

Coding Lab 1

- **Data:** Download the data file `nds_cl_1.csv` from ILIAS and save it in a subfolder `../data/`.
- **Dependencies:** You don't have to use the exact versions of all the dependencies in this notebook, as long as they are new enough. But if you run "Run All" in Jupyter and the boilerplate code breaks, you probably need to upgrade them.

Recommended folder structure:

```
.  
├── data/  
│   └── nds_cl_1.csv  
├── notebooks  
│   └── CodingLab1.ipynb  
├── matplotlib_style.txt  
└── requirements.txt
```

```
import numpy as np  
import pandas as pd  
import matplotlib.pyplot as plt  
from scipy import signal  
from sklearn.decomposition import PCA  
from __future__ import annotations # for Python 3.8 and below  
  
# %matplotlib qt  
  
# black is a code formatter (see https://github.com/psf/black).  
# It will automatically format the code you write in the cells imposing consistent Python  
%load_ext jupyter_black  
  
%load_ext watermark  
%watermark --time --date --timezone --updated --python --iversions --watermark -p sklearn
```

<IPython.core.display.HTML object>

Last updated: 2023-04-25 19:32:27CEST

Python implementation: CPython

Python version : 3.11.3

IPython version : 8.11.0

sklearn: 0.0.post1

scipy : 1.10.1

numpy : 1.24.3

matplotlib: 3.7.1

pandas : 1.5.3

Watermark: 2.3.1

```
# matplotlib style file
# Template for style file: https://matplotlib.org/stable/tutorials/introductory/customizing.html
plt.style.use("../matplotlib_style.txt")

# from plotstyle import PlotStyle
# ps = PlotStyle()
```

Load data

```
fs = 30000.0 # sampling rate of the signal in Hz
dt = 1 / fs
cols = ["Ch1", "Ch2", "Ch3", "Ch4"]
x = pd.read_csv("../data/nds_cl_1.csv", header=0, names=cols)
```

```
x.describe()
```

	Ch1	Ch2	Ch3	Ch4
count	1.920000e+07	1.920000e+07	1.920000e+07	1.920000e+07
mean	3.600331e+00	-8.850918e-01	2.864284e-01	2.210982e+00
std	5.824474e+02	6.014818e+02	6.464363e+02	6.126105e+02
min	-3.607000e+03	-3.739000e+03	-3.871000e+03	-3.750000e+03

	Ch1	Ch2	Ch3	Ch4
25%	-3.460000e+02	-3.610000e+02	-3.950000e+02	-3.640000e+02
50%	1.200000e+01	8.000000e+00	-1.000000e+00	1.000000e+01
75%	3.650000e+02	3.720000e+02	4.010000e+02	3.810000e+02
max	2.873000e+03	3.004000e+03	3.099000e+03	3.017000e+03

Task 1: Filter Signal

In order to detect action potentials, the first step is to filter out low frequency fluctuations (LFP) and high frequency noise. Determine appropriate filter settings and implement the filtering in the function `filter_signal()`. A typical choice for this task would be a butterworth filter. Plot a segment of the raw signal and the filtered signal for all four channels with matching y-axis. The segment you choose should contain spikes. When you apply the function also test different filter settings.

Grading: 2 pts

For our filter we chose the butterworth bandpass filter. This takes care of removing the high- and low frequency noise in a single operation.

```
def filter_signal(
    x: pd.DataFrame, fs: float, low: float, high: float, order: int = 3
) -> pd.DataFrame:
    """Filter raw signal x.

    Parameters
    -----

    x: pd.DataFrame, (n_samples, n_channels)
        Each column in x is one recording channel.

    fs: float
        Sampling frequency.

    low, high: float, float
        Passband in Hz for the butterworth filter.

    order: int
        The order of the Butterworth filter. Default is 3, but you should try
        changing this and see how it affects the results.
```

Returns

y: pd.DataFrame, (n_samples, n_channels)
The filtered x. The filter delay is compensated in the output y.

Notes

1. Try exploring different filters and filter settings. More info:
<https://docs.scipy.org/doc/scipy/reference/generated/scipy.signal.butter.html>
2. The output signal should be phase-shift compensated. More info:
<https://dsp.stackexchange.com/a/19086>

```
"""
# create empty list to store the filtered signal
signals = []
col_names = []
for col in x.columns:
    # get the data from one channel
    data = x[col].values

    # get the filter coefficients
    coeffs = signal.butter(order, [low, high], "band", fs=fs, output="sos")

    # pass the filter coefficients and the data to the filter function
    filtered_data = signal.sosfiltfilt(sos=coeffs, x=data)

    # append to empty list
    signals.append(filtered_data)
    col_names.append(col)

# convert list to pd.DataFrame
df = pd.DataFrame(np.asarray(signals).T, columns=col_names)

return df
```

Here we apply the filter and create a time axis for plotting the signal using its samplingrate.

```

xf = filter_signal(x, fs, 500, 5000)
time = np.arange(0, len(xf) / fs, dt)

mosaic = [
    ["raw: Ch1", "filtered: Ch1"],
    ["raw: Ch2", "filtered: Ch2"],
    ["raw: Ch3", "filtered: Ch3"],
    ["raw: Ch4", "filtered: Ch4"],
]
fig, ax = plt.subplot_mosaic(
    mosaic=mosaic, figsize=(8, 6), layout="constrained", dpi=100
)

for i, m in enumerate(mosaic):
    raw = m[0]
    filtered = m[1]

    sig = x[raw[-3:]].values
    sigf = xf[raw[-3:]].values

    ax[raw].plot(time, sig, lw=1)
    ax[filtered].plot(time, sigf, lw=1)

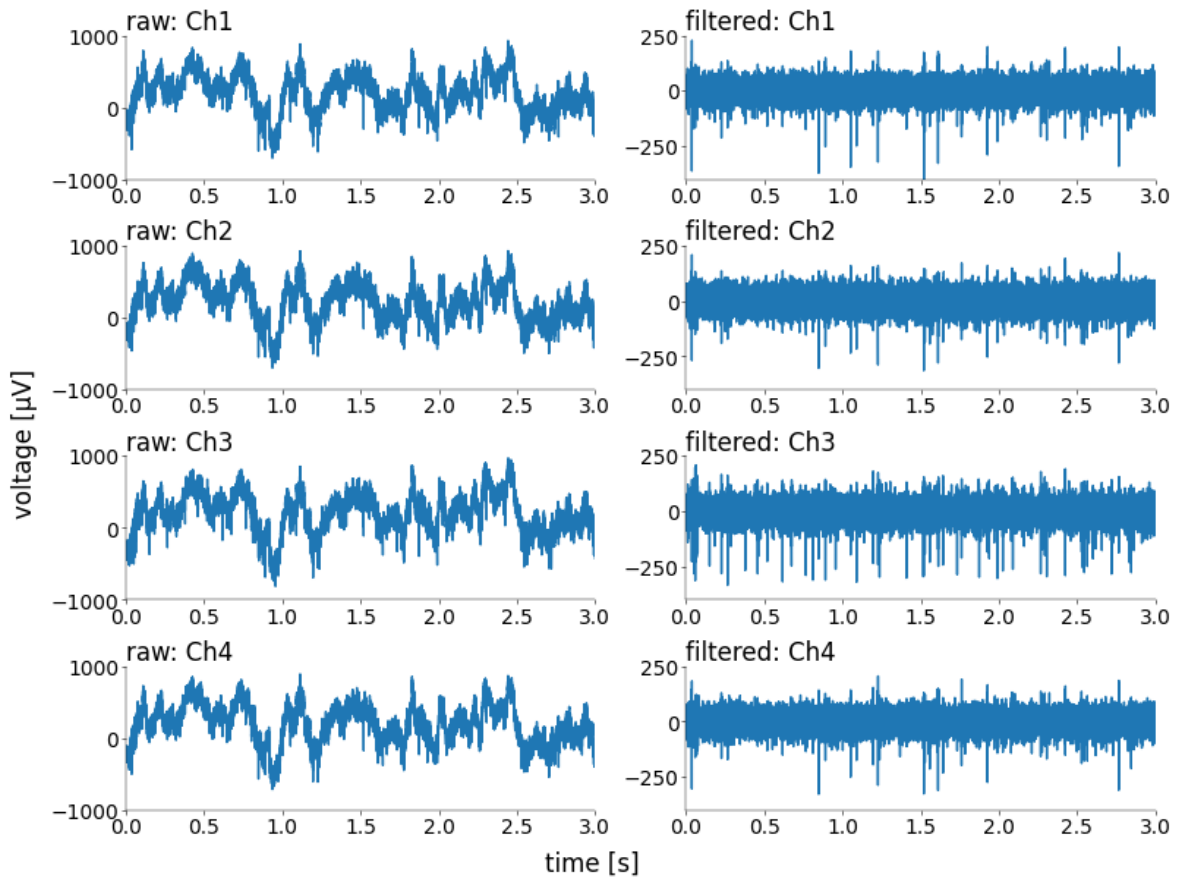
    ax[raw].set_xlim((0, 3))
    ax[raw].set_ylim((-1000, 1000))

    ax[filtered].set_xlim((0, 3))
    ax[filtered].set_ylim((-400, 250))

    fig.supylabel("voltage [\u03BCV]", fontsize=12)
    fig.supxlabel("time [s]", fontsize=12)
    ax[filtered].set_title(m[1], loc="left")

    ax[raw].set_title(m[0], loc="left")

```



Task 2: Detect action potentials

Action potentials are usually detected by finding large-amplitude deflections in the continuous signal. A good choice of threshold for detecting spikes is important. If it is too low, you will detect too many low amplitude events (noise); if it is too high, you run the risk of missing good spikes. Implement an automatic procedure to obtain a reasonable threshold and detect the times when spikes occurred in the function `detect_spikes()`. Plot a segment of the filtered signal for all four channels with matching y-axis and indicate the time points where you detected spikes. **Plot the threshold.** Are the detected time points well aligned with peaks in the signal?

Grading: 3 pts

For this we first need to convert the pandas dataframe into a np.ndarray.

```
xf = xf.values
print(xf.shape)
```

(19199999, 4)

A question that comes up is why there should be a lockout, from my understanding a refractory period. We are recording many cells simultaneously, that can fire whenever they want. So why should we lockout the detection of spikes? The lockout should be implemented in the sorting process instead of the peak detection.

We obtain a reasonable threshold by calculating the robust estimation of the threshold.

Here, in the following we will detect peaks on all electrodes separately and group closely indices afterwards. The threshold is same over all four channels. Spikes detected on one channel are labeled as spikes at the same time (± 3 samples) on all the other channels even if the amplitude at the time of the spike on the other channels is too small to cross the threshold.

```
def detect_spikes(
    x: np.ndarray, fs: float, N: int = 5, lockout: int = 10
) -> tuple[np.ndarray, np.ndarray, np.float64]:
    """Detect spikes, in this case, the relative local minima of the signal x.

    Parameters
    -----

    x: np.array (n_samples, n_channels)
        The filtered signal from Task 1.

    fs: float
        the sampling rate (in Hz).

    N: int
        An arbitrary number with which you multiply with the standard deviation
        to set a threshold that controls your false positive rate. Default is 5
        but you should try changing it and see how it affects the results.

    lockout: int
        a window of 'refractory period', within which there's only one spike.
        Default is 10 but you should also try changing it.
```

Returns

```
s: np.array, (n_spikes, )
    Spike location / index in the signal x.

t: np.array, (n_spikes, )
    Spike time in ms. By convention the time of the zeroth sample is 0 ms.

thrd: float
    Threshold = -N * sigma.
```

Tips

Use scipy functions to detect local minima.
Noted that there are four channels in signal x.

```
"""
spike_indices = []
thresh = np.median(np.abs(x - np.median(x))) / 0.6745) * N

# detect peaks for each channel
for ch in range(x.shape[1]):
    sig = -x[:, ch]
    sig[sig < thresh] = 0
    spikes, _ = signal.find_peaks(sig, distance=lockout)
    spike_indices.extend(spikes.tolist())

# remove all redundant duplicates
spike_indices = np.unique(np.sort(np.asarray(spike_indices)))

# group all close spike indices
tolerance = 3
spike_indices = np.split(
    spike_indices, np.where(np.diff(spike_indices) > tolerance)[0] + 1
)

# compute the rounded mean of each group and use it as the spike index
spike_indices = np.asarray([int(np.round(np.mean(s))) for s in spike_indices])
```



```

    spike_times = spike_indices / fs

    return spike_indices, spike_times, -thresh

s, t, thrd = detect_spikes(xf, fs, N=4)
print(f"Number of spikes: {len(s)}")
print(f"Threshold: {thrd}")

```

Number of spikes: 35694
Threshold: -144.16209293776222

```

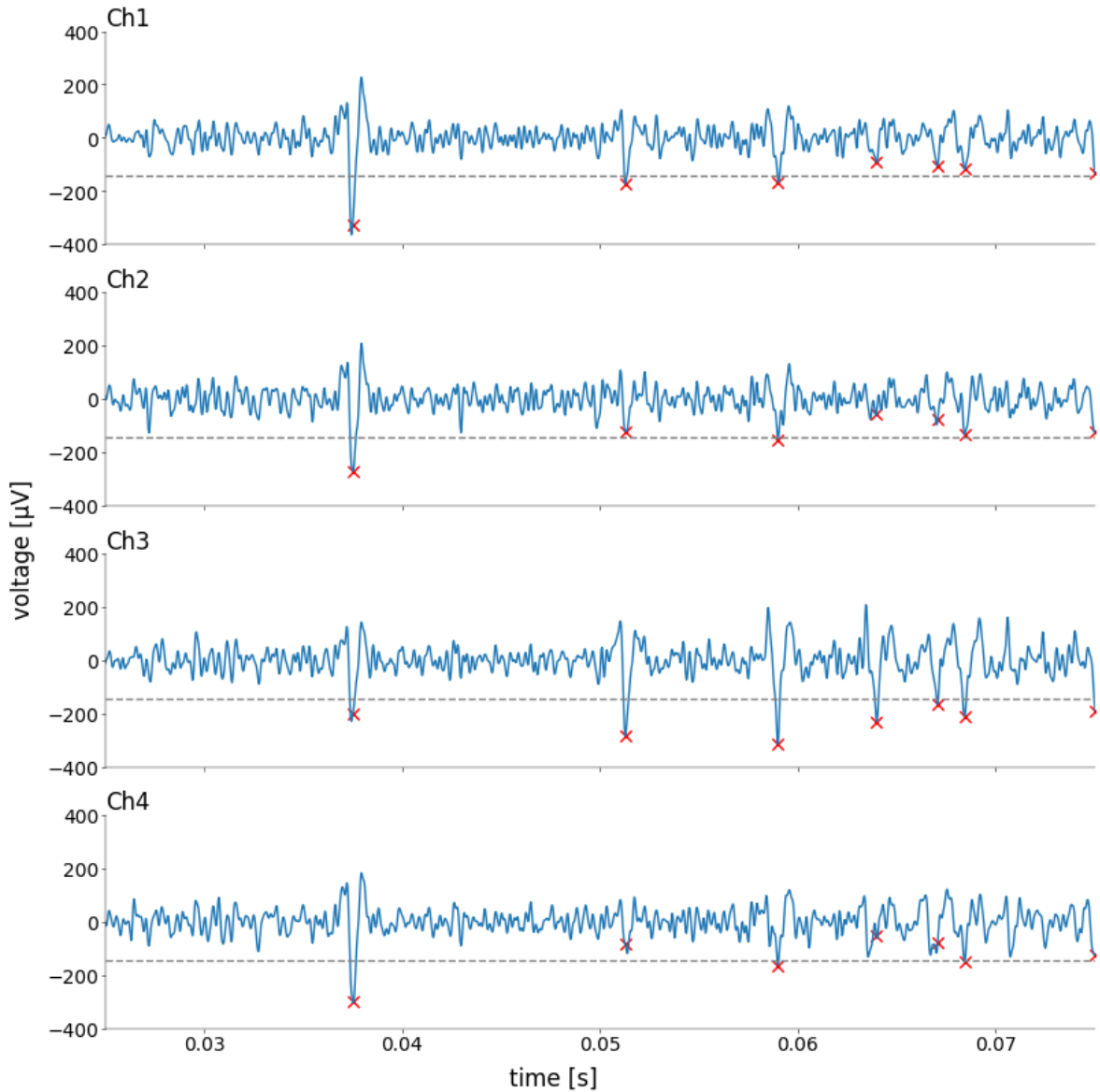
mosaic = [
    ["Ch1"],
    ["Ch2"],
    ["Ch3"],
    ["Ch4"],
]
fig, ax = plt.subplot_mosaic(
    mosaic=mosaic, figsize=(8, 8), layout="constrained", dpi=100, sharex=True
)

for i, col in enumerate(cols):
    x = xf[:, i]
    ax[col].plot(time, x, lw=1)
    ax[col].scatter(t, x[s], marker="x", color="red")
    ax[col].axhline(thrd, color="gray", ls="--", lw=1)

    ax[col].set_ylim((-400, 400))
    ax[col].set_xlim((0.025, 0.075))
    ax[col].set_title(col, loc="left")

fig.supxlabel("time [s]", fontsize=12)
fig.supylabel("voltage [\u03BCV]", fontsize=12)
fig.align_labels()

```



Task 3: Extract waveforms

For later spike sorting we need the waveforms of all detected spikes. Extract the waveform segments (1 ms) on all four channels for each spike time (as a result each spike is represented by a 4x30 element matrix). Implement this procedure in the function `extract_waveforms()`. Plot (a) the first 100 spikes you detected and (b) the 100 largest spikes you detected. Are there a lot of very small spikes (likely noise) among your detected spikes? If so your threshold may be too low. Can you see obvious artifacts, not looking like spikes at all?

Grading: 2 pts

```
def extract_waveforms(x: np.ndarray, s: np.ndarray) -> np.ndarray:
    """Extract spike waveforms at times s (given in samples)
    from the filtered signal `xf` using a fixed window around the
    times othrdf the spikes.

    Parameters
    -----

    x: np.array (n_samples, n_channels)
        The filtered signal.

    s: np.array, (n_spikes, n_channels)
        Spike time in samples.

    Return
    -----

    w: np.array, (n_spikes, length_window, n_channels)
        Waveforms. (You don't have to get the exact same
        shape as we noted here. It's just the shape of w
        that can be easily retrieved via broadcasting.)

    Notes
    -----

    More on Numpy Broadcasting
    https://jakevdp.github.io/PythonDataScienceHandbook/02.05-computation-on-arrays-broadc

    """
    # create a window of 10 samples before and 20 after the spike
    # window_index = np.arange(-10, 20)

    # Array broadcasting: Add window to indices. We need to add a new axis
    # because the window_index is 1D and the spike indices are 2D.
    # the np.newaxis just makes the window_index 2D.
    # window_indices = s[:, np.newaxis] + window_index

    # Now we can use the window_indices to extract the waveforms from the signal.
```

```

# output = x[window_indices]

# this can all be put in one line:
return x[s[:, np.newaxis] + np.arange(-10, 20)]

w = extract_waveforms(xf, s)
print(np.shape(w))

```

(35694, 30, 4)

```

mosaic = [
    ["Ch1", "Ch2"],
    ["Ch3", "Ch4"],
]
fig, ax = plt.subplot_mosaic(
    mosaic=mosaic, figsize=(8, 6), layout="constrained", dpi=100
)

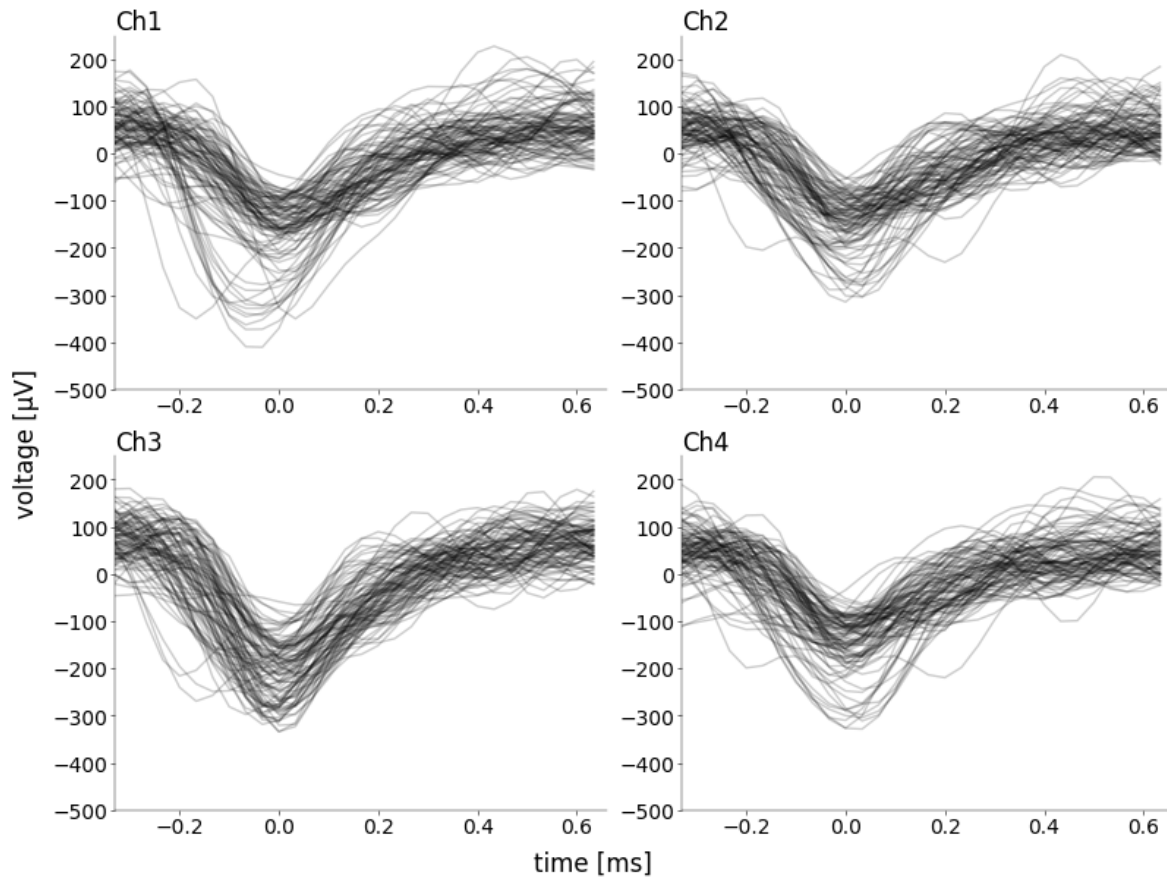
time = np.arange(-10, 20) / fs * 1000

# cols = ["Ch1", "Ch2", "Ch3", "Ch4"]
for i, col in enumerate(cols):
    ax[col].plot(time, w[:, 100, :, i].T, color="black", lw=1, alpha=0.2)
    ax[col].set_ylim((-500, 250))
    ax[col].set_xlim((-0.33, 0.66))
    ax[col].set_title(col, loc="left")

fig.supxlabel("time [ms]", fontsize=12)
fig.supylabel("voltage [\u03BCV]", fontsize=12)

```

Text(0.02, 0.5, 'voltage [V]')



Plot largest 100 spike waveforms

To get the largest 100 waveforms, we can use `numpy.argsort` if we can extract the maxima. The following command will take all values of the second and third axis (i.e. all samples and all channels) of the extracted waveforms in `w` and return the largest value for each waveform.

```
maxima = np.min(w, axis=(1, 2))
print(maxima[:100])
```

```
[-363.14603726 -281.92681059 -313.29734102 -228.70845381 -164.00587698
 -207.65115283 -189.26935792 -246.5894946  -157.19536367 -281.47667102
 -334.47064061 -158.99790895 -267.1223504  -282.16032643 -204.1581294
 -226.19268583 -318.38002623 -246.84100521 -289.69337732 -153.13391989
 -310.93376582 -372.97404989 -193.99928428 -310.33312055 -262.06724904
 -345.89762105 -320.50760207 -235.57695096 -322.59926443 -297.3801607
 -147.53239784 -158.32863104 -301.98199701 -150.56538866 -295.22045305]
```

```

-410.49185377 -193.87950531 -148.50746901 -230.47726903 -152.19128323
-328.19994114 -248.83035737 -248.83035737 -242.35482006 -286.14317662
-211.13900525 -152.75089431 -145.89161443 -297.98058979 -146.02818165
-290.39145143 -213.45875482 -304.11103456 -189.51399458 -269.34747485
-269.34747485 -154.37510925 -262.27338453 -158.20704498 -281.91616178
-174.00370635 -155.57133186 -199.61955389 -295.13212988 -167.99190233
-290.66815646 -192.84089175 -252.43323234 -157.9949674 -276.87739447
-248.92599194 -144.74803459 -214.3137758 -169.87089998 -177.34745301
-283.62383895 -144.84516497 -152.16316246 -341.93169061 -180.17298101
-231.23572932 -277.07560193 -177.80773057 -144.39958729 -215.699999
-154.01914486 -333.69239083 -152.73119285 -275.87377516 -166.24534129
-196.77157418 -184.42293186 -316.72015777 -322.32014373 -277.789026
-204.58458448 -216.92742227 -152.06863508 -350.20456519 -350.20456519]

```

Now we can easily get the indices of the largest waveforms `numpy.argsort`, which returns the indices that would sort the array.

```
sorted_indices = np.argsort(maxima)
```

Now we just need to index the waveforms using the `sorted_indices` to get the largest waveforms and plot them in the same way as before. The whole procedure can also be done in a single line:

```

sorted_waveforms = w[np.argsort(np.min(w, axis=(1, 2))), :, :]

mosaic = [
    ["Ch1", "Ch2"],
    ["Ch3", "Ch4"],
]
fig, ax = plt.subplot_mosaic(
    mosaic=mosaic, figsize=(8, 6), layout="constrained", dpi=100
)

# also add the mean
means = np.mean(sorted_waveforms[:100, :, :], axis=0)

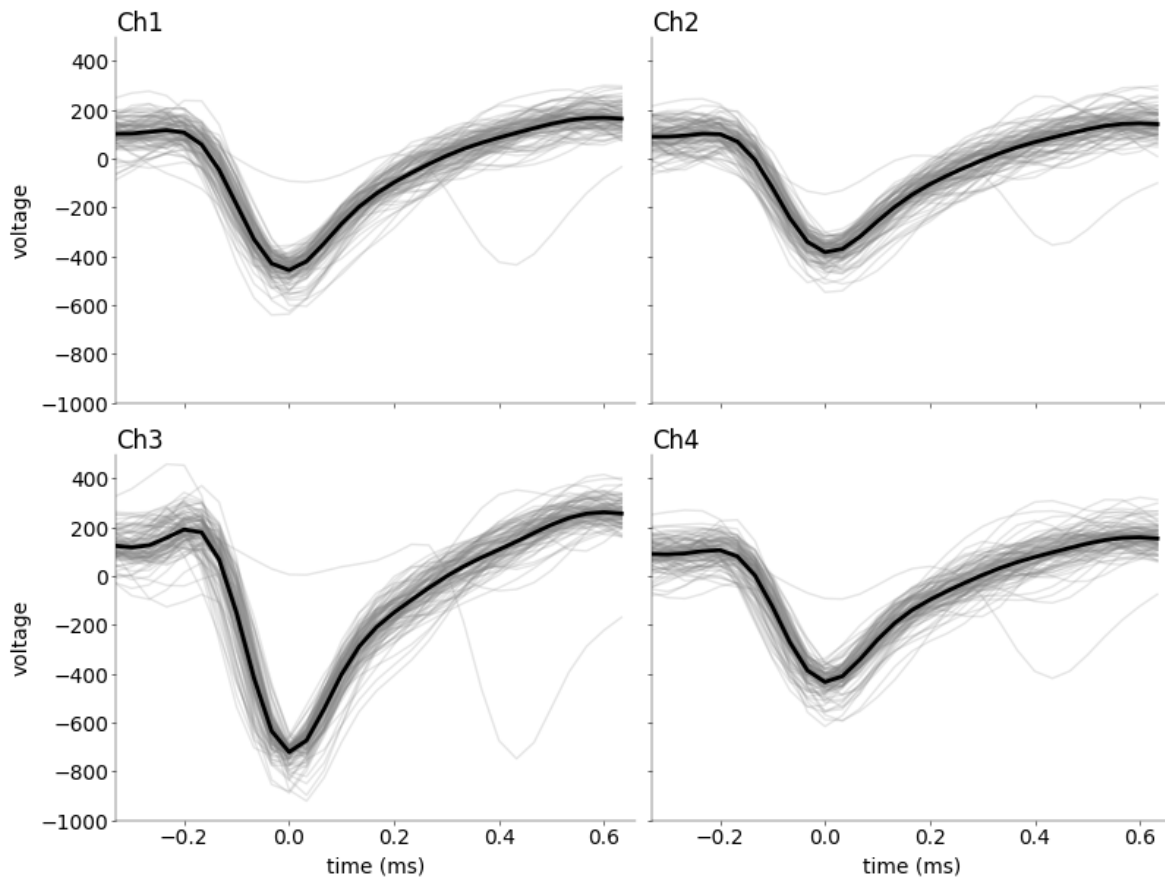
for i, col in enumerate(cols):
    ax[col].plot(time, sorted_waveforms[:100, :, i].T, color="gray", lw=1, alpha=0.2)
    ax[col].plot(time, means[:, i].T, color="black", lw=2, alpha=1)
    ax[col].set_ylim((-1000, 500))
    ax[col].set_xlim((-0.33, 0.66))

```

```

ax[col].set_title(col, loc="left")
if col == "Ch3" or col == "Ch4":
    ax[col].set_xlabel("time (ms)")
else:
    ax[col].set_xticklabels([])
if col == "Ch1" or col == "Ch3":
    ax[col].set_ylabel("voltage")
else:
    ax[col].set_yticklabels([])

```



Task 4: Extract features using PCA

Compute the first three PCA features on each channel separately in `extract_features()` (2 pts). You can use a available PCA implementation or implement it yourself. After that, each spike is represented by a 12 element vector. Compute the fraction of variance captured

by these three PCs. Plot scatter plots for all pairwise combinations of 1st PCs. Do you see clusters visually?

Grading: 2+1 pts

PCA:

- how to preprocess data?

(<https://stats.stackexchange.com/questions/385775/normalizing-vs-scaling-before-pca>)

```
from sklearn.preprocessing import scale, normalize, StandardScaler
```

```
def extract_features(w: np.ndarray):
```

```
    """Extract features for spike sorting from the waveforms w.
```

```
    Do PCA on the waveforms of each channel separately,
    then concatenate the first three principal components
    of each channels into one numpy array (`b`).
```

```
    Parameter
```

```
    -----
```

```
    w: np.ndarray, (n_spikes, length_window, n_channels)
        Waveforms from Task 3.
```

```
    Return
```

```
    -----
```

```
    b: np.ndarray, (n_spikes, n_feature)
```

```
    Notes
```

```
    -----
```

```
    You can use PCA from sklearn.
```

```
    More on PCA
```

```
    https://jakevdp.github.io/PythonDataScienceHandbook/05.09-principal-component-analysis
```



```

"""
# use the PCA class from sklearn and reduce the matrix to 3 dimensions
pca = PCA(n_components=3)
# calculate the PCA for every channel
for i in range(w.shape[2]):
    input = w[:, :, i]
    output = pca.fit_transform(input)
    # combine the output to one array
    if i == 0:
        b = output
    else:
        b = np.concatenate((b, output), axis=1)
# print the explained variance
print(
    f"Explained variance ratio for channel {i+1}: {pca.explained_variance_ratio_}"
)

return b

b = extract_features(w)
print(b.shape)

```

```

Explained variance ratio for channel 1: [0.49543188 0.22374809 0.07320718]
Explained variance ratio for channel 2: [0.40652697 0.22460507 0.09447735]
Explained variance ratio for channel 3: [0.43913007 0.23038406 0.09023702]
Explained variance ratio for channel 4: [0.42429928 0.22073761 0.09471362]

```

```

mosaic = [
    ["Ch2 vs Ch1", ".", "."],
    ["Ch3 vs Ch1", "Ch3 vs Ch2", "."],
    ["Ch4 vs Ch1", "Ch4 vs Ch2", "Ch4 vs Ch3"],
]
fig, ax = plt.subplot_mosaic(
    mosaic=mosaic, figsize=(8, 8), layout="constrained", dpi=100
)

# indices of the 1st PC in `b`
i = {"Ch1": 0, "Ch2": 3, "Ch3": 6, "Ch4": 9}

```

```

for m in np.ravel(mosaic):
    if m == ".":
        continue
    # get the indices of the channel for the first channel vs second channel
    firstch = i[m[:3]]
    secondch = i[m[-3:]]
    ax[m].scatter(b[:, firstch], b[:, secondch], s=1, color="black", alpha=0.2)

    y, x = m.split(" vs ")

    ax[m].set_xlabel(x)
    ax[m].set_ylabel(y)
    ax[m].set_xlim((-1500, 1500))
    ax[m].set_ylim((-1500, 1500))
    ax[m].set_xticks([])
    ax[m].set_yticks([])

fig.suptitle("Pairwise 1st PCs", fontsize=20)

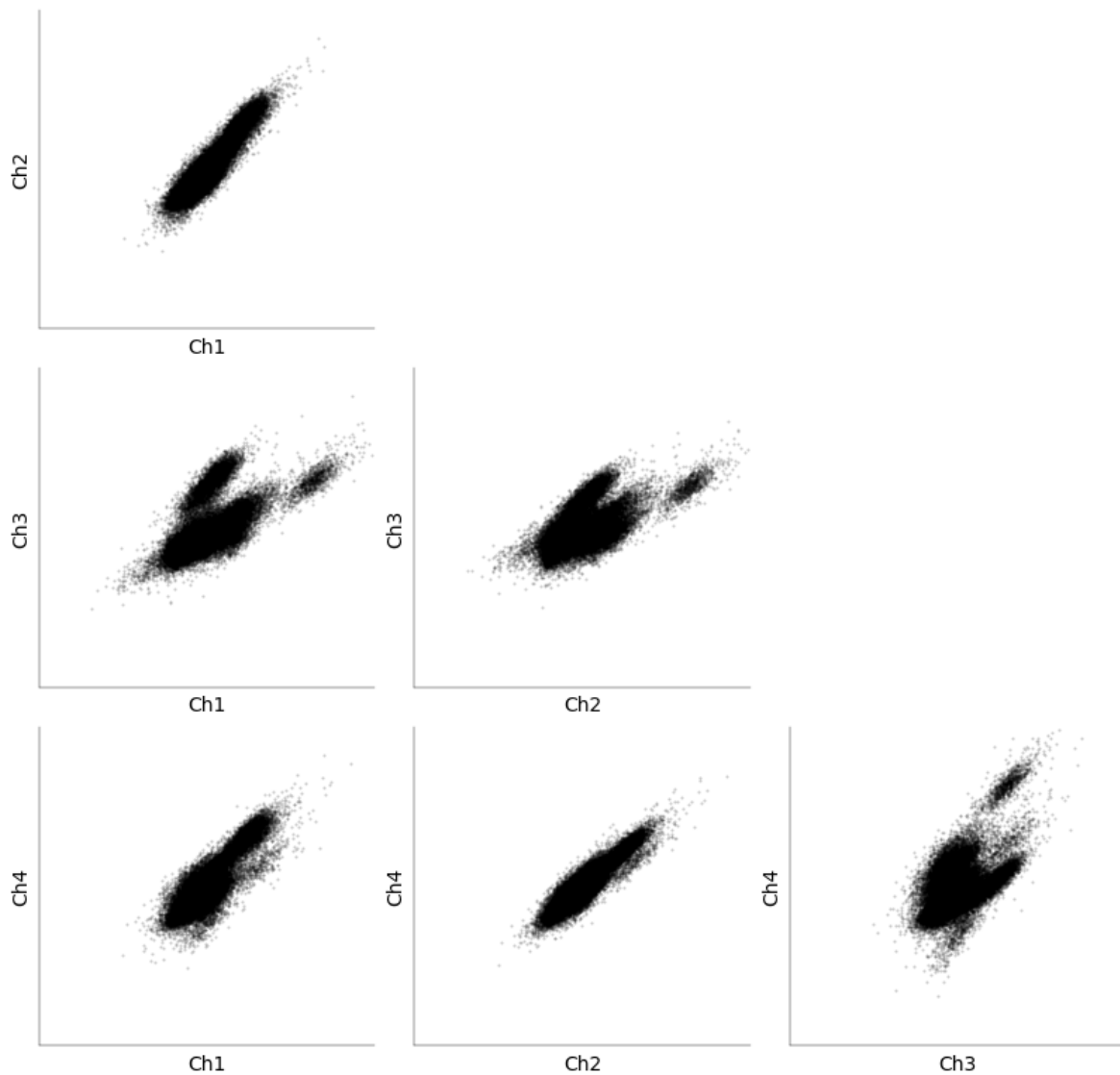
```

```

Text(0.5, 0.98, 'Pairwise 1st PCs')

```

Pairwise 1st PCs



```
# # save data for the next Coding Lab

np.save("../data/nds_cl_1_features", b)
np.save("../data/nds_cl_1_spiketimes_s", s)
np.save("../data/nds_cl_1_spiketimes_t", t)
np.save("../data/nds_cl_1_waveforms", w)
```

We can estimate 3 clusters between the first PC in channel 1 vs 3 and channel 2 vs 3. And 2

cluster in channel 3 vs 4.