Lab 3. Neural signal analysis – Neural spikes

1. Objective

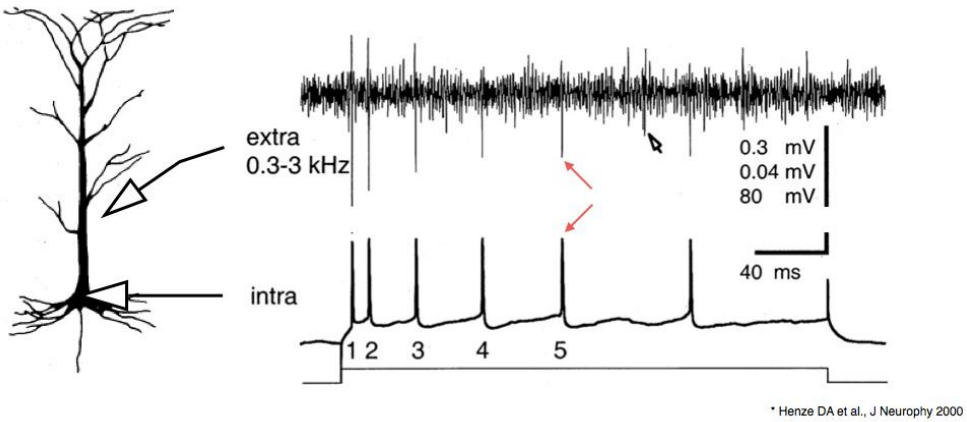
Understand the statistics of extracellular spike trains and perform multichannel spike train analysis for spontaneous activity and evoked responses

- In order to understand the neural data analysis from real neurons, we will use an in vitro model system using primary neuronal cultures.
- From a rat brain, neurons from hippocampus are obtained and seeded on a neuro-sensor chip (‘planar-type microelectrode array (MEA)’) that contains multiple microelectrodes.
- Multi-channel neural data are obtained from an MEA and you will build your own program to analyze neural data.

2. Backgrounds

2.1. What is extracellular spike recording?

In neuroscience and engineering research, it is often required to measure electrical activity from individual neurons. Extracellular recordings are currently the only practical choice in experiments that intend to establish correlations between neural ensemble responses and behaviors involving awake animals. Extracellular spikes are measured by microelectrodes placed in extracellular spaces. Extracellular recordings involve the activity of many adjacent single neurons which can usually be resolved into independent action potentials. Extracellular spike is the time-varying potential that is recorded at a single location around the extracellular space of a single neuron during one of its action potentials. While the amplitude of intracellularly recorded action potentials ranges between 60 and 100 mV, extracellular spikes can be two orders of magnitude smaller from hundreds of uV to a few mV, making their observation difficult under large background noises. (figure 1)



< Figure 1. Simultaneous recording of intracellular and extracellular recording from the same cell. (Top:

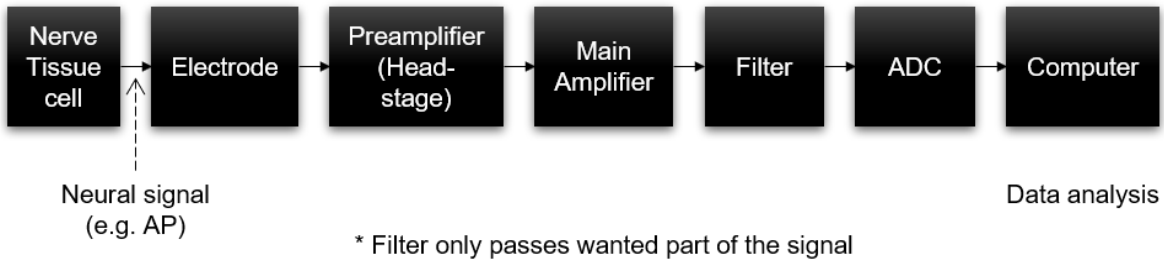
Filtered extracellular trace, Middle: intracellular trace, Bottom: electrical stimulation for depolarizing step, each scale bar presented at same bar.)>

Extracellular recordings measure electrical voltages outside the cell membrane when neurons generate action potentials. The loop current that flows around the cell makes a small voltage that is in the range of a few tens to hundreds of microvolts. In the brain, neurons are densely packed. If the microelectrode is sufficiently small, it would be possible to recording spikes reflecting a single cell activity ('single-unit recording'). Otherwise, extracellular spikes inevitably contain neural activity from nearby multiple neurons, which reflects activity from multiple cells ('multi-unit recording'). In case of multi-unit recordings, spikes should be separated based on certain signal features such as waveform shapes (e.g., peak amplitude, duration, valley amplitude, etc.).

2.2. Neural signal processing

① Bioinstrumentation of extracellular recordings

Metal microelectrodes (diameter 10 ~ 30 μm) are used as a voltage sensing element. The microelectrode is connected with a cascade of amplifiers. The overall gain of the system ranges from 1000 ~ 10000. The recorded signals are passed through a bandpass filter to remove various noise sources (electrode drift, power-line noise). We can also remove unwanted biological signals such as local field potentials that is much slower than spikes. The amplified and filtered signals are converted into digital signals through analog-to-digital converter and further processed in the computer. In this lab, you will only practice the data processing in the computer.



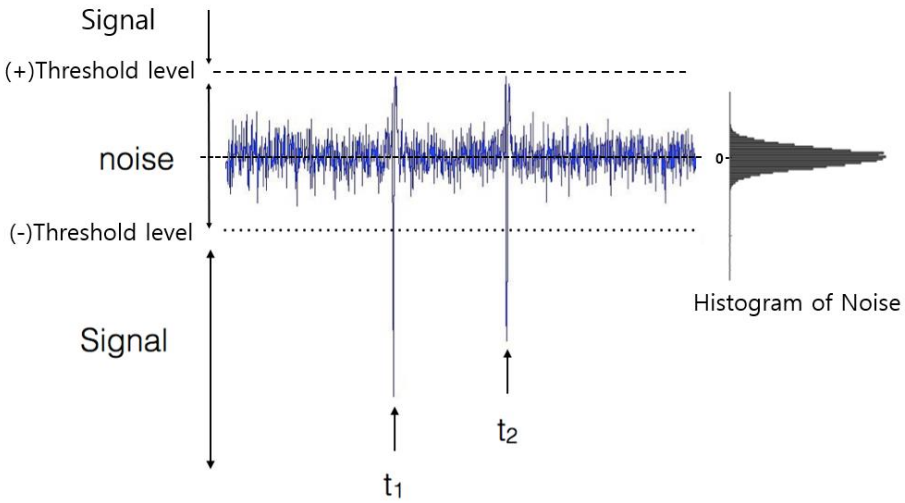
< Figure 2. Functional block diagrams of neural recording system >

② Digital signal filtering

If the recorded signal contains unwanted noises or signal sources, it can be further filtered using digital filters. The function of the digital filter is same as analog filters, but it is implemented by programming. In this lab, you need to design digital filter that consists of 200Hz high-pass filter cut-off to remove low frequency noise including 60Hz noise that was not sufficiently attenuated.

③ Spike detection using thresholding method

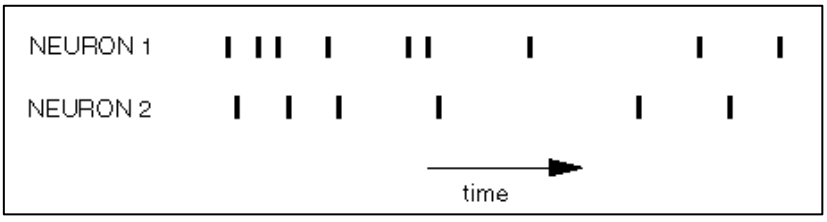
Spikes are detected based on the assumption that their amplitudes are much larger than background noises. To detect spikes from background noises, a threshold level should be calculated. Calculating the statistics of the background noises, we can find a proper amplitude for threshold level that is higher than noises. Then, the signals that is greater than threshold in amplitude will be regarded as spikes.



< Figure3. Setting the threshold level and spike detection >

④ Time stamp: extraction of spike timing

Once we detect spikes, one can extract the timing information of the spikes. The series of timing events collected from spikes contains the most of the neural information in neural systems. Spike train is a plot of spikes as a function of time and a raster plot is a graphical representation of spike trains with a single spike depicted as a vertical bar.

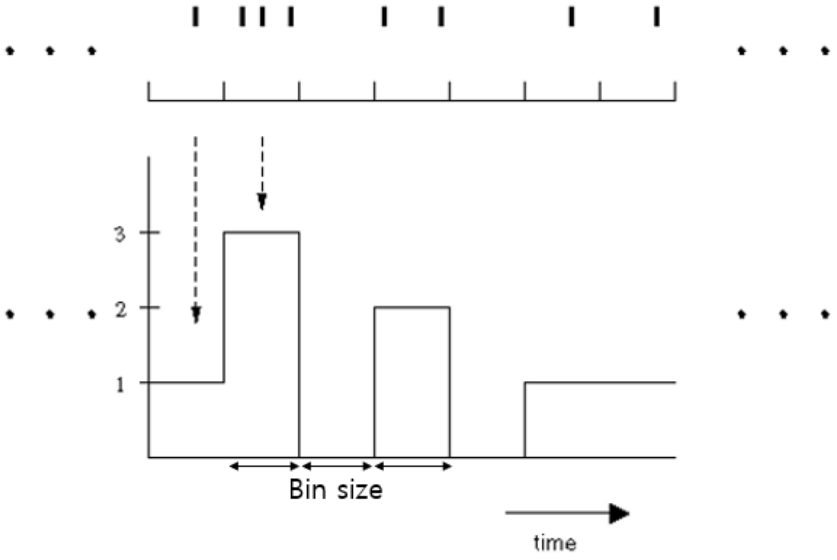


< Figure 4. Raster plot of two spike trains >

2.3. Spike train analysis

① Spike rate histogram

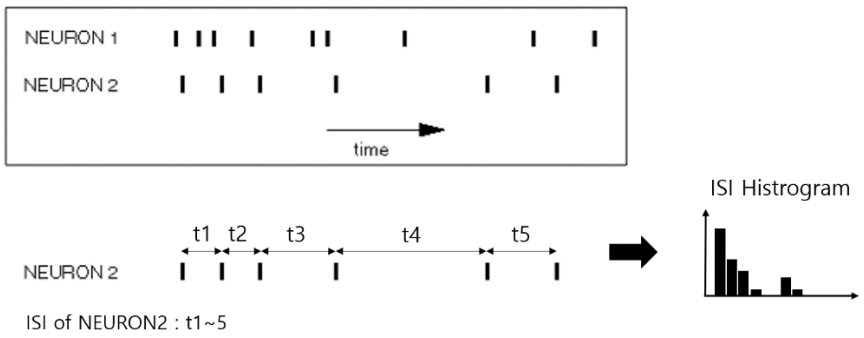
- Spike rate histogram is used to show neural firing rates as a function of time
- Time axis is comparted(bin)and corresponding spike counts are plotted



< Figure 5. Construction of rate histogram by binning the time axis >

② Inter-spike interval histogram

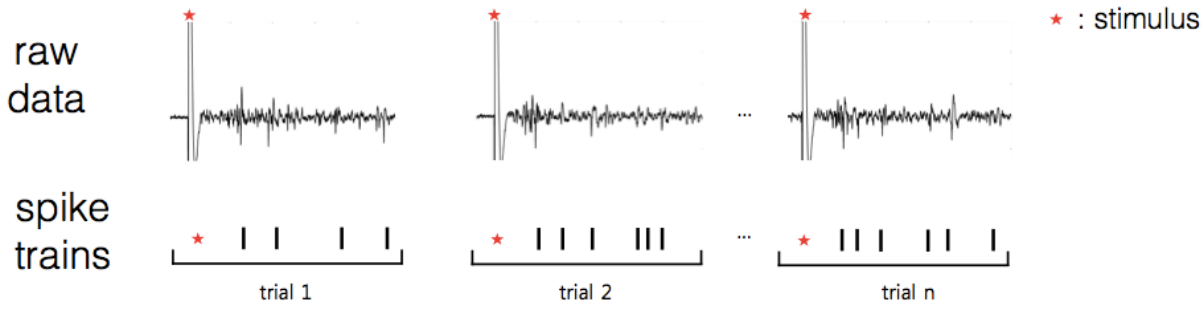
- The temporal pattern of a spike train can be quantified by constructing a histogram with inter-spike intervals



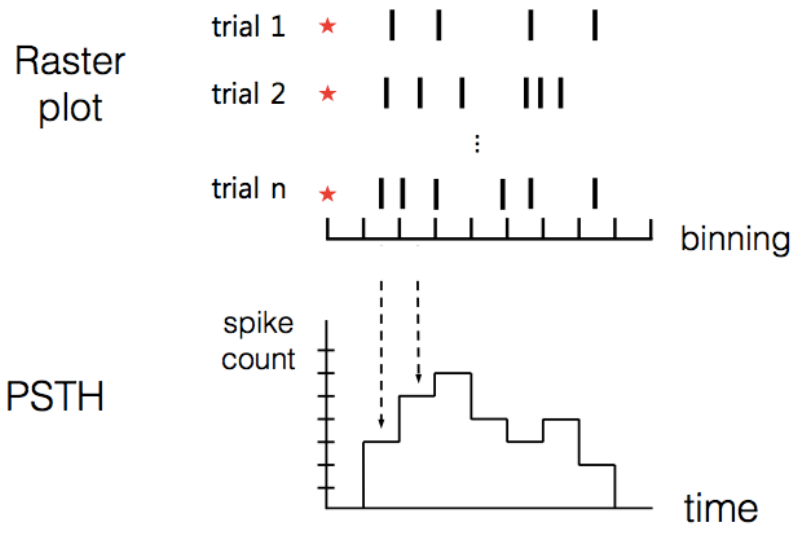
< Figure 6. Calculation of inter-spike interval of a spike train >

③ Post-stimulus time histogram (PSTH)

- In order to measure neural responses under a certain stimulation condition, stimulation (e.g. electrical stimulation) is applied to one electrode and extracellular recordings are done in other electrodes. After multiple trials, each spike train is stacked with respect to the stimulus time point (t = 0) and a post-stimulus raster plot is constructed. In this way, it is easy to visualize and identify strong neural responses induced by the stimulation.
- PSTH shows conditional probability of a spike at time t1 on the condition that there is a stimulus at t = 0. To construct the PSTH, time window is binned and spike counts in each bin are plotted.



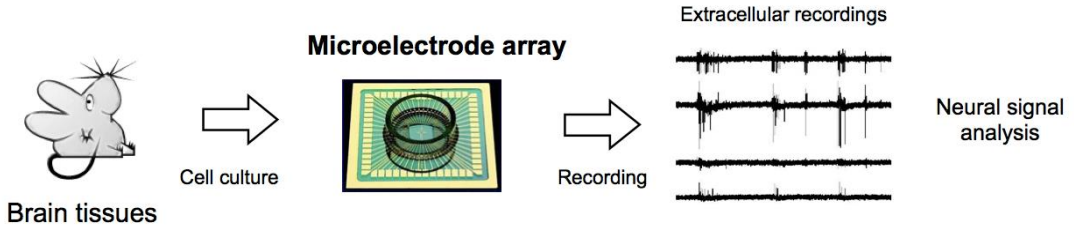
< Figure 7. Neural recording under electrical stimulation. * show stimulation artifact >



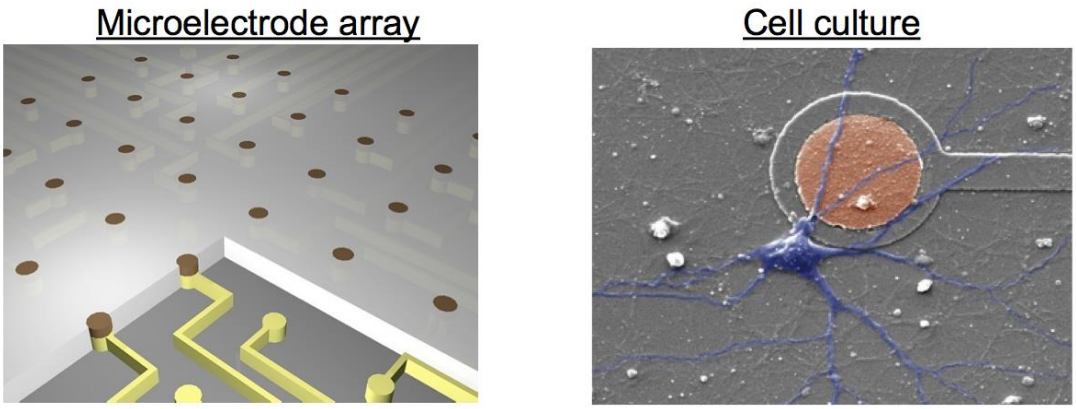
< Figure 8. Post-stimulus raster plot and time histogram>

2.4. Planar-type microelectrode array technology

A planar-type microelectrode array (MEA) is composed of microelectrodes embedded in a substrate. Its main functions are electrical recording and stimulation. As it can record extracellular spikes from multiple sites simultaneously, it is extremely beneficial when studying neural circuits in brain slices or cell cultures. One can record extracellular spikes or field potentials from multiple neurons or sites and extract network information. There are a few commercially available MEAs that are composed of 60 or 64 microelectrodes.



<Figure 9. Diagram for neural signal analysis>



<Figure 10. Diagram for microelectrode array(left), a neuron on a microelectrode(right)>

In this lab, we will obtain extracellular spikes from biological neural networks prepared from primary neuronal cell cultures. Neurons are isolated from an embryonic rat brain, and cultivated on an MEA for two to three weeks inside humidified incubator (37 degree C, 5% CO2). When neurons are cultivated for a few weeks, they generate action potentials and form functional synapses that resemble those of neurons developing in vivo. We can monitor coordinated neuronal activity using an MEA and analyze the activity by spike train analysis.

* See JOVE video from Dr. Steve Potter's lab in Georgia Tech. [3]

3. Prelab activity

3.1. Watch JOVE video and summarize the experimental procedure for an MEA experiment. Ref. [3]

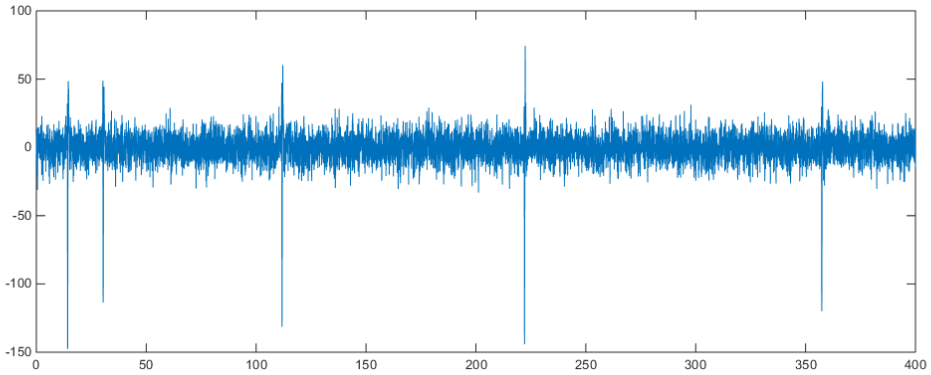
① Cell culture preparation

② Electrical recording from an MEA chip

* Please summarize the procedure of electrical recording from MEA. Note that you should write it by yourself (using your own words) with a 1 page limit.

3.2. Design a spike detector through the following procedure. You need to submit the code and the resulting plots(code will be counted as appendix).

① Plot raw data. (filename: Prelab_training_data.txt, MATLAB command: importdata( ))

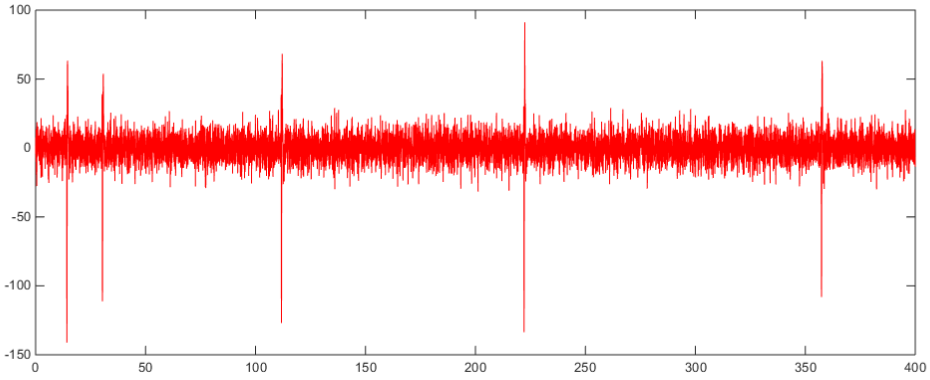


<Figure 11. Raw data plot>

② Confirm that the sampling interval is 0.04 ms (or 25 kHz). You need to understand the sample frequency to do part ③.

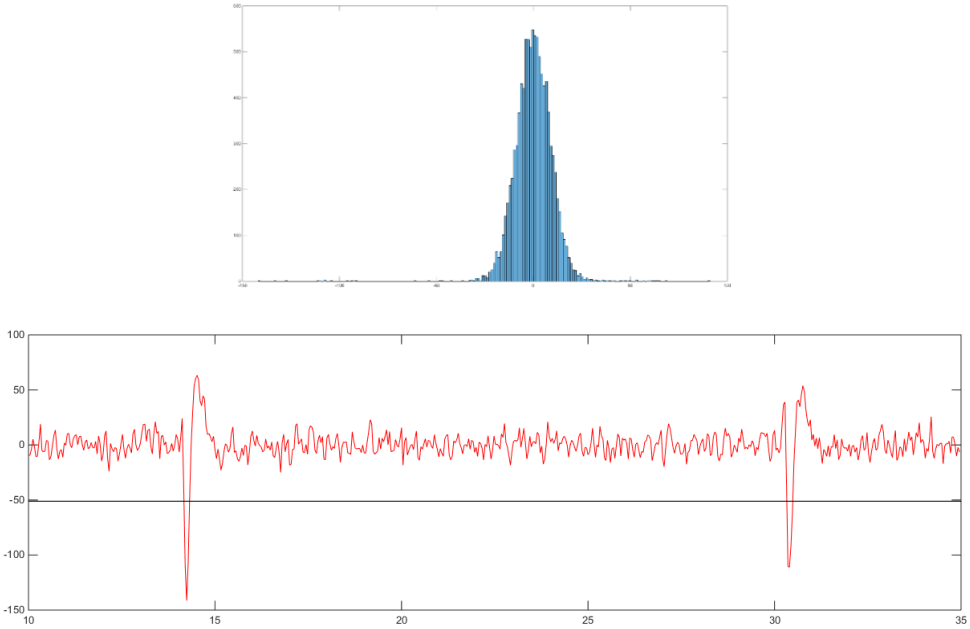
③ Use 2nd order Butterworth high-pass filter which has 200Hz cut-off frequency in order to remove noises from raw data, and plot it. [MATLAB commands: butter( )]

- [B, A]=butter(order, cut-off freq/(sampling freq/2)), filter type);
- filtered_signal = filter (B,A, unfiltered_signal);



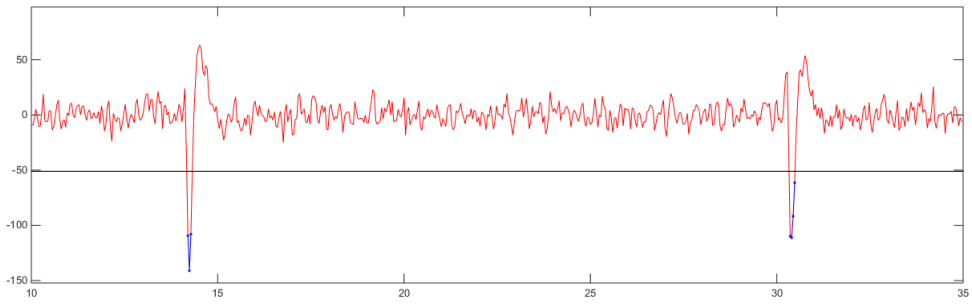
<Figure 12. Filtered data plot>

④ Make the histogram using amplitude values. Calculate mean and standard deviation of the amplitude distribution, and set the threshold level as (Mean – 5 * S.D). [histogram( ), mean( ), std( )]



<Figure 13. Histogram of background noise and threshold>

⑤ Function ‘find( )’ will return the index for the blue parts.



<Figure 14. Detecting signal under threshold>

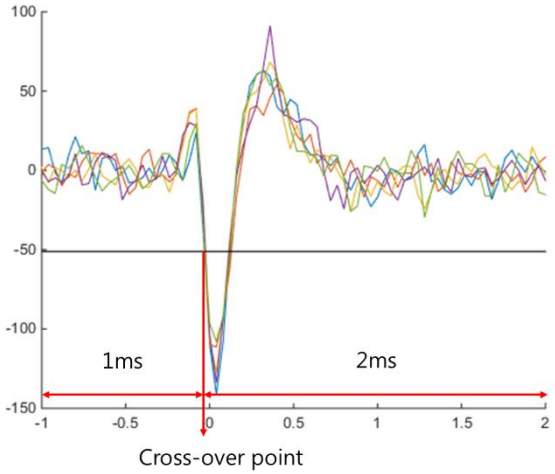
⑥ Find valley peak for each spike using min().

⑦ Extract spike time and construct spike train composed of spike timing.

	1	2
1	14.2400	
2	30.4000	
3	111.8000	
4	222.1200	
5	357.3600	
6		

<Figure 15. Extracted spike timing>

⑧ Extract detected spike waveforms, using the following criteria. Display all spike waveforms in one plot panel by overlaying each spike. [hold on]



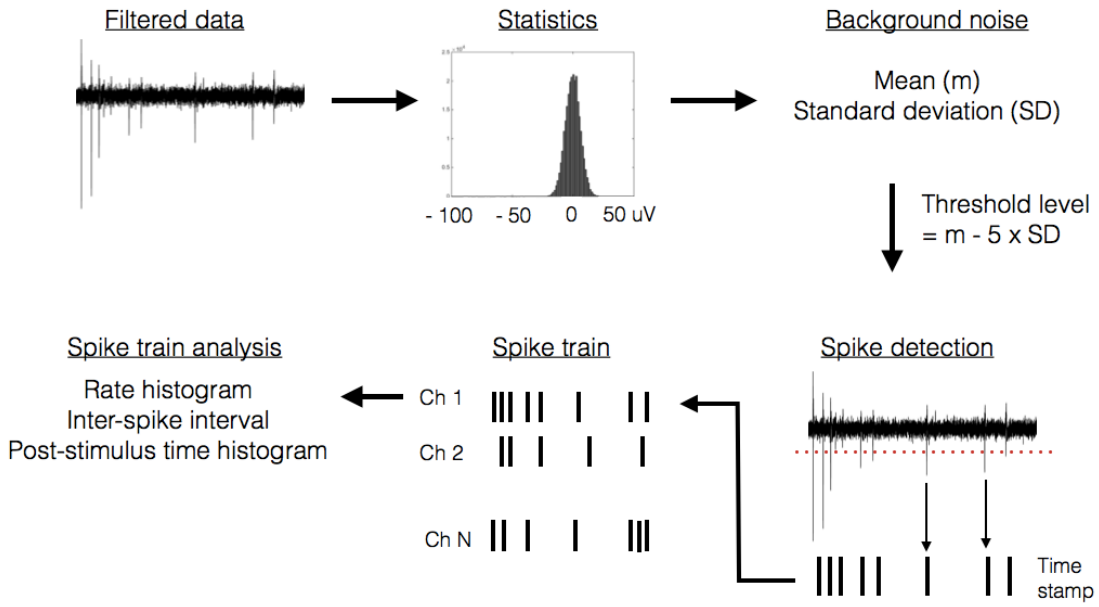
<Figure 16. Overlaid spike waveform>

⑨ Use your detector to find spike train for the test data (Pre_lab_test_data.txt).

* To achieve these activities, refer to the list of matlab functions below.

importdata, plot, butter, filter, mean, std, find, min, hold on, line, xlim, ylim, zeros

4. Experimental procedures



< Figure 17. Finding spike trains from multichannel extracellular spike recordings>

4.1. Spontaneous activity analysis

① Convert real data file (*.mcd) into an ASCII text file (*.txt) using MC_DataTool.

② Apply digital high-pass filter using 200 Hz cut-off frequency (Butterworth 2nd order filter)

- Draw raw data and filtered data of 10 channels at the same time.
- Why do you use 200 Hz high-pass filter?

③ Detect spike with – 5 x SD for each channel

- Use 'subplot' function for drawing 10 graphs and the threshold at the same time.
- Why do you use -5 x SD for threshold? What is the meaning of 5 x SD?
- What happens if you use -3 x SD for threshold?

④ Construct raster plot (x-axis: time, y-axis: channels)

- Draw raster plot for 10 channels at the same time. (top: ch 1, bottom: ch 10)

- How can you manipulate ytick label of figure? (‘Ch 1’, ‘Ch 2’, …)

⑤ Construct spike rate histogram
- Draw spike rate histograms with bin size of 0.1, 1, 10 sec.
- Calculate mean firing rate of each channel.
- How do those histograms look different?
- Which bin size is the best? Why?

⑥ Construct inter-spike interval (ISI) histogram.
- Draw ISI histogram with bin size of 1, 10 , 50, 100 msec
- Which bin size is the most proper between 1 ms and 100 msec? Why?
- What is the meaning of analyzing ISI of spike trains?

4.2. Evoked response analysis

① Convert real data file (*.mcd) into an ASCII text file (*.txt) using MC_DataTool. Please convert only three channels about Ch 36, Ch 48, Ch 56. Analysis using these three channels data in the next steps.
- What is the meaning of each column of text file?

② Apply digital high-pass filter using 200 Hz cut-off frequency (Butterworth 2^{nd} order filter)
- Overlap filtered data on raw data for only one trial.
- What is the big signal appeared right after the stimulation?

③ Display the evoked response data by aligning each trial with respect to the stimulation onset time at t = 0. Generate separate evoked response displays for every stimulation level. (Figure 18. (a))

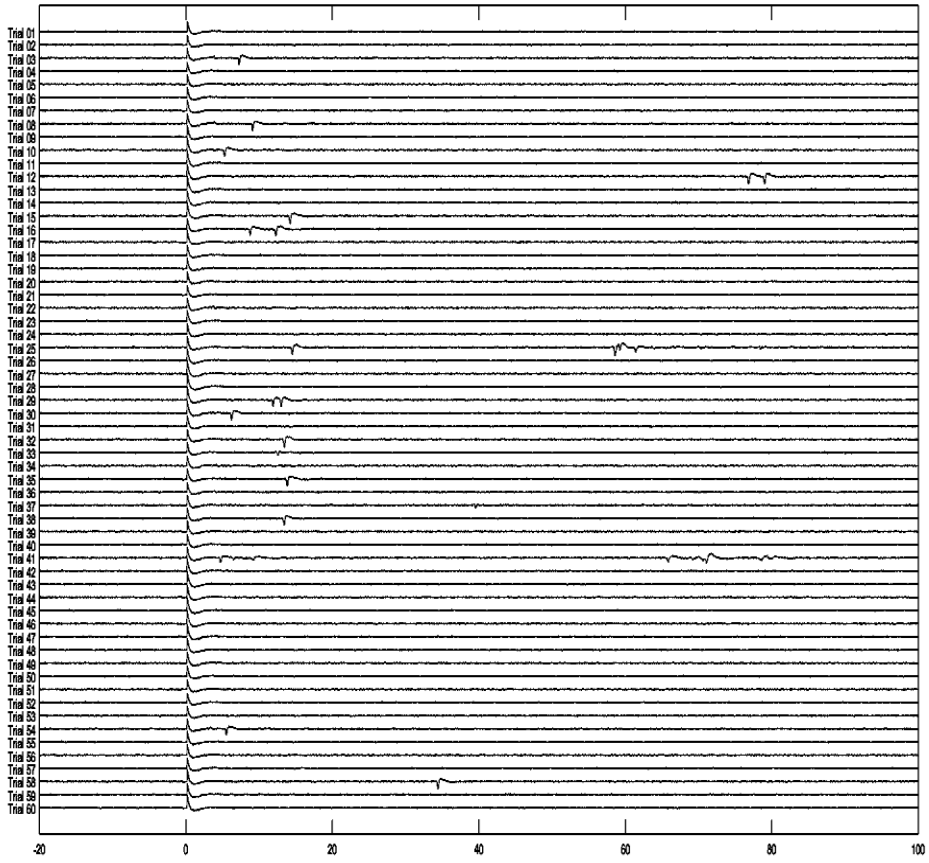
④ Detect spikes with a proper threshold level. There are unwanted large spurious spikes (‘stimulation artifact’) at near the stimulation onset time points which will bias (overestimate) the spike detection level when you use the threshold level calculation you used in section 4.1. For this exercise, you are free to determine the threshold level. Display the detected spikes point on figure of the evoked response data (4.2.3).
- How can you remove the stimulation artifact?
- What is the proper time range in order to remove the stimulation artifact?

⑤ Obtain post-stimulus time raster plot after aligning each trial with stimuli (or trigger)
- Draw raster plot starting with -20 msec.

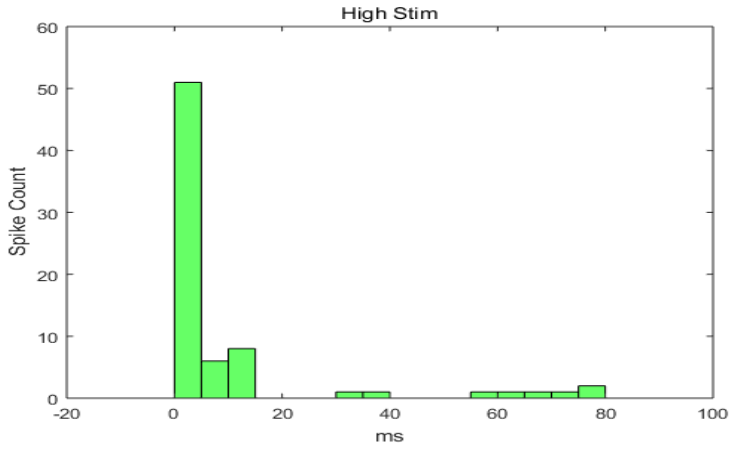
⑥ Construct PSTH with bin size of 5 msec
- What is the mean that trend of PSTH?

⑦ Obtain strength-response curve by measuring the response with the spike counts within 50 msec in PSTH. Strength-response curve is a graph between electrical stimuli of different intensities and spike count recorded by each stimulus. (x-axis: stimulus intensity, y-axis: spike counts(/trial)).

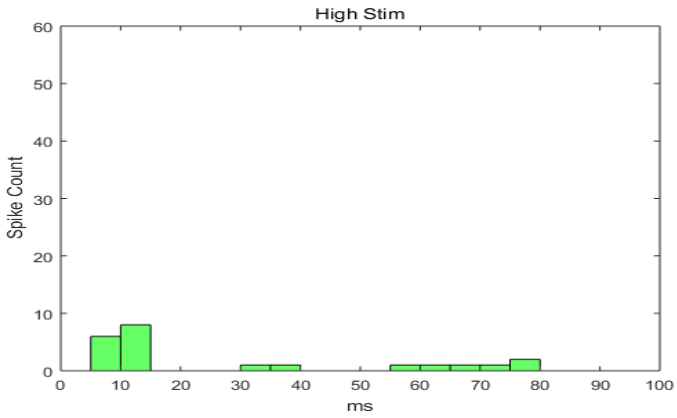
- Normalize strength-response curve with the number of trials.
- How does the curve look like? Why?



(a)



(b)



(c)

<Figure 18. Evoked response analysis. (a) Display of 50 evoked response data aligned by stimulation onset (t =0). Each trial is from -20 ms to + 100 ms with respect to the stimulation onset. Large stimulation artifact is shown at t = 0. Spikes could be found in some trials. x-axis: time [ms], y-axis: uV. (b) PSTH constructed from data shown in (a). Artifact (non-biological spikes) as well as neural spikes are counted, which is indicated by data value 50 at the bin 0 – 5 ms. (c) PSTH after resetting the bin 0 – 5 ms to zero to exclude non-biological spike (artifact) counts.>

5. References

[1] Introduction to spike train analysis:
http://www.med.upenn.edu/mulab/analysis.html#Introduction

[2] Planar-type microelectrode array technology articles: Y. Nam and B. C. Wheeler, "In vitro microelectrode array technology and neural recordings," Crit Rev Biomed Eng, vol. 39, pp. 45-61, 2011. (pdf)

[3] Video clip: How to prepare cell cultures for planar-type microelectrode arrays (http://www.jove.com/video/2056/how-to-culture-record-stimulate-neuronal-networks-on-micro-electrode)