_{seal} < 100\ M\Omega$ → counting spikes

- cell-attached patch
  - formation of Gigaseal ($R_{seal} > 1\ G\Omega$)
  - allows measurement of currents through single or few ion channels
  - intracellular intact, little disturbance of cell
  - pull patch pipette away from Gigaseal configuration
  - inside is now exposed to extracellular solution

- allows measurement of currents with varying solutions at intracellular
- whole cell patch
  - starts with cell attached configuration
  - by application of strong suction the membrane is ruptured → access to intracellular space
  - current can be injected to see cell response
  - pipette solution must be adjusted to keep cell alive
- outside - out patch
- perforated patch
- inside - out patch

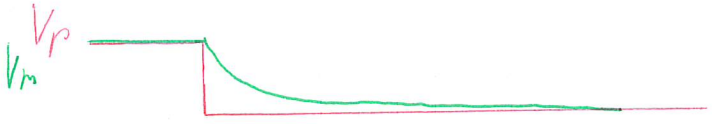
patch clamp - current clamp

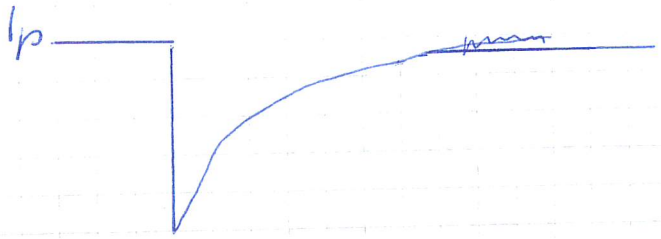
- Summary
  - application of const. current (can be 0)
  - measurement memb. voltage / potential
  - estimation of passive memb. properties R_m and C_m
  - number and shape of AP can be analyzed
- challenges
  - compensation of electrode resistance and capacitance
  - cellular damage induced by the electrode (in whole cell configuration only)
  - filtering of the recorded signal by the electrode



patch clamp - voltage clamp

- Summary
  - application const. voltage across cell membrane
  - measurement of membrane current
  - estimation of active membrane properties (ion channel conductance)
- challenges
  - compensation of electrode resistance and capacitance
  - cellular damage induced by the electrode
  - space clamp problem / imperfect clamping





volt. clamp

- defined memb. volt. steps are applied
- current is measured
- ion channel properties are estimated

current clamp

- defined current is injected into cell
- memb. volt. is measured
- threshold voltage, spike width, spike height etc. are measured

biophysics - Tech 2

one neuron - patch clamp
many neurons - MEA (micro electrode array)

applications

broad field
from subcellular [µm] over spatial scale/network complexity to large brain structures [cm]
cell culture, retina, brain organoids, brain slices, cortical array
in vitro - in vivo (neuroprosthetics)

"distant" electrode

- no tight contacts
- in vivo: recordings
- in vitro: recordings from tissue

"close" electrode (seal)

- point contact model
- in vitro: cell cultures, recordings from tissue

MEA application - in-vivo (in a living organism)

- vision restoration
- electrical stimulation electrodes implanted into brain
- communication locked in patients
- flexible MEA

simplified example (extracellular recordings from distant neurons)

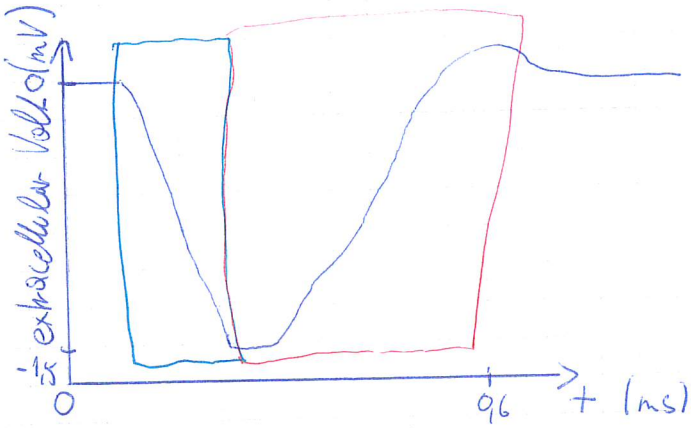
sodium influx - ~~potassium efflux~~, ~~AP = Spike~~

distance is crucial

measured signal is AP outside of cell

measured signal: $V(r) = \frac{I \rho}{4\pi r}$

ρ... resistivity of the medium through which the current flows



what does the electrode record?

- waveform inverted compared to an intracellular AP
- signal amplitude is much smaller compared to intracellular recording (mV range)
- neuron - electrode distance influences the signal amplitude measured at the electrodes
- an extracellular AP is generally referred as a spike

MEA application - in-vitro (outside a living organism)

neuronal culture

close contact, electrodes ~ 10-20µm in diameter

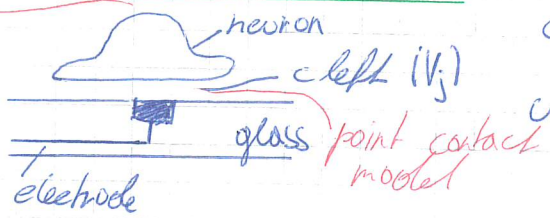
- neuron from tissue are dissociated and placed over MEA
- neurons grow to form a network
- neurons can be dissociated (isolated) from different

nervous structures (also cardiomyocytes) or from stem cells (iPSCs)

- in combination with HD MEA, it is possible to record from subcellular structures (dendrites, axons)

point contact model

HH model



cleft is difference
want to measure V membrane

extracellular voltage V_j can be calculated by applying Kirchhoff's law to the node formed at the junction (cleft)

$$g_j V_j = \underbrace{c_M \frac{dV_M}{dt}}_{\text{memb. C volt.}} + \underbrace{\sum_i g_{jM}(V_M - V_{eg}^i)}_{\text{memb. ionic voltage}}$$

$g_j V_j$: junction volt. (at cell-electrode)

intracellular voltage $V_M(t)$ can be calculated by applying Kirchhoff's law to the node formed in the cell interior, and considering the possibility of current injection by the patch-clamp

$$\underbrace{c_M \frac{dV_M}{dt}}_{\text{memb. C volt.}} + \underbrace{\sum_i g_{FM}^i (V_M - V_{eg}^i)}_{\text{memb. ionic volt.}} = \frac{I_{inj}}{A_{FM}\text{-free memb.}}$$

$A_{jM} \approx 2\% < A_{FM}$ - free memb.
docked to electrode

The combination of both equations enables the resolution of the problem of the coupled dynamics of the extracellular and intracellular potentials $V_M(t)$ and $V_j(t)$.

The neuronal excitation (action potential) drives ionic and capacitive current through the membrane attached to the chip. That current is squeezed through the cleft between cell and chip, and gives rise to the transduction extracellular potential.

$$C_{j,\mu} = C_{\mu} \qquad \text{free membrane}$$

$$g_j V_j - \sum_i \left(g^i_{j,\mu} - g^i_{F,\mu} \right) \left(V_{\mu} - V^i_{cp} \right) + \frac{I_{i,j}}{A_{F,\mu}}$$

junction membrane

Neuron - MEA interface: resume

neuron side: Hodgkin Huxley model (membrane)

| point contact model

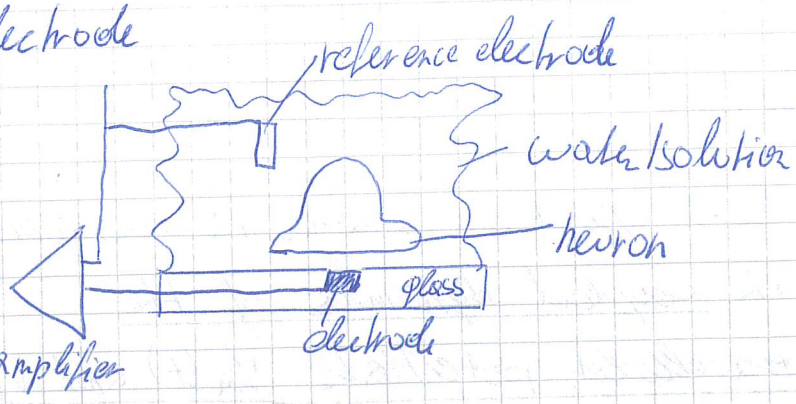
electrode side (microelectrode)

signal is visible on multiple electrodes, depending on distance to AP (far away → smaller), can be color coded

electrochemical recording

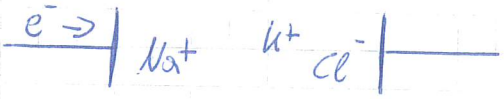
measure voltage in a solution - need reference electrode

$$V(r) = \frac{I \rho}{4\pi r}$$



electrode in solution (electrochemistry)
water is good insolator → therefore need ions

electrode - electrolyte interface



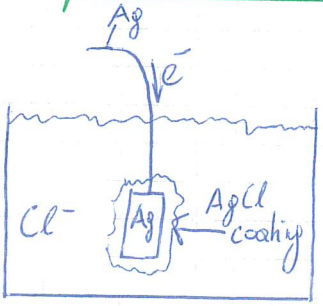
perfectly polarizable electrodes

- electrodes behave like capacitor
- there is no charge which may cross the electrode - electrolyte interface
- when voltage is applied to one contact side. the current across the interface is a displacement current.
- examples: platinum electrode, fully insolated electrodes

perfectly non-polarizable electrodes

- electrodes behave like resistor
- electrodes in which current passes with little resistance electrode - electrolyte interface
- for biomedical application current is carried by ions
- examples: Ag / AgCl electrode

non-polarizable electrodes: ground electrodes in electrophysiology



charge moves across electrode-electrolyte interface via Red-ox reactions

electrode reaction:

$Ag + Cl^- \rightleftharpoons AgCl + \text{electron}(e^-)$

or

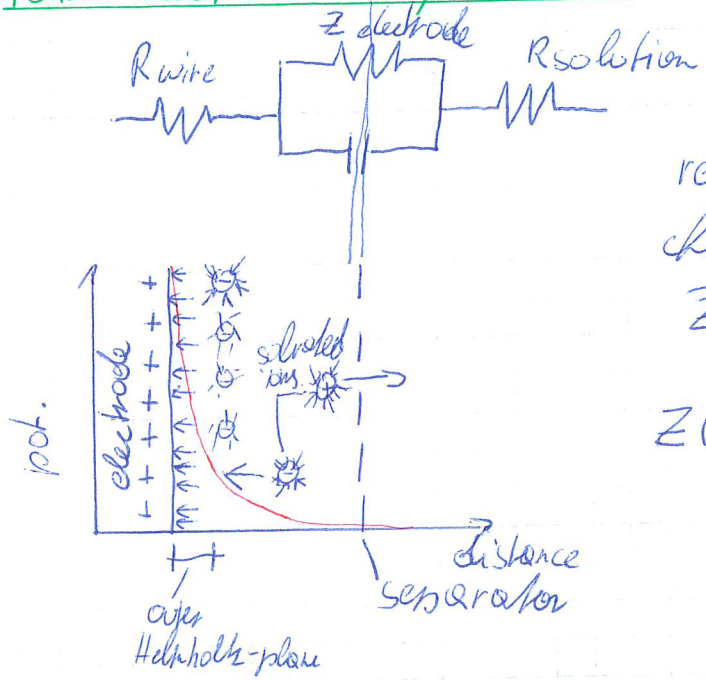
$AgCl \rightleftharpoons Ag^+ + Cl^-$

$+e^-$ ↓ ↑ $-e^-$

Ag

1. reversible electrode, current flows in both directions
2. the Ag/AgCl performs well only in solutions containing chloride ions
3. If the AgCl is exhausted by the current flow, bare silver could come in contact with the solution. Silver ions leaking from the wire can poison cells.

real electrode: impedance



double layer

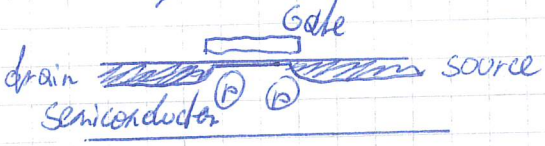
real electrodes are characterized by the impedance

$$\vec{Z} = R + jX$$

$$Z(\omega) = \frac{R}{1 + j\omega RC}$$

active / capacative electrode

EOSFET (electrolyte oxide semiconductor field effect transistor)



MEA design

design and materials are application driven.
→ there is no perfect MEA, there is the best MEA for each application

system requirements
- spatial sampling (electrode size and spacing)
- temporal sampling
- material, mech. properties, biocompability ...

System requirements for the detection of single cell and of single axons

AP extracellular ~1 ms → 1 kHz
→ temporal sampling min. 10 kHz (10 points per AP) to 25 kHz
→ always trade of, no point of storing useless data

spatial sampling, cell body 5-50 µm
→ do I measure cells or axons?
→ for cell 25-30 µm
→ for axon 8 µm

"standard" MEA

- electrode pitch "30-200" µm
- tens - few hundreds of electrodes
- photolithography (Maske, wegätzen) on glass/insulator substrate

MEA Types:

- passive MEA ("standard"), connecting is problem for grid
- MEA with integrated CMOS circuit, less problem with connecting (one layer lower, more space to connect grid), can achieve higher spatial resolution

result is electrical imaging
retina: all axons in one direction - optic nerve

data analysis - spike sorting

problem 1: a single channel can record signals from multiple neurons
problem 2: a single neuron is recorded by multiple channels (especially in HD MEA)
goal: spikes from single neuron
filter: bandpass 300 - 3,5 kHz to isolate spikes
AP lasts ~1ms → fgc. is 1000 Hz = 1 kHz
reduces noise
small spikes means signal is further away, take neurons apart by spike size
use principal component analysis - in this case spike sorting (computational heavy)

spike sorting

- is a name for a series of algorithm that allows to identify single cell activity from MEA recordings
- common approaches uses features extraction techniques, i.e. PCA (principal component analysis), in combination with clustering methods to seperate signals from different cells

- for HD MEA more modern approaches have developed to reduce the computational load and improve performance (speed)

alternative to MEA: optical imaging of neuronal activity

common in neuroscience

pros: easy, just need microscope, less invasive

cons: slow, not single spike

MEA in vivo: retina implants

In-vivo / neuroprosthetics applications have specific challenge:

- power supply
- long term stability (encapsulation)
- optimal contact with neural tissue
- material and mechanical properties, i.e. flexibility or bio compability