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Course Module

Analysis of synaptic events in intracellular voltage clamp recordings

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Synopsis

This course module introduces the analysis of data from intracellular patch-clamp recordings in the voltage clamp mode, focusing on the detection and statistical description of spontaneous as well as evoked post-synaptic currents (PSCs). After having completed the module, students should be able to detect PSCs by setting appropriate thresholds and analyze amplitude distribution, reliability and amplitude variability of the detected PSCs. Data from whole-cell patch-clamp recordings from layer-V pyramidal cells of rat somatosensory cortex are provided, where spontaneously occurring PSCs are used to train detection and assessment of amplitude distribution, while PSCs evoked via glutamate uncaging at presynaptic sites are used for reliability and amplitude variability measures.

Supplemental Material

With this course module, we provide an Introductory Lecture *Intracellular_signals.pdf* and additional data files for practical analysis. The supplemental material for this teaching module is available online at the following URL: www.g-node.org/teaching/material/modules

Citation

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Requirements

Practical work on this course module requires that you run Matlab Version 7 or higher. Supplemental data files are required for data analysis.

Introduction

Over the past two decades electrophysiological experiments in vitro have contributed strongly to our knowledge of neural function on the level of single neurons (e.g. dendritic integration and computation), synaptic transmission (e.g. synaptic strength, variability and connectivity) and synaptic plasticity (e.g. LTP, STP, STDP). There are different types of in vitro preparations, divided into two broad classes: cultured nerve tissue and acute brain slices. Compared to experiments in vivo these preparations have the major advantage of very good experimental accessibility with a variety of different electrophysiological techniques.

This exercise covers the analysis of intracellular whole-cell patch-clamp recordings from layer V pyramidal cells in the acute slice of rat somatosensory cortex. The patch-clamp electrode is a glass pipette filled with an electrolyte, a solution that resembles the intracellular medium in its ionic composition, to provide for electric coupling of the amplifier equipment to the cytoplasm of the cell. With this electrode, it is possible to measures the membrane potential relative to the reference electrode, which is connected to ground. This way, the extracellular potential outside the neurons is defined as zero. The recordings for today's exercise were made in the voltage-clamp mode, which means that the trans-membrane voltage is kept constant by means of injecting currents that counteract all synaptic currents. By measuring the dynamic current that is injected we effectively measured the (dynamic) synaptic current that flows into the cell in reaction to vesicle release at synaptic sites, which may have occurred spontaneously or in reaction to a presynaptic action potential.

Part I : spontaneous synaptic activity

In neocortical acute brain slices, there is only little spontaneous activity compared to the in vivo situation due to the de-afferentiation (cutting off the inputs from the rest of the brain). This is an advantage if we want to determine the size of single excitatory postsynaptic currents (EPSCs). Goal of the first part of the exercise is to first determine the average time course and peak amplitude of synaptic events that arrive at one particular cell, and, secondly, to determine the distribution of peak amplitudes that stem from different synaptic release sites.

1. load data file: "spontaneous_recording.mat"
    - file contains matlab structure "data". important structure fields are:
    - I : unfiltered current trace
    - FI : filtered current trace
    - TimeResolutionS : temporal resolution of recording in seconds, inverse of:
    - SamplingFrequencyHz : sample frequency of recording in time steps per second.
2. open figure and plot the raw signal (I) and the band-pass filtered signal (FI) in 2 different colors on top of each other (graph 1). Use an appropriate time scale (s or ms) on the x - axis and choose a time window that allows

to observe the structure of the data (e.g. 1s length). Be careful: the data array is long (check with “size”) and you might not want to display the complete data array.

3. use a threshold to detect PSCs in the band-pass filtered data. The threshold should be chosen such that only obvious synaptic events cross the threshold, while noise fluctuations do not reach this threshold. Add the horizontal threshold line to the previous graph 1. To detect the instances of threshold crossing you need to find those data points below threshold, for which the previous data point was above the threshold. Assign a variable “onset_idx” to the vector indices at which threshold crossing occurred. How many PSCs did you detect? How many did your neighbors detect? What is the reason for differing numbers?
4. Make data cutouts of stretches of the raw signal around the onsets of the detected PSCs. Choose a reasonable time interval before and after the onset such that the time course of the PSCs is well captured. Assign the variable “PSCs” to the matrix of cutouts.
5. Graph 2: plot all PSCs in one axis. On top plot the average PSC with a stronger line. Use a second axis (graph 3) to plot the average PSC and two additional lines indicating the standard deviation in positive and negative direction with respect to the average PSC. What is the peak amplitude of the avg. PSC?
6. Statistics: Determine the peak amplitude of each single PSC and produce a histogram (i.e. show the distribution) of PSC peak amplitudes (graph 3). Is this distribution symmetric or skewed? Produce a histogram on the logarithmic axes, or, equivalently, produce a histogram of the logarithmic values of peak amplitude. Distribution of synaptic strength is well approximated by the so-called log-normal distribution (i.e. normally distributed on the logarithmic axis).
7. Do all your graphs have proper axes labeling and units? You should save your result figures using the “print” command and the option ‘-dpdf’ for PDF output.

Part II : Reliability of synaptic transmission

In this part we analyze a different experimental paradigm. Again, voltage clamp measurements from a layer V pyramidal neuron were performed to observe membrane currents. To measure the repeated postsynaptic response to synaptic transmission, a presynaptic neuron was repeatedly stimulated by means of phototlytic release of the excitatory neurotransmitter glutamate using short pulses (4-10ms) of UV light [1]. For generating the pulses a shutter (similar to that in a photo camera) was used to start and end illumination of small spots of the slice by a continuous UV laser beam. The movement of the mechanical shutter was recorded along with the physiological data. This allows to determine the exact times at which the shutter was opened. These shutter opening times will serve us as the trigger (instead of the threshold crossing in Part I) for cutting out the data pieces. The goal is to determine the trial-to-trial variation of the peak amplitude of the PSCs and the temporal precision of PSC onset.

1. load data file “synaptic_response_variability.mat”
    - file contains matlab structure “data”. important structure fields are:
    - I : current trace
    - Z : shutter command voltage
    - I_TimeResolutionS: temporal resolution of current recording in seconds

- Z_ TimeResolutionS: temporal resolution of shutter command voltage in seconds.

2. Detect the stimulation onset index/times from the variable “Z”, the shutter is closed when the shutter control voltage is +5 V, and open when it assumes -2V. Note that shutter command voltage was sampled with different time resolution than the current trace! There are 42 shutter openings corresponding to 3 different presynaptic stimulation sites (3 different presynaptic neurons that were targeted) and 14 repetitions for each target site. The stimulation sequence was as follows: target 1, target 2, target 3, target 1, … and so on. You thus you need to group the 14 appropriate indices for each of the sites: first collect all time indices of opening, then “reshape” your array (42 x 1) such that you receive a matrix of 3 x 14 entries.
3. Amplitude variability: For each presynaptic site, extract the responses to the stimulation during 100 ms following the stimulation (cutouts) and make for each target a graph (graph 1-3) that shows all 14 repetitions in different colors. Measure the peak amplitude for each single repetition and calculate mean and standard deviation of peak amplitudes across repetitions, as well as the coefficient of variation CV (std / mean). Add the numbers as text to your graph. How does the CV depend on the avg. peak amplitude for the three cases? Do your results fit to the results given in Figure 5C in [1]?
4. Temporal precision. Assess the temporal precision with which the PSCs occurred in repeated trials. An easy way to do this is to detect the PSC onset again by using a threshold and then measure the standard deviation of onset times (in milliseconds). The smaller this standard deviation, the more precise is the response.

Suggested reading:

[1] Boucsein C, Nawrot M, Rotter S, Aertsen A, Heck D (2005) *Controlling Synaptic input Patterns In vitro by Dynamic Photo Stimulation.* J Neurophysiol 94: 2948-2958

[2] Markram H, Lubke J, Frotscher M, Roth A, Sakmann B (1997) *Physiology and anatomy of synaptic connections between thick tufted pyramidal neurons in the developing rat neocortex.* J Physiol 500 (2):409-440.

[3] Nawrot MP, Schnepel P, Aertsen A and Boucsein C (2009) *Precisely timed signal transmission in neocortical networks with reliable intermediate-range projections*. Frontiers in Neural Circuits 3:1 http://frontiersin.org/neuralcircuits/paper/10.3389/neuro.04/001.2009/pdf/

Matlab functions which can be helpful:

for calculations:

```
mean
std
find
diff
hist
zeros
reshape
```

for plotting:

```
bar
squeeze
xlabel
ylabel
line
text
set
XTickLabel
print
```