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Course Module

Measurement of interval and count variability in neural spike trains

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Synopsis

The present course material is designed to introduce students to the empiric measurement of variability in neural spike trains. We deal with two different aspects of spike train variability, (1) the variable length of a finite sample of inter-spike intervals (ISIs) as quantified by the *coefficient of variation* (CV); this measure captures the temporal *irregularity* of spike occurrences, and (2) the variability of the spike count across repeated measurements as quantified by the *Fano factor* (FF). In point process theory, interval and count statistics are closely related. We therefore suggest the co-analysis of both aspects, count variability and spiking irregularity, under the null-hypothesis $FF=CV^2$ which is the prediction for a renewal process. We specifically address statistical issues of estimating both, CV and FF, in stationary and non-stationary spike train data. In particular, we address the estimation bias for short observation intervals and introduce the method of firing rate demodulation in order to pursue interval statistics in rate-modulated data. Finally, we explore the effect of serial interval correlation (non-renewal properties) in neural spike trains on interval and count variability. This course material is designed to provide hands-on experience with data analysis. We provide sample data sets of intracellular patch clamp recordings *in vitro* and *in vivo* from rat neocortex and extracellular data from the honeybee mushroom body as well as a set of point process simulated data which are made publically available. This course addresses graduate students with either experimental *or* theoretical background, and with limited experience in statistical data analysis. Ample programming skills in Matlab are required.

Supplemental Material

Together with this course module we provide an Introductory Lecture *Estimating Variability in Neural Spike Trains – Theory and Practice*. Supplement data files are provided for practical analysis. The supplemental material for this teaching module is available online at the following URL: www.g-node.org/teaching/material/modules

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Requirements

Practical work in this course module is designed for Matlab Version 7 or higher. Supplemental data files are required for data analysis.

Course Format

The current format of the module separates practical taks into three parts A, B and C. Part A commences basic data handling and statistics. Part B deals with the analysis of rate-modulated data and involves rate estimation and time transformation. Part C introduces the co-analysis of count and interval statistics and interprets the influence of serial correlations in single neuron spike trains. For a one day course that comprises an introductory lecture, it is thus recommended to split into two groups that complete *either* parts A and B *or* parts A and C. Students with good programming skills and some experience in spike train analysis will be able to complete all three parts. This course module was taught for the first time during one full day of the *1st G-Node Winter Course on Neural Data Analysis* at G-Node, Ludwig – Maximilans – Universtität München, Jan 26 – 30, 2009.

Introductory reading

For an introduction to the point process theory relevant for this practical course module refer to the supplement introductory lecture and to chapter 1.4 of Dayan and Abbot (2005). The analysis methods taught in this course are described in detail in Nawrot (2010), Nawrot et al., (2008), and Meier et al., (2008). For a general introduction to the issue of variability in cortical activity you may read through the introduction and discussion sections in Nawrot et al., (2008), and refer to Shadlen & Newsome (1998).

General Instructions

Comment your code while scripting so that you will later be able to rehearse your script. You may also comment within your code questions and answers that arise during discussion with the tutors. Include for each script a header with date and name of author. When producing figures with your results make sure that you label all axes appropriately and that you add the correct units. Print all result figures to file for presentation and documentation. Choose an appropriate image format that you can use with the presentation software of your choice.

1 Spike train irregularity – the coefficient of variation (CV)

1.1. Definitions

We assume an empiric sample of interspike intervals X_i, i= 1, …, n. Then we denote with μ the mean interval, and we denote with $\sigma = (Var(X))^{1/2}$ the standard deviation of the interval distribution. The empiric coefficient of variation is then given by

$$CV = \sigma / \mu$$

which is a dimensionless statistical quantity (hence termed a 'coefficient'). Thus, the empiric CV of the ISI distribution represents the normalized standard deviation of ISIs. It represents the empiric estimate of the theoretical CV that is associated with the underlying stochastic spiking model. In point process theory, the CV is well defined only for stationary point processes (see Lecture).

1.2. Analysis of stationary Data

We start our analyses with the empiric measurement of the ISI distribution and CV in a sample of stationary spike train data. There exist different theoretical definitions of the 'stationarity' of a point process. In practical terms, we mean by 'stationary' a constant firing rate of action potentials, i.e. we only consider recordings, or parts thereof, during which the firing rate of a neuron does not change. However, statistical testing for stationarity is tricky (for a discussion of this problem see e.g. Johnson, 1996; Farkhooi et al., 2009). Here, instead of using a rigorous statistical test, I will introduce a simple method of visualization that supports a decision upon visual inspection.

Tasks (A)

Open a new matlab script for tasks 1 – 5. When you later run this script it should reproduce Figures 1-3. Make notes of your observations. For tasks 1 – 5 we provide two data sets, A1 (*in vivo* intracellular) and A2 (*in vitro* intracellular noise current injection). Choose one of these two data sets.

1. Load data set A1 (or A2). This contains an *in vivo* intracellular recording from a cortical neuron (see section Data for detailed information). Open a new figure (Figure 1) and plot only a small piece of 2s length from the complete voltage trace. Set the range of the yaxis to (-75mV,20mV). Compare to Fig. 1A in Nawrot et al., (2007) which shows the same neuron. How long is the complete voltage trace in seconds?
2. Determine all time points at which an action potential occurred, this list of time points defines the so-called spike train. spike tIn the intracellular voltage trace detect all spike times in milliseconds. Define a suitable threshold Θ and detect the spike times as positive threshold crossings (i.e. the first data point of a spike that is higher than the threshold). Assign the variable *s* to the resulting list of spike times. How many action potentials did you detect? In Figure 1, mark all detected spike times within the previous 2s interval in with a red star. What is the average firing rate of this neuron?
3. Open a new Figure 2. For visual inspection of the stationarity of the spike train, plot the spike times *t1, t2, …, tn* against the spike index (i.e. *1, …, n*) as data points (scatter diagram). If the process is stationary you expect to find the data points distributed along a straight line. However, if firing rate changes, you will observe a deviating curvature of the data points.
4. Now compute the interspike intervals and assign to it the variable isi. Open a new Figure 3 and make a histogram of the ISI distribution. To do this, use the 'histc' and the 'bar' command. This requires that you first define a vector of edges for the histogram classes with a predefined bin width, e.g.

   ```
   bin = 10; ISImax=300; edges = [0:bin:ISImax];
   ```

   Increase/decrease the bin width and repeat the procedure. Graphics: Assign a handle to the graphics object: `ba=bar(…)`. Type `set(ba)` this will give you all parameters that are associated with your graphics object, in particular 'facecolor' and 'edgecolor'.
5. Finally compute the CV of the empiric ISI sample. Add the CV in the axis of the interval distribution in Fig. 3 using the 'text' command.

Take your time to finalize your figures and to prepare for a final presentation of your results. Comment your code such that later you can understand what you did and how you achieved it.

1.3 Estimation bias

In the classical experimental approach, we repeat the identical stimulation protocol several times, or we repeat the same behavioural task several times, and thus we obtain a number of repeated measurements (trials) under fix experimental conditions. We here investigate the observation bias of the CV in a long recording of spontaneous activity (A1) and in a long recording under controlled stationary input conditions (A2). From such an ongoing recording we may construct non-overlapping (i.e. independent) repeated observations by simply cutting a long experimental observation into shorter pieces. In this setting of independent observations we may pool all intervals from all trials and then compute the CV in dependence on the length T of the independent observations.

Tasks (A)

6. Load data set A1 (or A2) into memory. Define a time interval T such that it comprises on average $\tau = 10$ intervals. Divide the array s of spike times into a maximum number of N observation intervals of length T: $\Omega_j = (t_a^j, t_a^j+T]$, j=1, …, N. From the spike times of each interval Ω_j subtract the start time t_a^j of observation to obtain relative spike times. You may use a cell array for representing lists of spike times.
7. New Figure 4 will comprise 2 axis (use 'subplot'). Produce a raster display (or 'dot display') of all spike times in all observation intervals where each row represents one observation (trial), and in each row a tick mark or point represents a single spike time (cf. e.g. Fig. 7a in Nawrot et al., 2008).
8. Pool all intervals from all trials and calculate the CV. Now repeat this procedure for different integer values of $\tau < 10$. Plot the CV in dependence on τ into the second axis of Figure 4. This function describes the estimation bias of the CV in dependence on the duration of the observation.
9. Discuss with your neighbours why the CV reduces for shorter observation intervals. For which value of τ does the curve saturate?

Discussion: What do we assume when we pool intervals from different trials? What could be an alternative approach? If you were fast in completing tasks 6 – 9 you may try out this alternative approach.

2 Measuring CV for non-stationary firing rates

The CV is a useful statistical measure only if the analyzed sequence of events has a stationary rate of occurrences. If, however, a neuron's firing rate changes during the course of an observation, the interval distribution becomes more or less meaningless. Increasing the firing rate reduces the ISI length, and reducing the rate increases ISIs. Thus, the interval statistics is determined not only by the assumed random nature of spike generation, but also

by some deterministic (or stochastic) change in the firing rate. Here we apply the method of 'time-warp' that is designed for a repeated measurements under identical experimental conditions. We first estimate the firing rate by averaging across trials. We then use the estimated rate function to demodulate the spike train. For this we use some custom-written algorithms that are implemented in the FIND toolbox for neural data analysis with Matlab (Meier et al., 2008; http://find.bccn.uni-freiburg.de/).

Tasks (B)

This analysis proceeds in 3 steps. First we need to estimate the neuron's (trial-averaged) firing rate function on the bases of repeated measurements. Then we use this rate function to transform the original spike trains to the so-called operational time axis where the rate is now constant. Finally we may estimate the CV from the intervals in the transformed spike trains.

10. Choose a data set from B1 (or B2) and load it into memory (see section *Data Sets* for details). B1: This data set is analyzed in Fig. 8 of Nawrot et al., 2008 and contains repeated trials recorded intracellular during an *in vitro* current injection paradigm. The data file contains the Matlab struct 'DCS' with various fields. To explore the struct type `>>DCS` at the command line. The relevant spike train data is accessible as the field 'SpikeMatrix'. This 2-d array contains columns which represent 40 trials. Each column represents a single trial spike train of 5s duration in 0/1 format with at time resolution of 1ms. The total of 40 trials separates into two different input paradigms with 20 trials each. The two stimulation paradigms were presented in an interlaced fashion, i.e. we alternated paradigm 1 (columns 1,3,5, …) and 2 (columns 2,4,6, …). Why did we alternate the different injection paradigms in the course of the experiment? B2: This data set is analyzed in Fig. 3 of Meier et al., 2008 and consists of a total of 66 trial recorded under a single experimental paradigm.
11. Construct separate matrices for both paradigms. Open a new Figure 5. Into two separate axes, plot spike raster diagrams for the two paradigms. Make sure that your time axis is correct.
12. Rate Estimation 1: We concentrate on experimental paradigm 1 for which the firing rate is modulated. To estimate the dynamic firing rate we use the method of linear kernel convolution (Nawrot et al., 1999; cf. Lecture) which we apply to the trial-averaged (or pooled) spike train. First, pool or average the spike matrix. Now construct a kernel function using the matlab function **makeKernel**. You need to specify (1) the kernel shape, e.g. 'TRI' for a trianglular shape, and (2) more importantly the time resolution (or width) of the kernel function. Open a new Figure 6 and plot kernels of different shape but with the same time resolution. Use different colors for different kernels.
13. Rate Estimation 2: Now estimate the firing rate by convolving the average spike train with the kernel function. Matlab's built-in 'filter' or 'conv' function. Try out different time resolution for the kernel function and plot the resulting rate functions into Figure 7. Suggest a reasonably good time resolution. There also exist heuristic and model-based ways to automatically approximate the optimal kernel width, (e.g. Nawrot et al., 1999; Meier et al., 2008).
14. Time Transformation: We now use a non-linear transformation of the time axis to produce single trial spike trains with a statistically constant rate from the original (rate-modulated) spike trains. To do this, we 'unwarp' the time axis according to the estimated rate function. Intuitively, we stretch time, where the rate is high, and we compress time where the rate is low. This is performed with the function **unWarpTime**.
15. Measure the CV from the unwarped spike trains. Compare this to the CV of the

original spike trains and to the CV in the control paradigm with stationary firing rate.

3 Count versus interval variability – a combined analysis

Thus far we have considered only the variable length of interspike intervals and computed the CV as a measure for spike train irregularity. We now also measure the variability of the spike count. We calculate mean M and variance *Var* of the spike count and define the Fano factor as

$$FF = Var(C)/M$$

which is again a dimensionless index that quantifies the dispersion of the spike count distribution. In point process theory, interval and count statistics are related. In particular it holds for the class of renewal processes (see Lecture) that $FF=CV^2$. We formulate this renewal prediction as our null-hypothesis and test for deviations in our data sets. We may then interpret observed deviations in the light of possible causes (Nawrot 2010).

Tasks (C)

16. Load data set C1. This contains a struct 'SIM' with several independent simulation units. Each simulation unit consists of a set of repeated trials and represents repeated experiments in one neuron. Measure CV and FF for each unit. Figure 8: Two axis. Left: Scatter plot FF vs CV^2 for data set C1. You may possible use logarithmic axes. Add the identity line in your plot – this represents the renewal hypothesis.
17. Repeat the analysis for either data set C2 or C3 and plot the result in the right axis. Interpret your results during the final presentation.

We discuss and interpret your results in the final presentation.

Data Sets

A1 – intracellular recording of spontaneous activity in a cortical cell of the anaesthetized rat. Details are given in Nawrot et al., (2007). These experiments were performed by Clemens Boucsein, University of Freiburg, Germany and Dymphie Suchanek, University of Freiburg, Germany.
Filename: A1_data_set_040528_boucsein.mat

A2 – intracellular current patch clamp recording from a layer V pyramidal neuron in the acute cortical brain slice from rat somatosensory cortex. Stimulation with stationary noise current injections, balanced condition. Details are given in Nawrot et al., (2008). These experiments were performed by Victor Rodriguez-Molina, University of Freiburg, Germany and Martin Nawrot.
Filename: A2_data_set_2003_rodriguez_nawrot.mat

B1 – intracellular current patch clamp recording from a layer V pyramidal neuron in the acute cortical brain slice from rat somatosensory cortex. Stimulation with stationary noise current injections under balanced condition, and with modulated excitatory input. Details are given in

Nawrot et al., (2008).
Filename: B1_DCS_041020_cortex1_2cell008.mat

B2 – extracellular single unit recording from extrinsic neurons in the mushroom body of the honeybee during odor stimulation. During repeated trials the neuron strongly modulates its firing rate in response to the stimulus. Data is described in more detail in Meier et al., (2008). Recording techniques and neural responses of mushroom body extrinsic neurons are described in detail in Strube-Bloss, Nawrot & Menzel (2011).
Filename: B2_BeeExampleData.mat

C1/C2/C3 – point process simulations without (C1) and with (C2/C3) serial correlations. The model marginal interval distribution is log-normal. Details of the model are given in Farkhooi et al., (2009).
Filenames: C1_Simulation.mat;
C2_Simulation.mat;
C3_Simulation.mat;

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