BB511 Manual Ion Transport Lab Exercise

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April 2025 v2

Contents

Chapter 1

About

This manual contains the background information for the exercise (Chapter 2) as well as the instructions for each experiment and the data analysis going with it. I am planning to add a markdown template to write your report, but that hasn't happened yet :)

1.1 Usage

This book will take you through the entire exercise step by step and also contain example data so you can analyze and the results after the course. All code to work with the data is written in code blocks like this:

```
print("BB511 is awesome!")
```

You can directly copy the code from these blocks using the copy button in the upper right corner of the block.

Chapter 2

Background information

Please refer to the Ion transport compendium.

2.1 A bit of history

2.2 About Ussing

2.3 The frogs problem

Chapter 3

Setting up your equipment

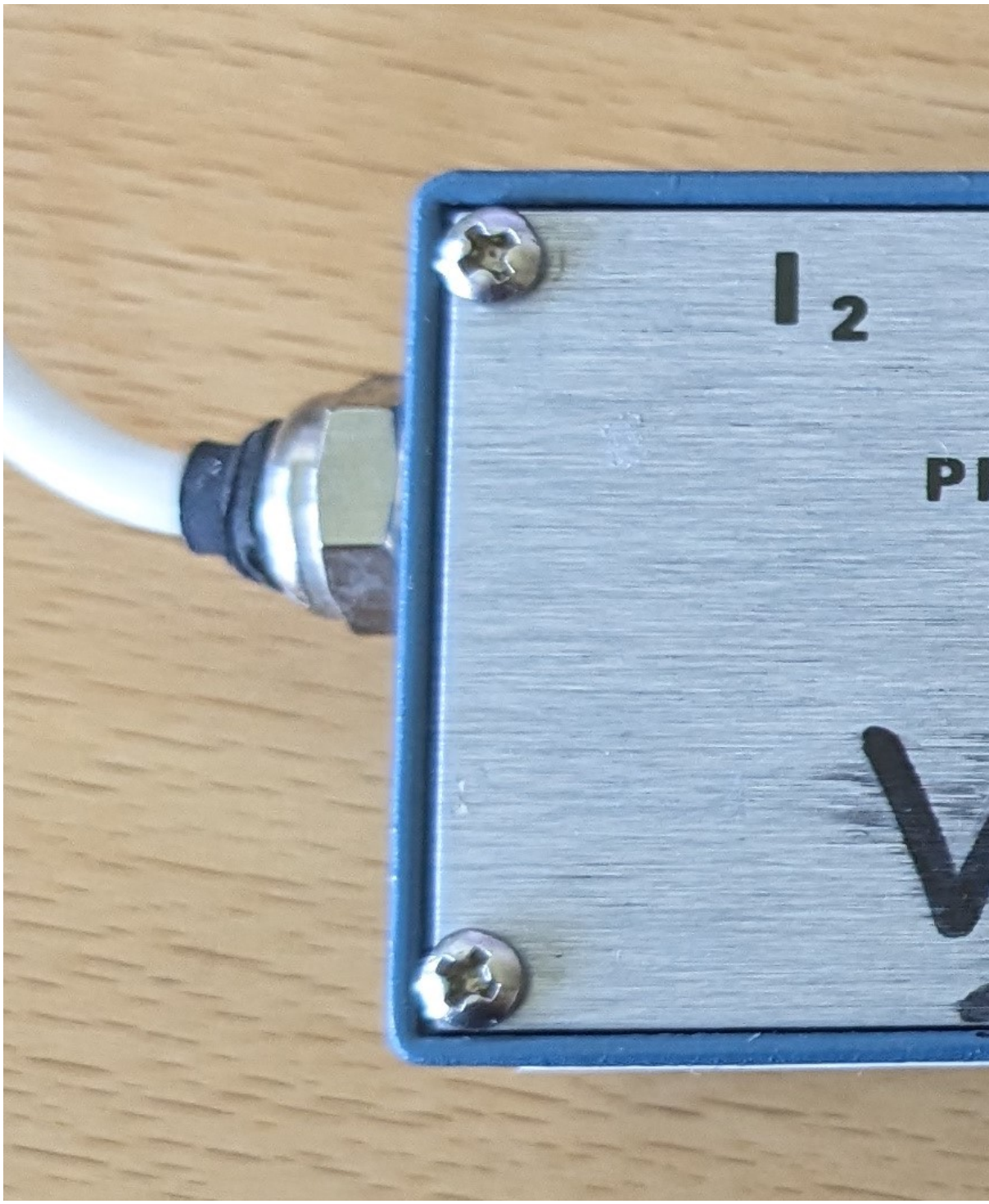
Switch on the equipment and wait 5 minutes. This is necessary for all recording hardware as resistances and capacitors on the circuit boards change with temperature and make the measurements drift while the hardware is heating up.

3.1 Building and powering up your setup

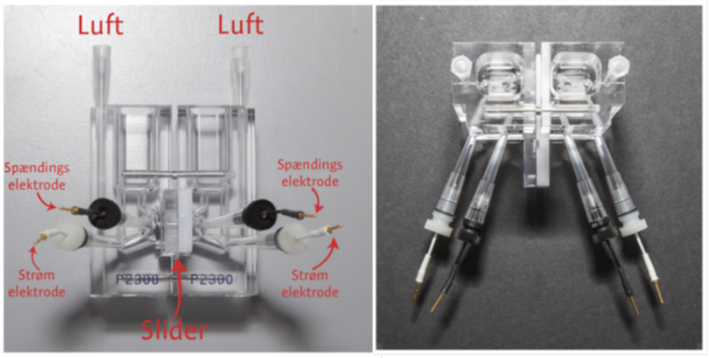
1. Assemble your chamber by:

- inserting the slider in between the two chamber halves
- inserting the chamber in the chamber holder
- tightening the screw on the side

2. Connect **Voltage** (**V**, black cables) and **Current** (**I**, red and white cables) electrodes.


I 2

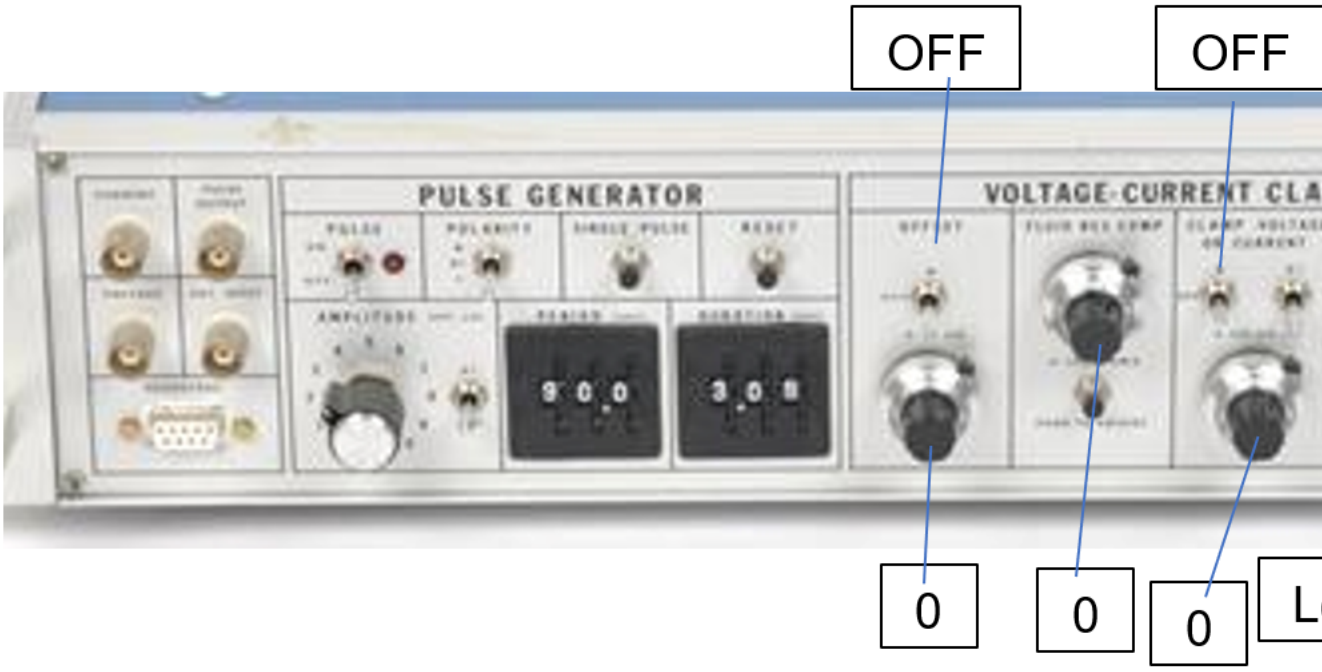
3. Connect electrodes to headstage.



4. Make sure V1 and I1 and V2 and I2 are on the *same side* of the chamber.

5. Fill chamber with Ringer's solution, insert tubing that feeds air and start the pump.

6. Check that all instrument settings are in the rest setting like on the image below:



3.2 Get familiar with the two measuring modes

Your equipment has to be on the rest setting at all times, unless you want to measure something. We will continuously measure voltage and current. For that you have to switch between two instrument settings. Familiarize yourself with both procedures.

3.2.1 Measure Voltage

1. Set the FUNCTION switch to OPEN and METER to VOLTAGE
2. Read the value in the display

3.2.2 Measure Current

1. Set the FUNCTION switch to CLAMP and METER to CURRENT
2. Read the value in the display

3.3 Account for electrode asymmetry

1. Switch FUNCTION to OPEN, and METER to VOLTAGE. The value that is now displayed reflects the asymmetry of the electrodes.
2. Use the OFFSET SWITCH in the plus or minus position and turn the OFFSET DIAL to bring the number on the display to zero.
3. If it doesn't work, get help. Check for air bubbles in your voltage electrodes (black).

3.4 Account for fluid resistance

1. Function switch still on OPEN, place METER to CURRENT and hold the FLUID RESISTANCE COMPENSATION button down.
2. The values should now be between 60 and 68 µA.
3. If it is lower, your resistance is too high. Get help.
4. Still holding the button switch to voltage mode (OPEN + VOLTAGE). Adjust the fluid resistance compensation dial to bring the number on the display to zero.
5. The value on the dial represents the fluid resistance of your system. I.e. the resistance all fluid parts of the setup together have.
6. Note down the fluid resistance.

3.5 Mount skin sample

OBS: After mounting the skin sample, you have to immediately start measuring I and V, so get ready for that first

1. Empty your chamber using the provided syringe.
2. Get a skin sample that was dissected from green frogs (*Rana kl. esculentus*). The skin was dissected from different parts of the frog (e.g. belly, back, leg etc). Note down from which part your skin was.
3. Identify the outer (mucosal) and inner (serosal) side.
4. Mount the skin on the slider of the Ussing chamber. Try to have the mucosal side point to the left halfchamber. It is important to keep track which side points to which half-cell of the chamber!
5. Note down to which side the mucosal side points.
6. Fill fresh Ringer's solution into both half chambers and ensure that both sides are bubbled. The stream of air bubble serves two purposes: it provides oxygen and mixing of the Ringer's solution.
7. Check for leaks.
8. Check for air bubbles in the chamber and specifically on the skin.
9. Start your stopwatch.

Chapter 4

Experiment 1: Determine the epithelial resistance

You will now measure the potential difference over the skin (from now on referred to as V) as well as the current needed to even out the potential difference between both sides (from now on referred to as I). This current is also called "short circuit current". I is a measure of ion transport across the skin.

4.1 Take measurements

Measure V and I every minute for 10 minutes after mounting the skin.

1. Measure V and note it in your excel sheet.
2. Measure I and note it in your excel sheet.
3. Repeat for 10 minutes in 1 minute intervals.

When not taking measurements, always switch to ZERO.

4.2 Data analysis

The value we would like to measure is the resistance of the skin, so we need to calculate the measurements you made.

Use Ohm's law to calculate the electrical resistance R of the skin. Make sure to get the unit right.

4.3 Discussion

Once you have calculated the reistance, try to answer the questions below. We will discuss them in the group, and you will use them to guide your data discussion for the report.

1. What does the epithelial resistance reflect?
2. What is the unit of the resistance?
3. How can we make it more comparable across experiments?

- How are these parameters called?

4. Do the values change over time?
5. What can we conclude from that?
6. What is the mechanism behind our observations? Why?

Chapter 5

Experiment 2: Concentration dependence of ion transport

In this experiment, you will investigate whether the ion transport process depends on the concentration of sodium ions on the mucosal side. To achieve this, you will measure I for a series of diluted Ringer's solutions. The 1x Ringers you used so far was **112 mM NaCl**.

5.1 Preparation

What is the concentration of Na^+-ions in 1x Ringer's?

To test if the ion transport depends on the concentration of Na^+-ions, we will need the following solutions:

Calculate the different concentrations of Na^+-ions yourself and enter them in the **excel sheet**. Plan how to prepare 20 mL of each of these solutions from 1x or 2x Ringer's. Once you have a plan find an instructor to help you make the solutions.

5.2 Take measurements

1. Empty chamber
2. Using the 0x solution, wash the chamber on the mucosal side 3 times (wait 2 minutes in-between).
3. Measure I and note it in your excel sheet.

4. Empty the chamber and add the solution with the next higher concentration.
5. Wait 1 minute
6. Measure I and note it in your excel sheet.
7. Repeat step 4-6 for each of the solutions in order of increasing Na^+-ion concentration.
8. **At the end go back to 1x Ringer's!**

5.3 Data Analysis

1. Convert I to the flux of Na+-ions (J) using the following relationship (F: Farraday constant): $J_{Na^+} = \frac{I_{Na^+}}{F} = \frac{1\frac{C}{s}}{96500\frac{C}{mol}}$
2. Plot J as a function of Na^+-concentration.

5.4 Discussion

1. Have you seen a curve like that before? What is it called?
2. What kind of information can you extract from these curves?

Chapter 6

Experiment 3: Influence of chloride ions

In this experiment, you investigate whether overall ion exchange over the membrane includes chloride ions. To characterize the anion transport, you will use "Sulfate - Ringer's solution" on the mucosal side of the skin. In "Sulfate - Ringer's", chloride ions are replaced with SO_4^{2-} ions.

To compare normal ringers to Sulfate-Ringers, we will take the average of 5 measurements

6.1 Take measurements

1. Empty chamber on mucosal side.
2. Using 1x Ringer's, wash the chamber on the mucosal side 3 times (wait 2 minutes in-between).
3. Measure V and note it in your excel sheet.
4. Measure I and note it in your excel sheet.
5. Repeat (step 3-4 for) 4 times in 1 minute intervals.
6. Empty chamber on mucosal side.
7. Using Sulfate Ringer's, wash the chamber on the mucosal side 3 times (wait 2 minutes in-between).
8. Measure V and note it in your excel sheet.
9. Measure I and note it in your excel sheet.
10. Repeat (step 8-9 for) 4 times in 1 minute intervals.

6.2 Discussion

1. What happened after changing the buffer?
2. Why?
3. What are the underlying mechanisms?

Chapter 7

Instructions for your lab report

Introduction: Describe how a frog osmoregulates in its natural habitat. Pay special attention to describing how the cells mechanistically function. Name and highlight the most important ion transporters the frog is using to stay in homeostasis. Remember to include a brief objective at the end. Max. 1 page.

Methods: with drawing/pictures/figures of the arrangement. Provide a general ovewrview of the experiment and pay attention to describe and mention any deviations from the manual. The figures can be made in the program BioRender (www.biorender.com).

Results: relevant figures/tables (from all experiments) with accompanying explanation; the main trends/effects in the figures are described in words. It is good practice to end the paragraph related to a figure with the main take home message of the figure. Remember that you do not discuss/explain anything in the results section, you only must describe.

Discussion: of results and conclusion (see guidance questions below). What have you shown? How does this fit in with the theoretical background? Assess and discuss uncertainties and sources of error.

Conclusion

Include your R-script as *.R file and your excel sheets in the upload.

Hand in as group and remember to include all members' full names and email addresses.

The hand-in date for the report is indicated in the assignment on ItsLearning.

General form For this lab report I expect you to adhere to all good practice regarding figures, quantitative reporting, scientific language, writing and prac-

tice that you have learned since you started studying Biology. As a guide you can refer to the guidelines for report writing you have been using many times before (linked in the assignment).

7.1 Questions and points that should be adressed in the report

Experiment 1:

1. What does the epithelial resistance reflect?
2. What is the unit of the resistance? (Make sure to have that right in the report)
3. How can we make it more comparable across experiments?

- How are these parameters called? (Make sure to use these throughout the entire report)

4. Do the values change over time?
5. What can we conclude from that?
6. What is the mechanism behind our general observations? Why?

Experiment 2:

1. Have you seen a curve (Na-flux over Na-concentration) like that before? What is it called?
2. What kind of information can you extract from these curves?
3. What does K_m express?
4. What does V_{max} express?
5. Based on your results: Give an estimate of how fast the transport (in % of V_{max}) takes place when a frog is sitting in its watering hole, with a Na^+-concentration of approximately 5 mmol/L.

Experiment 3:

1. What happened after changing the buffer?
2. Why?
3. What are the underlying mechanisms?

Chapter 8

Analyzing your data

In order to strengthen your programming and data analysis skills, we will analyze the data for this course in R. If you are proficient enough in another programming language or environment and can produce the same outcome there, that is fine with me too.

This chapter will take you through all the steps analyzing and visualizing your data and link to little instructions how do do this in R. You do not get a prewritten script, but should write your own with the instructions provided here as well as your knowledge from previous courses (eg statistics).

8.1 Experiment 1:

The value we would like to measure in this exercise is the resistance of the skin, so we need to calculate it from the measurements you made.

1. Read your data into R. See 9.1.1

2. Use Ohm's law to calculate the electrical resistance R of the skin. Make sure to get the unit right. For example:

```
data1$R <- (data1$V*1000)/data1$I
```

3. Calculate resistivity and current density.

```
data$R_cm2 <- data$R / 0.71
data$I_cm2 <- data$I / 0.71
```

4. Plot resistivity and current density over time see 9.1.2

8.2 Experiment 2:

1. Read your data into R. See 9.1.1
2. Convert I to the flux of Na+-ions (J) using the following relationship (F: Farraday constant): $J_{Na^+} = \frac{I_{Na^+}}{F} = \frac{1\frac{C}{s}}{96500\frac{C}{mol}}$
3. Plot J as a function of Na^+-concentration. 9.1.2

8.2.1 Extract V_{max} and K_m using nonlinear curve fitting.

One of the advantages of having modern computers is that we can do curve fitting to non-linear functions. We will apply this to find the V_{max} and K_m of your data.

1. First we have to define the equation we want to use. We are using the Michaelis Menten formula:

$V_0 = \frac{V_{max} * S}{K_m + S}$

```
MMcurve <- formula(J ~ Vmax * Na_concentration / (Km + Na_concentration))
```

2. Next we will use the R curve fitting function nls() to find the V_{max} and K_m that will best fit our measured values to the Michaelis-Menten-equation. For that we have to provide some start values for the function to use.

```
bestfit <- nls(MMcurve, data2, start = list(Vmax = 50, Km = 2))
```

3. Now we will calculate the fitted curve for Na^+-ion concentrations from 0 mM to 170 mM.

```
#create many x-values from 0 to 170
Na_conc <- seq(0,170,0.1)
#calculate the y-values using the fitted function
J_fitted <- predict(bestfit,list(Na_concentration = Na_conc))
```

4. Now we plot our real data and add the fitted values

```
plot(FILL THIS OUT YOURSELF)
test <- lines(Na_conc, J_fitted)
```

5. Now we only need to extract the values for V_{max} and K_m from the fitted equation. The values are stored in "bestfit" and you can extract them like this:

```
Vmax <- coef(bestfit)[1]
Km <- coef(bestfit)[2]
Vmax
Km
```

8.3 Experiment 3

1. Read in your data. See 9.1.1
2. Calculate resistivity, current density and resistance for both conditions.
3. Take the average of all values from one condition. See 9.1.3
4. Plot potential difference, current density and resistivity of the two groups. See @ref{plotting}

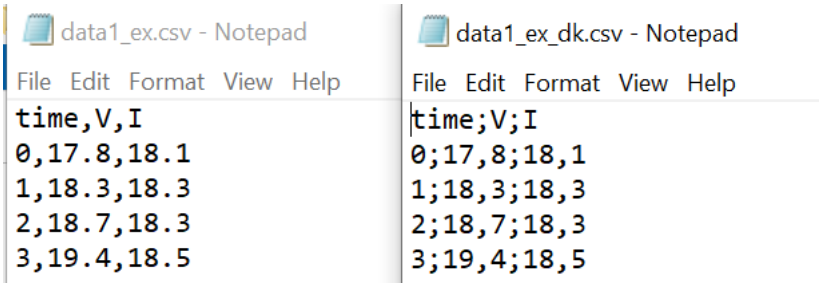
Chapter 9

R instructions and help to solve p-R-oblems

9.1 How to...

9.1.1 Read in data

Depending on how your computer is set up, csv files require different settings to read in. A CSV file is simply a text file, where columns are separated by one sign and the decimal point is indicated by another. In English speaking countries and most scientific data you can download from the internet, columns are separated by a comma "," and the decimal point is a ".". The default in other European countries, including Denmark, is to use a semikolon ";" to separate columns and a comma "," as decimal point.



Depending on which settings your computer follows, you will have to read in csv files using two different functions:

Reading in data from "Danish files":

```
data <- read.csv2("folder/myfile.csv")
```

Reading in data from "English files":

```
data <- read.csv("folder/myfile.csv")
```

Many errors are related to this step. See 9.2.1

9.1.2 Plot your data

The basic plot command in R is plot(). You HAVE to tell the function: - what to put on the x-axis, - and the y-axis, And you CAN tell the function additionally: - which type of plot you want, - what the axis labels should be and - the min and max points of the axes.

```
plot(x = data1$time,
     y = data1$V,

     type = "l",
     xlab = "Time (min)",
     ylab = "Potential difference (mV)",
     ylim = c(0, max(data1$V)))
```

9.1.3 Averaging by group

Often you want to calculate the average and standard deviation of a dataset by group. Below is one way of doing this for all data in one command using the aggregate function. You tell it how the average should be named (av_V), what it should be calculated from (data$V), and if there is a column that informs about the grouping (by = data$condition) and which function you want to apply(FUN = mean).

```
#calculating all averages
data_av <- aggregate(list(av_V = data$V,
                          av_R_cm2 = data$R_cm2,
                          av_I_cm2 = data$I_cm2),
                     list(data$condition),
                     FUN = mean )

#calculating all standard deviations
data_sd <- aggregate(list(sd_V = data$V,
                          sd_R_cm2 = data$R_cm2,
                          sd_I_cm2 = data$I_cm2),
                     list(data3$condition),
                     FUN = sd )

#then we attach the sd columns to the table that has the means
data_mean <- cbind(data3_av, data3_sd[2:4])
```

9.1.4 Set your working directory to a different folder

The working directory is the place "where R works from" at the moment. Files that are in that folder can be used by simply using their file name. If you need to change to a different folder, there are two ways: 1. commandline

```
setwd("C:/Users/new/path/to/folder/you/want")
```

2. Using R studio interface

a) Go to "Files"
b) Navigate to the folder you want to use as new working directory
c) Click on the settings wheel
d) Click "Select As Working Directory"
e) Done :)

9.1.5 Write and troubleshoot a new script

1. Think about what you want the code to do.
2. Identify the functions that you need.
3. Start writing your code.
4. Check if the code did what you expected after every step. The last step is really important. You can run scripts without ever seeing your data and R will do whatever you tell it, no matter whether it makes sense or not. When writing new code, it is vital to check the result after each step.

Usefull check are: Simply view the data table or object you work on to see the result of your code

```
View(df)
```

Check if the table or column has the right type of data in it.

str() tells you what kind of table df is, how many rows (the number of objects) and columns (the number of variables) there are, and what type of data is in the columns. It is a very useful tool for troubleshooting: - Does your data frame have the right amount of columns? Lets say you calculated a new variable and wrote it into a column, then there should be one more than before - Does your data frame have the right amount of rows? - Is the data in the right format? "chr" means that the column is a character column, which means it is regarded as text. Even if the signs are numbers, R will not be able to use the column for calculations.

```
str(df)
```

9.1.6 Change the type of data in a column

Most commonly you want to do this if R has read in data as "character" (text), but you need it to be "numeric" (numbers). Of note: Typically, if you have a

column that should be numeric but isn't, then there is a problem when reading in the data or creating the column taht should be solved.

Changing a column from "character" to "numeric":

```
df$col <- as.numeric(data$col)
#Takes the column "col" in the table df, formats it as number and overwrites it with i
```

9.2 Common error messages and how to solve them

9.2.1 Cannot open the connection

The error

When you try to read in a csv file you get the error message below:

```
read.csv("data2.csv")
```

```
## Warning in file(file, "rt"): cannot open file 'data2.csv': No such file
## or directory
```

```
## Error in file(file, "rt"): cannot open the connection
```

Why is this happening?

You just told R to open a file in your working directory that is called data2.csv. R is telling you it can't do that because the file doesn't exist. The two major reasons for this are: 1. The file is in a different folder. 2. You mistyped the filename.

Troubleshooting

1. Check where your current working directory is set to.

```
getwd()
```

Is the file in the working directory?

Yes: check for correct spelling.

No: Either use the path to the file in the command OR change the working directory to the folder where the file is in as described in 9.1.4.

9.2.2 My code doesn't do what it should or fails with cryptic message

1. Google
2. If you are taking BB511 at the moment: Ask Iris :)