

Analyse des séquences de potentiels d'action Tutorial

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1 The idea motivating this development

The idea here is to implement the Unix/Linux "philosophy"—as exposed for instance in the article of Arnold Robbins *What's GNU*—to the analysis of neuronal spike trains. Since spike trains make not too voluminous data, they can be stored as text files (ASCII) and most operations on them can be designed as "filters", that is programs (usually but not always written in C) that read their input in text format from the "standard input" (`stdin`) and send their result in text format to the "standard output" (`stdout`). For graphical displays, we are going to use *gnuplot*.

2 Required software

The code will be written mostly in C. If you want a clear and quick introduction to this language, check Ben Klemens: [Modeling With Data](#). To compile the code you will need a C compiler like [gcc](#). If you are using Linux or MacOS it's in a package from your favorite distribution, if you are using Windows you will have to install [Cygwin](#). The heavy computational work is going to be performed mainly by the [gsl](#) (the *GNU Scientific Library*) that is easily installed through your package manager (from now on, for windows users, the "package manager" refers to the one of Cygwin). The graphs will be generated with [gnuplot](#). Windows user who want to use the interactive plotting capabilities of the library (recommended) will also have to install [Cygwin/X](#).

For now the compilation requires either `=make` or [Scons](#).

2.1 Getting and compiling the code

The code is hosted on [GitHub](#). The easiest is to clone or download the repository (there is a button for that on the GitHub page). Once you have the repository on your hard drive, go to the code sub-directory and, if using `make`, type:

```
1 make all
```

or with SCons:

```
1 scons -Q
```

This will compile the library `libaspa.a` as well as a bunch of user programs all starting with `aspa_`, like `aspa_read_spike_train`. As mentioned previously, you need the `gsl` to be installed in order to compile the code.

Once the compilation is done you should move the user programs to one of the directories listed on your `PATH`, that is on one of the directories appearing when you type:

```
1 echo $PATH
```

After that, you're in business.

3 Data used

We are going to use spike trains obtained from the antennal lobe—first olfactory relay—of locust, *Schistocerca americana*. These spike trains can be found on the [zenodo-locust-datasets-analysis](#) GitHub repository. You can also find there a complete description of the sorting procedure used to go from the raw data, that are available on [zenodo](#), to the spike trains. We will mostly use the spike trains from experiment `locust20010214` that can be found at the following address: https://github.com/christophe-pouzat/zenodo-locust-datasets-analysis/tree/master/Locust_Analysis_with_R/locust20010214/locust20010214_spike_trains.

3.1 Getting a spike train

We will start by downloading the spike train from unit 1 from group Spontaneous_1. This is done by typing in the shell (I'm using the "line continuation character, \" to fit my lines on a single page of the PDF version of this document, when typing directly to the shell you don't need these line breaks):

```
1 wget https://raw.githubusercontent.com/christophe-pouzat/\
2 zenodo-locust-datasets-analysis/master/Locust_Analysis_with_R/\
3 locust20010214/locust20010214_spike_trains/\
4 locust20010214_Spontaneous_1_tetB_u1.txt
```

This "spike train" contains in fact the result of 30 consecutive continuous acquisitions, each 29 s long with a 1 s gap in between, as is made clear in the [detailed sorting description](#) of this data set.

4 Preliminary analysis

4.1 Reading the data

It is not expected that the data (spike trains) one wants to work with will be obtained in any standard format. That means that a usually slightly "painful" work will be required (but that's always the case when dealing with actual data) to read the data and reformat them in the text (or binary) format used by aspa. Looking at the source code of `aspa_read_spike_train` is the way to proceed (more specifically, look at the code of `aspa_raw_fscanf` that is called by `aspa_read_spike_train` and that is found in `aspa_single.c`).

The data we just downloaded are collections of spike times in "sample times"—the time unit is therefore not the second but 1/15000 second—with one spike time per line. This can be seen by calling first the `head` function (showing by default the first ten lines of the file):

```
1 head locust20010214_Spontaneous_1_tetB_u1.txt
```

```
4364.629
49876.8
50529.95
50988.26
51371.66
51769.29
52703.77
54772.34
56472.7
71766.51
```

Calling `tail` shows the last lines of the file (by default the last ten lines):

```
1 tail locust20010214_Spontaneous_1_tetB_u1.txt
```

```
13442792
13455679
13458610
13460049
13460517
13461154
13464139
13470059
13471539
13472243
```

Function `aspa_read_spike_train` will read these times from the `stdin` and output them in a "nice" format (still a text file by default) to the `stdout`. You can get a description to arguments accepted by the function by calling it with the `--help` argument:

```
1 aspa_read_spike_train --help
```

That will give you:

Usage:

```
--in_bin: specify binary data input
--out_bin: specify binary data output
--sample2second <positive real>: the factor by which times
in input data are divided in order get spike times in seconds
used only when reading 'raw' data (default 15000)
--inter_trial_interval <positive real>: the inter trial
interval (in s) used only when reading 'raw' data
--trial_duration <positive real>: the recorded duration
(in s) of each trial used only when reading 'raw' data
--stim_onset <real>: the stimulus onset time
(in s) if that makes sense, used only when reading 'raw' data
--stim_offset <real>: the stimulus offset time
(in s) if that makes sense, used only when reading 'raw' data
```

For demonstration we can call it on the data file we just downloaded (`locust20010214_Spontaneous_1_tetB_u1.txt`), writing the result into a new text file `locust20010214_Spontaneous_1_tetB_u1.aspa` for further inspection:

```
1 aspa_read_spike_train --inter_trial_interval=30 --trial_duration=29 < \
2 locust20010214_Spontaneous_1_tetB_u1.txt > \
3 locust20010214_Spontaneous_1_tetB_u1.aspa
```

We can then look at the first 25 lines of our new file with:

```
1 head -n 25 locust20010214_Spontaneous_1_tetB_u1.aspa
```

```
# Number of trials: 28
# Number of aggregated trials: 1
# Stimulus onset: 0 (s)
# Stimulus offset: 0 (s)
# Single trial duration: 29 (s)
```

```
# Start of trial: 0
# Trial start time: 0 (s)
# Number of spikes: 94
0.290975
3.32512
3.36866
3.39922
3.42478
3.45129
3.51358
3.65149
3.76485
4.78443
5.06381
5.11507
5.24077
5.28448
5.31933
```

We see that the "non-data" element are on lines starting with a "#" character. The "head" of the file specifies how many trial are in the file and gives some other information. The data from trial 0 (we start counting at 0) com next after two blank lines. To see the whole file interactively you can type:

```
1 less locust20010214_Spontaneous_1_tetB_u1.aspa
```

4.2 Basic statistics

Program `aspa_mst_fns` (`mst` stands for "multiple spike trains" and `fns` for "Five-number summary") return elementary statics related to a spike train data set. A description of its use is obtained by calling the program with the `--help` argument:

```
1 aspa_mst_fns --help
```

```
Usage:
  --in_bin: specify binary data input

Returns five number summary and additional stats.
```

We can call this function directly on the output of `aspa_read_spike_train` using a `pipe` with:

```
1 aspa_read_spike_train --inter_trial_interval=30 --trial_duration=29 < \
2 locust20010214_Spontaneous_1_tetB_u1.txt | \
3 aspa_mst_fns
```

```
Data from 28 trials.
The mean rate is: 4.10222 Hz.
The inter spike interval statistics are:
  The sample contains 3303 elements.
```

```

The mean and SD are      : 0.2333 and 0.4660.
The median and MAD are: 0.0546 and 0.0359.
The five number summary:
Min.    1st qrt Median 3rd qrt Max.
0.0157 0.0369  0.0546 0.1491  4.5264
A 95% confidence interval for the lag 1 Spearman rank correlation is: [0.400336,0.443483].

```

4.3 Basic plots

There are several plots one might want to create at an early stage of spike train data analysis. Since these plots are more "attractive" when built from data with a response to a stimulus, we will start by getting one such case (from the same experiment and same neuron):

```

1 wget https://raw.githubusercontent.com/christophe-pouzat/\
2 zenodo-locust-datasets-analysis/master/Locust_Analysis_with_R/\
3 locust20010214/locust20010214_spike_trains/\
4 locust20010214_C3H_1_tetB_u1.txt

```

This file contains the responses to 25 stimulations with *cis-3-hexen-1-ol*. The classical way of displaying such data is the raster plot. This plot as well as several over ones we will shortly see is generated by calling `aspa_mst_plot`. As usual, calling the function with argument `--help` gives us a basic explanation on how to use it:

```

1 aspa_mst_plot --help

```

Usage:

```

--in_bin: specify binary data input
--text: specify text output
--what <string>: one of 'raster', 'cp_rt', 'cp_wt',
'cp_norm', the type of plot (see bellow)

```

An interactive lot is generated.

If what is set to 'raster' a raster plot is generated.

If what is set to 'cp_rt' the observed counting process in 'real' time is generated, that is trial appear one after the other.

If what is set to 'cp_wt' the observed counting processes corresponding to each trial are shown on the 'within trial time'.

If what is set to 'cp_norm' the normalized aggregated counting process is displayed (normalization means here that the step size due to each spike in each trial is 1/number of trials; in a sense the 'mean' counting process is displayed).

4.3.1 Raster plot

Here, to get the classical raster we do:

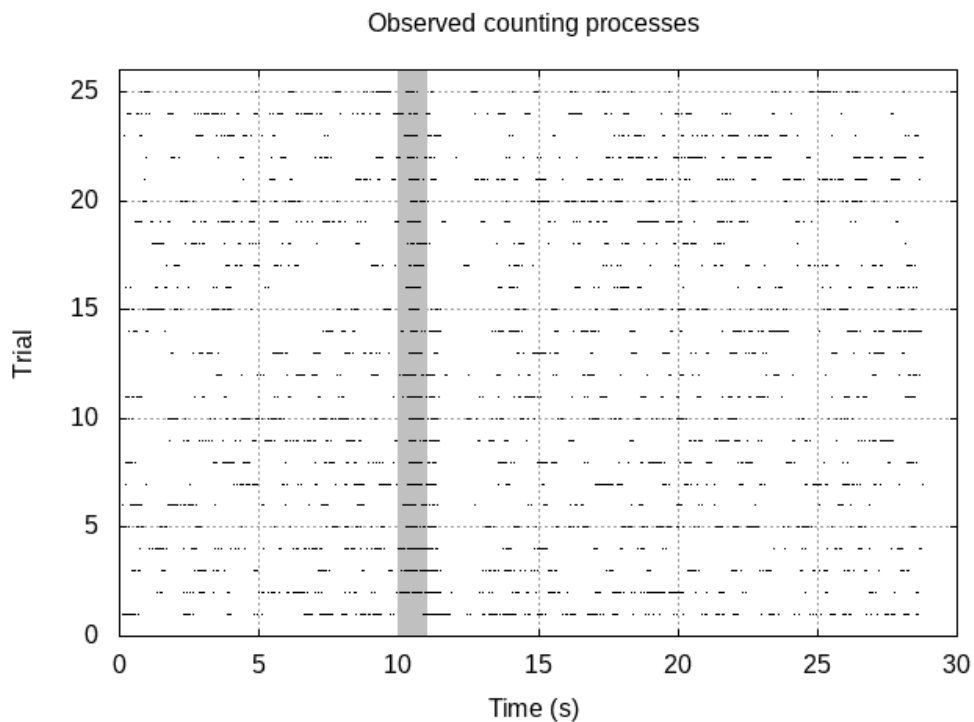
```
1 aspa_read_spike_train --inter_trial_interval=30 --trial_duration=29 \  
2 --stim_onset=10 --stim_offset=11 < \  
3 locust20010214_C3H_1_tetB_u1.txt | \  
4 aspa_mst_plot --what=raster
```

This will make a new window appear with a plot similar to the one we will now construct after calling the function with an additional argument (you can type q to kill the plot window):

```
1 aspa_read_spike_train --inter_trial_interval=30 --trial_duration=29 \  
2 --stim_onset=10 --stim_offset=11 < \  
3 locust20010214_C3H_1_tetB_u1.txt | \  
4 aspa_mst_plot --what=raster --text > \  
5 locust20010214_C3H_1_tetB_u1.raster
```

Here instead of the "new window output" we generated at text output (that's what the --text argument means) sent to the stdout and redirected this stdout to a file called locust20010214_C3H_1_tetB_u1.raster. We can now build "by hand" with gnuplot the same figure as the one we directly got (we have now more control on the output):

```
1 set grid  
2 unset key  
3 set xlabel 'Time(s)'  
4 set ylabel 'Trial'  
5 plot [0:30] [0:26] 'locust20010214_C3H_1_tetB_u1.raster' \  
6     index 0 using 1:2 with filledcurve closed lc 'grey',\  
7     '' index 1:25 using 1:2 with dots lc 'black'
```



4.3.2 A fancy trick

We can also make the raster plot and get the basic stats printed at once with the `tee` command as follows:

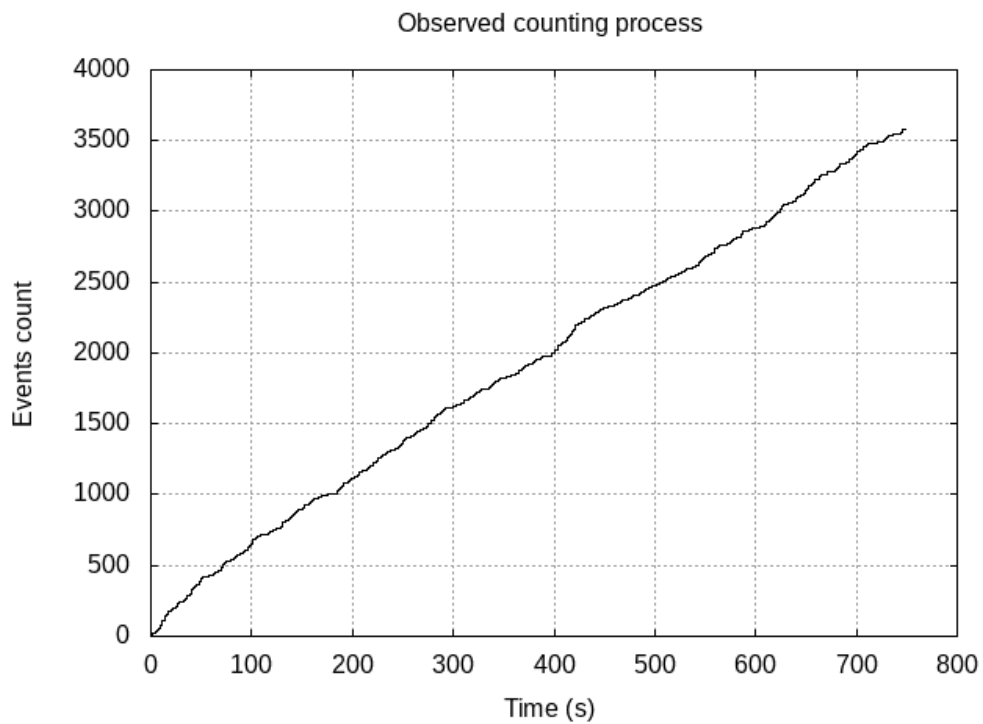
```
1 aspa_read_spike_train --inter_trial_interval=30 --trial_duration=29 < \
2 locust20010214_C3H_1_tetB_u1.txt | tee >(aspa_mst_plot --what=raster) | \
3 aspa_mst_fns
```

4.3.3 Counting process plot

There are several ways to create a "counting process" plot. The first one, used mainly for checking data stationarity is building the "true" observed counting process plot, that is at each spike time the step function jumps by one unit and successive trials are shown one after the other as they *actually* occurred. This is what is specified with argument `cp_rt` to option `what`:

```
1 aspa_read_spike_train --inter_trial_interval=30 --trial_duration=29 \
2 --stim_onset=10 --stim_offset=11 < \
3 locust20010214_C3H_1_tetB_u1.txt | \
4 aspa_mst_plot --what=cp_rt
```

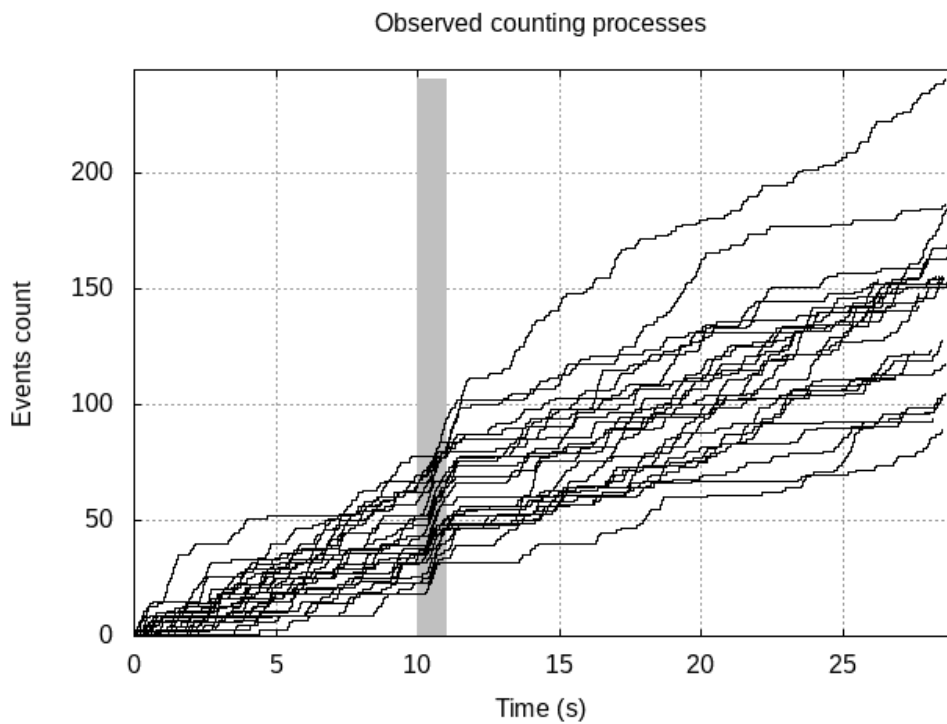
Giving a plot looking like:



We might also want to look at the individual observed counting processes after realigning them on the stimulus onset. This is obtained with argument `cp_wt` to option `what`:

```
1 aspa_read_spike_train --inter_trial_interval=30 --trial_duration=29 < \  
2 locust20010214_C3H_1_tetB_u1.txt | \  
3 aspa_mst_plot --what=cp_wt
```

resulting in a plot looking like:



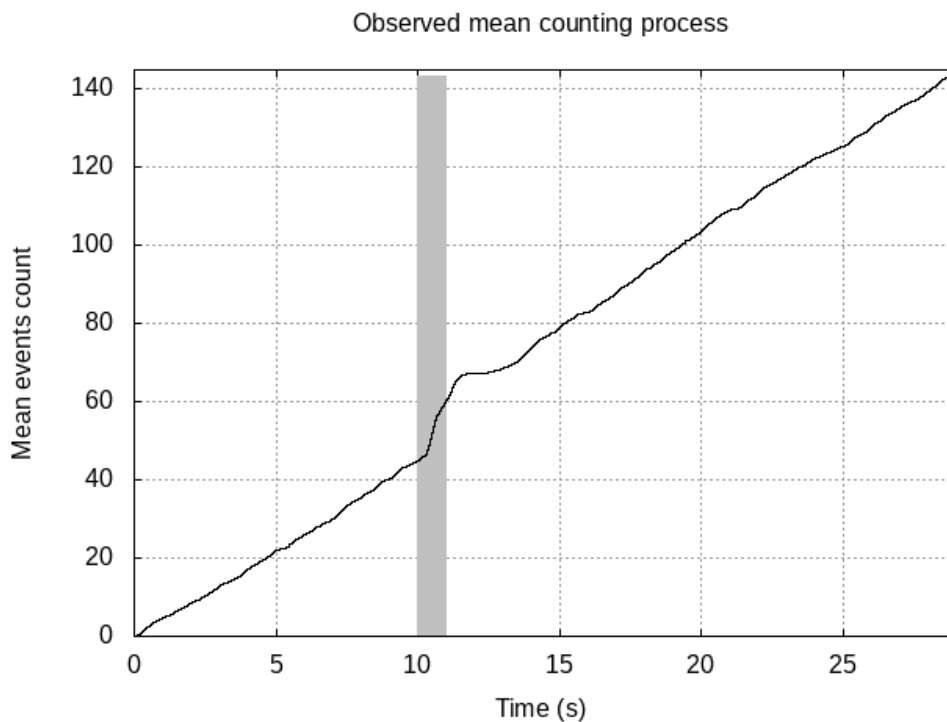
We can also decide that to see if there is a response or not, we can construct the average step function. That is, we replace the step size in the previous plot by $1/N$ (N is the number of trials) and we sum all these resulting step functions. This is done with argument `cp_norm` to option `what`:

```

1 aspa_read_spike_train --inter_trial_interval=30 --trial_duration=29 \
2 --stim_onset=10 --stim_offset=11 < \
3 locust20010214_C3H_1_tetB_u1.txt | \
4 aspa_mst_plot --what=cp_norm

```

resulting in a plot looking like:



One might want to prepare a more sophisticated plot for, say, a publication showing both the individual observed counting processes and their average. This could be done as follows, by creating first some files with the "properly" formatted data:

```

1 aspa_read_spike_train --inter_trial_interval=30 --trial_duration=29 \
2 --stim_onset=10 --stim_offset=11 < \
3 locust20010214_C3H_1_tetB_u1.txt > \
4 locust20010214_C3H_1_tetB_u1.aspa
5
6 aspa_mst_plot --what=cp_norm --text < \
7 locust20010214_C3H_1_tetB_u1.aspa > \
8 locust20010214_C3H_1_tetB_u1.cp_norm
9
10 aspa_mst_plot --what=cp_wt --text < \
11 locust20010214_C3H_1_tetB_u1.aspa > \
12 locust20010214_C3H_1_tetB_u1.cp_wt

```

Then within `gnuplot`:

```

1 set grid
2 unset key
3 set xlabel 'Time(s)'
4 set title 'Observed counting processes'
5 set ylabel 'Events count'
6 plot [0:29] [0:245] 'locust20010214_C3H_1_tetB_u1.cp_wt' \
7     index 0 using 1:2 with filledcurve closed lc 'grey', \
8     '' index 1:25 using 1:2 with steps lc 'black', \

```

```
9 'locust20010214_C3H_1_tetB_u1.cp_norm' index 1 \  
10 using 1:2 with steps lc 'red' linewidth 2
```

