PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE

INSTITUTE FOR BIOLOGICAL AND MEDICAL ENGINEERING

TECHNICAL REPORT

Spikes & Nets (S&N): Documentation

Authors

Carlos Valle-Araya
María Rodríguez-Fernández
Antonio Eblen-Zajjur

Contents

Contents

List of Figures

List of Tables

1 Starting

Spikes and Nets (S&N v1.2) presents an easy to use GUI (Graphical User Interface) and friendly environment for data analysis. It was developed in Matlab 2017a and uses its compiler for a standalone version. Given that Matlab is a multi-platform software, S&N can be used in almost any machine running: **Windows**, **MacOS** or **Linux** for the compiled or the code versions which offer the same functions.

1.0.1 System requirements

1. Matlab Code version: S&N requires at least Matlab 2016b to work in a Windows, MacOS, or Linux computer.

2. Stand-Alone software: S&N requires of Matlab Runtime 2017a (9.2) in a Window, MacOS, or Linux computer.

3. S&N Suggested Hardware: quad core CPU or better, 8GB RAM or more, 7.5 Krpm disk drive or solid state disk drive.

Since S&N is based in Matlab 2017a, the computer must full fill the requirements of Matlab 2017a, exposed in *https://www.mathworks.com/support/sysreq.html* for the operating systems previously mentioned.

1.0.2 Additional software for S&N

Spikes and Nets utilize the software **ghostscript** http://www.ghostscript.com version 9.21 to compile the .pdf files generated by the methods of analysis. Ghostscript becomes essential to take advantage of the fully potencial of S&N.

1.0.3 Making use of code version

The use of the code version is straight forward. When downloading S&N you will get 3 elements: S_and_N.fig ,S_and_N.m and a folder with the name "Functions". These 3 elements must always be on the same folder for the correct working. To run S&N the file S_and_N.m need to be open in Matlab, this can be archived double clicking the file or using the file explorer on Matlab. Finally, click the Run button as show in **Figure 1.1** or press F5 key to open the software.
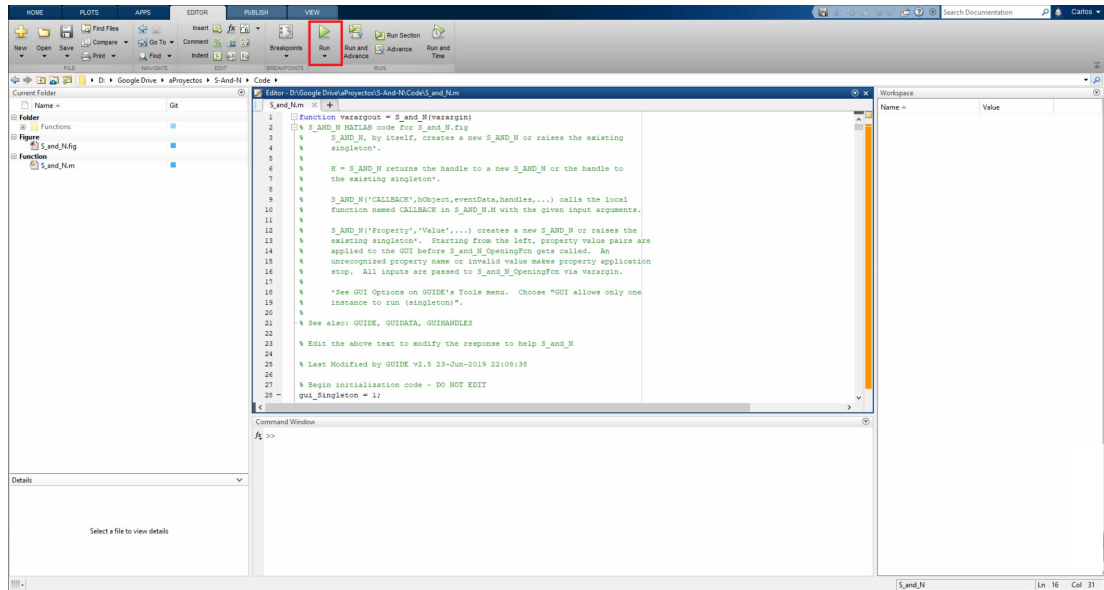
Figure 1.1: **Run button**.The run button will open the GUI.

Do not close Matlab meanwhile you are using S&N (code version), otherwise, the toolbox will close. If you are a new user of Matlab, we strongly recommend the use of the S&N compiled version. Now you are ready to start the analysis.

1.0.4 Making use of standalone software

To use the standalone software, it is necessary to install *"Matlab runtime"*, freely available at: `https://es.mathworks.com/products/compiler/Matlab-runtime.html` for Windows, MacOS and Linux platforms. In this versions list please select the 2017a (9.2) version. Matlab provides the corresponding documentation for the installation process.

After the runtime module installation, you must open the file S&N.exe (Windows) or the S&N application (MacOS and Linux) to initialize the GUI.

1.1 Setting up S&N

After the welcome window,the software presents 4 tabs: **Files selection**, **Groups generation**, **Run and parameters modification** and **Analysis progress** . When you run the software, the Files selection will be the first tab displayed (**Figure 1.2**).

Figure 1.2: **Methods and files selection**

1.1.1 First tab: Files selection

On this tab you will be able to select all the files needed for your analysis. To select them, you must click on the button "Select Files", in that way S&N will get the location but will not load them to the software until they are needed. The result of this process can be seen in **Figure 1.3**.

1. S&N offers 2 types analysis focus on the number of neurons:Discharge Patterns (DP) and Connectivity [Cross-correlation (CC) and Joint Interspike Scatter Diagram (JISD)] methods, that require one or several single neuronal files (SN), two or three simultaneously recorded neuronal files (CC and JISD respectively). You can use any combination of this 3 methods. Additionally, a time-segmentation of the neuronal files is also available to all methods to detect time-depended changes. DP analysis allows 9 different methods i.e., Raster single or multi-neuronal plot (RP), Interspike Intervals (ISI) statistics, Interspike Intervals histogram (ISIH), Auto-correlation (AC), Auto- spectrum (AS), Poincaré (PO), Phases (PA), Fractal (FA) and multiparametric Burst analysis (BA) thus, together with CC, JISD and time-dependency, offer a total of 11 methods. All or any combination of these analyses can be selected to be performed on every neuron file (tab 3).

2. To select the files, press the button *Select Files* and choose the files. Since the files are load to the software when they are needed, S&N can handle nominally an unlimited number of files. S&N use the standard .txt or .csv file format **Table 1.1** for the neuronal spikes occurrence times (please see below for more details). The

names of the available neuronal files will appear in the window below the button, as shown in **Figure 1.3**

3. Figure reader: This option allows you to load and displays saved .fig files from previous analyzes, including those generated by CC and JISD method, allowing to use all Matlab 3D graphics functions (zoom in, zoom out, rotate, data cursor, file format conversion, and many others).

The Home button will be available for the first 3 tabs, allowing to return to the home tab. The Help button is available only in the home tab and will provide the link to *http://marodriguezf.sitios.ing.uc.cl/S&N.html* where you can download all the information about S&N as well as the software itself, updates and contact information.
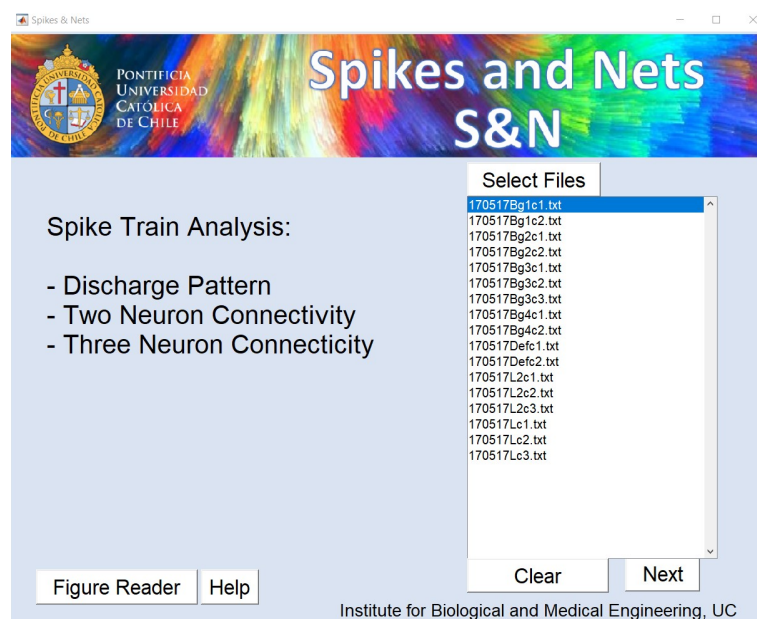
Figure 1.3: **Files selected** . It's important to remark that S&N, at this step, do not load the selected neuronal files. It just saves the name and directory into an operational file for later load depending on selected file and methods.

1.1.2 Second Tab: Groups generation

This tab is particularly useful to perform analysis from different experimental epochs allowing specific interpretations. In this window, you will be able to select which files do you want to analyze as a group. Due to that, some methods require a minimum number of files, this step is crucial to determinate the desired pair of file combinations. Figure 1.4 represents the second tab of groups generation.

Table 1.1: .

The neuronal files are a single column spike occurrence times recorded from a specific cell and obtained after the spike sorting method (see Eblen-Zajjur (1995) for a concise review of the widely used method). The time values can be expressed in milliseconds, seconds, minutes or days. The data time units can be specified in tab 3, default time format is milliseconds. In the example (Table 1.1) the first value is interpreted such as the first neuronal spike occurs at 4707.59 milliseconds from the beginning of the experimental recording time and so on. Note that every down list value should be greater than the previous value. The flexibility of S&N to use different time units allows its use on non-neuronal point processes data with long time scales in minutes, hours and even days scales.

4707.5918
10341.9092
21069.1855
28333.1348
35573.0078
35575.6953
$\vdots$
79391.5703
89393.668
85822.957
99557.1445
99559.25
109561.5937
12103.7734
41106.668
5444935.3828
9090162.6953
102030920.9687
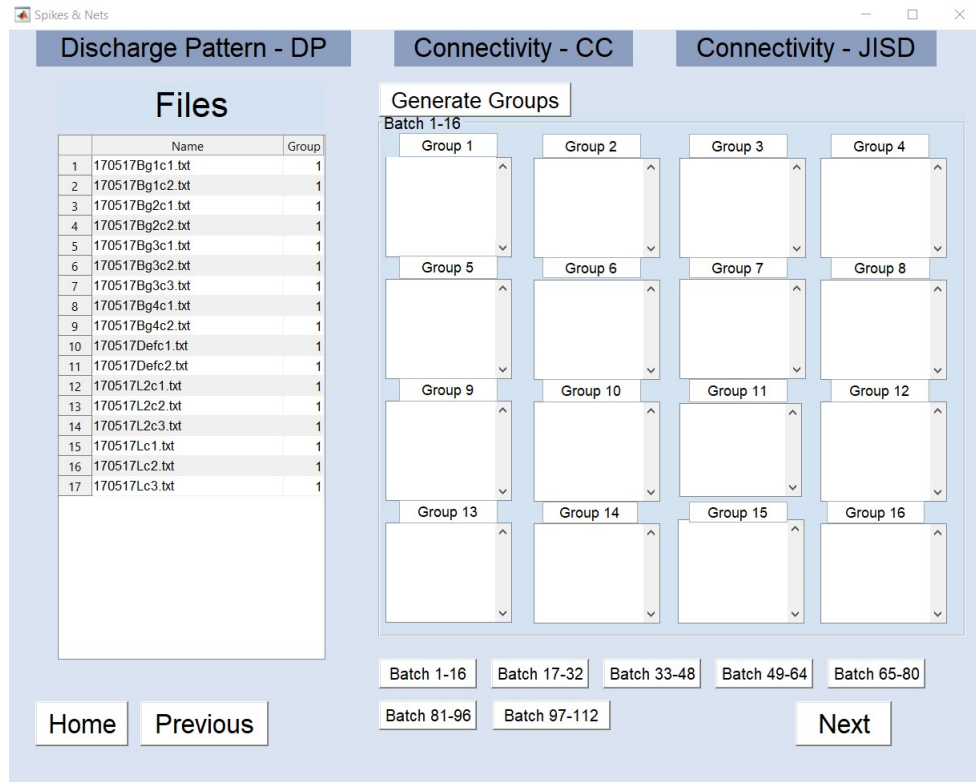
Figure 1.4: **Second tab.**

This tab is divided into 3 principal parts: selected methods, files and generated groups (**Figure 1.5**)

1. **Selected Methods:** This tab shows which analysis methods will be done as well with the number of total analysis for single, double and triple neuron methods.

2. **Files:** This window displays the selected neuronal files for any of the 3 analysis methods. It contains two columns, the first one contains the names of all selected neuronal files which at this stage are not modifiable. The second column contains the "file group" which can be modified. By default, all neuronal files are assigned to group 1 i.e., they are part of the same batch of files whose results will be saved in the same folder. It can be defined up to 112 different groups. For the creation of groups you must modify the second column with the number of the group (1 to 112) for each file. For example, if you have 5 files: "neuronA", "neuronB" "neuronC", "neuronD" and "neuronE", and you give the number 1 to the first 3 files and number 2 to the rest. then two groups will be created, the first one composed by: "neuronA, neuronB and neuronC" and the second one by: "neuronD and neuronE". It is possible to create any combination of groups e.g., all files in just one group, all files in different groups, 4 files in one group and 1 alone, etc. It is also possible to not
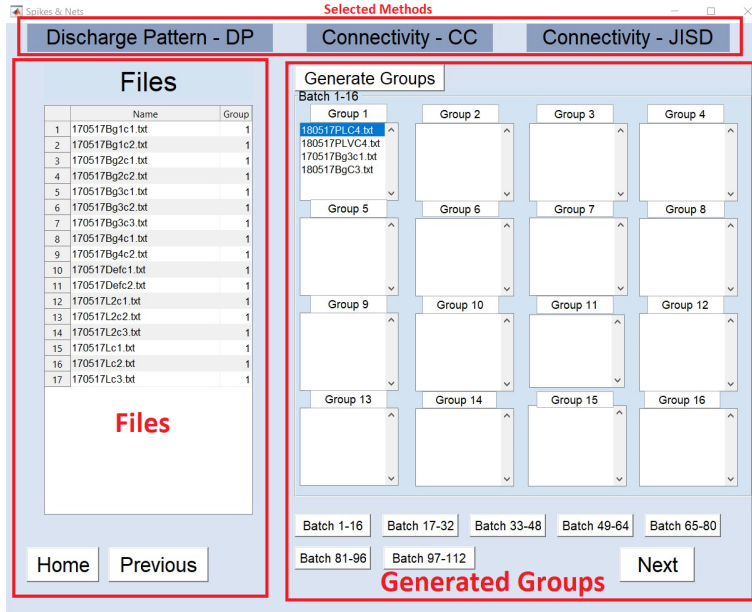
Figure 1.5: **Parts of the second tab**.

include some file, in this case, you can put to the group number 0. A real example is presented in Figure 1.6.

Group assignation of neuronal files has an impact in the CC and JISD analysis. These methods need at least two or three neuronal files respectively. CC analysis of 2 selected files will generate just one result file, for 3 files it will generate 3 result files, and for 4 selected files it will generate 6 result files. S&N generates automatically **all possible combinations from the selected neuronal files belonging to the same group**. This also applies to JISD analysis..

For CC, the number of possible combinations is given by:

$$C_2^N = \frac{N!}{2!(N-2)!} \tag{1.1}$$

and for JISD, the number of possible combinations is given by:

$$C_3^N = \frac{N!}{3!(N-3)!} \tag{1.2}$$

Where N is the total number of neuronal files belonging to the group. As shown in Table 1.2, the number of result files increases a lot, although S&N can handle any number of combinations, is important to consider this for calculation times.

Figure 1.6: **Assign group number to file** .The first 4 files are not assigned to any group, meanwhile the rest is in groups from 1 to 5. To apply changes is necessary to press "Generate Groups".

Table 1.2: **Number of possible combinations for CC and JISD**.

N of files	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
CC	-	1	3	6	10	15	21	28	36	45	55	66	78	91	105
JISD	-	-	1	4	10	20	35	56	84	120	165	220	286	364	455

3. **Groups generated:** in this tab you will be able to see the generated groups. As previously mentioned, you have to assign a group number to a neuronal file and then press the button "generate group", the group number should be between 1 and 112. Figure 1.6 shows the distribution of some neuronal files and Figure 1.7 shows the result after the group generation process. Each box represents a group whose files will be saved in the same folder with the name show in the box above each group. Each box has a default group name at its upper banner, i.e., "Group N" which can be changed at will. There are seven buttons below panel boxes e.g., "Batch 1-16", "Batch 17-32", "Batch 33-48", "Batch 49-64", "Batch 65-80", "Batch 81-96" and "Batch 97-112", any of this buttons represent a group of 16 boxes that can be used to make a group, i.e., "Batch 1-16" means the first 16 groups, "Batch 17-32" the second 16 groups and so on.

All 112 boxes are not always needed. If a box does not have any neuronal files, then no folder will be generated. In a similar way if a box (or group) do not meet the file requirements for CC and/or JISD, i.e., two and three files respectively, S&N will generate a folder with only one pdf file containing the names of the files used.

Finally, if the name of the box is empty then it will generate a folder called "No name" saving the result files of that box in that folder. The same process will occur for all empty boxes.
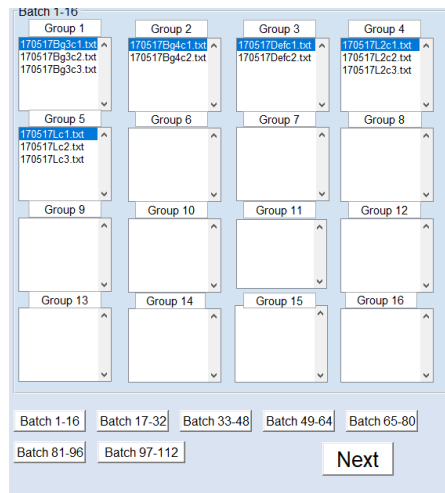
Figure 1.7: **Distribution of groups of the Figure 1.6**

Once the group distribution is ready, the "next" click should be pressed to continue to the third tab.

1.1.3 Third tab: run and parameters modification

In the **Figure 1.8** run and parameters modification window is shown.

1. Parameters modification: S&N provides a way to modify the default parameters for every analysis method. For Discharge Pattern methods RP, ISI, AC, AS, PO, PA, FA, and BA can be selected in any possible combination.
   Raster plot (RP) for up to 3 simultaneous neuronal files is also available after checking it. If the single raster plot is selected, then S&N will generate its graphic results in the folders of the single neuron analysis. For two or three neuronal files the resulting graphs will be saved in the folder of CC and JISD, respectively.

   The segmentation option offers an interesting file segmentation for all analysis methods by dividing every neuronal file into a user-defined number of sequential files with the same time length. This is a relevant option if time-related changes are suspected to take place in the spike train, i.e., if a spike train file has 6 minutes of recording time, the segmentation number with a 4 will split it into 4 sequential files with the same time duration each (in this case, 90 seconds each). Time-related changes can be detected by differences (evaluated with several methods) on one or more segmented results. It is important to remark that file segmentation is based

9
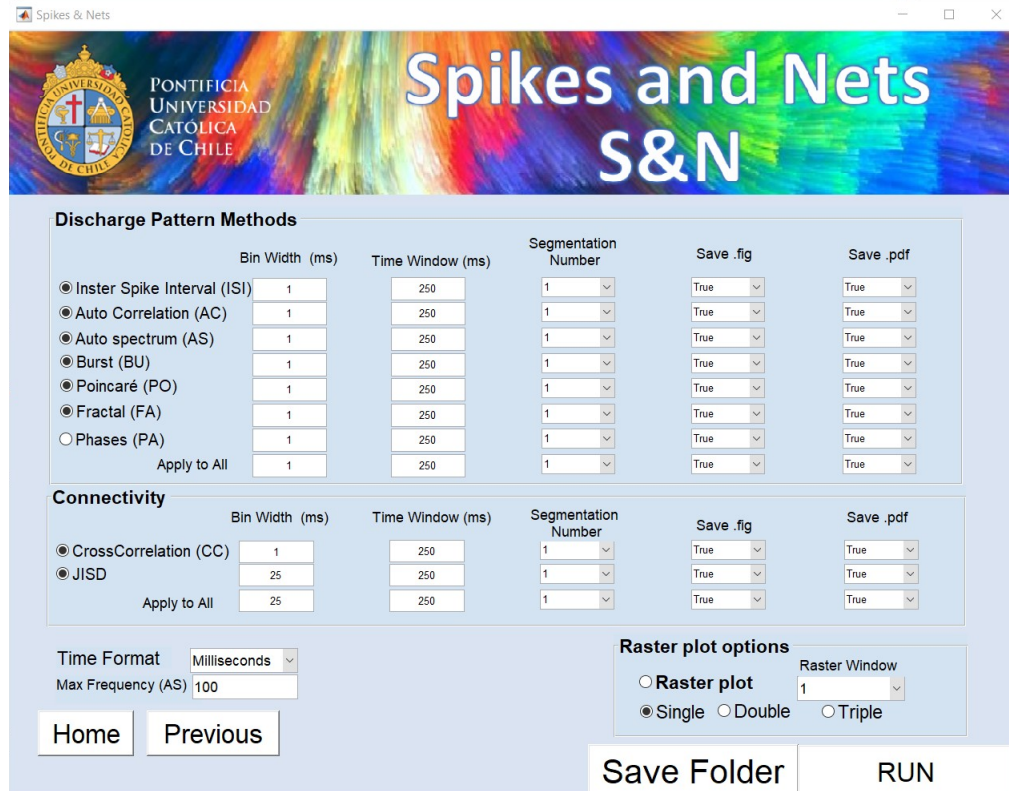
Figure 1.8: **Parameters modification and run.**

on the recording time and not on time events. A realistic example is shown in **Figure 1.9**

For each method is possible to save or not the .fig and .pdf files, set the segmentation number , time window and bin width. Time format must be the same for all the files used.

2. Run: This button starts the analysis.

1.1.4 Fourth tab: Analysis progress

On Run button click the window will change to the Analysis process monitoring the progress of the analysis according to the previously selected options and showing real-time updates about already processed file and folder name as shown in **Figure 1.10**.

It is important to be noted that both processed folders and files are displayed by nfo and nfi which represents the current number of folder and file respectively and NFO and NFI represent the total number of them. The nfi and NFI, do not represent the total number
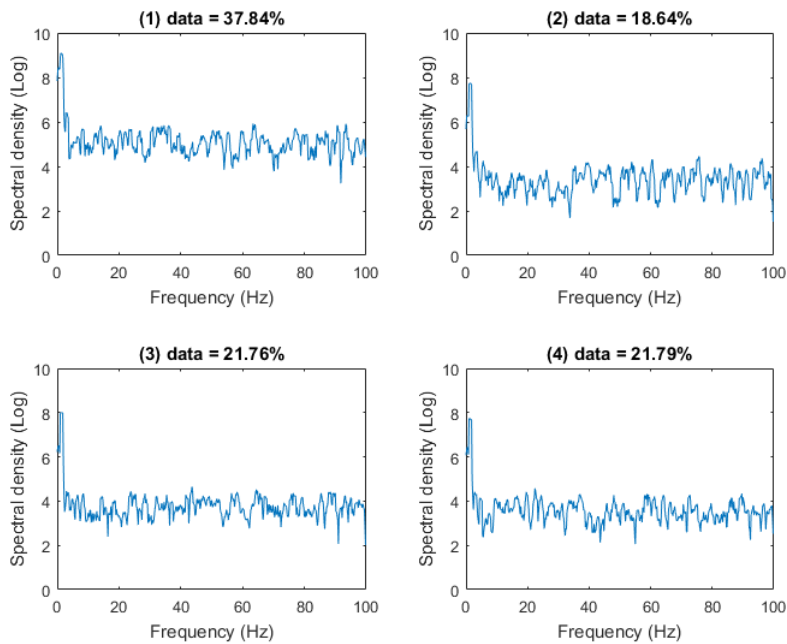
Figure 1.9: **Example in spectral analysis (AS) with a segmentation number of 4.** Note that the data is not event but recording time equally segmented. The above percentage indicates the amount of spikes in relation to the total, in that time window.

of files in all folder, but the number of files in that specific folder, NFI number may differ from folder to folder.

Once all files are processed, the "Finished" box will appear and the program will reinitialize to the first tab.

S&N can make use of parallel computing capabilities offered by Matlab, allowing the use of all the processor cores of the Machine. When using the parallel computing toolbox, it is not possible to show a specific file and folder name being processed, because, while using this toolbox S&N is processing multiple files at the same time, in this case, the message "File viewer is disabled using Parallel Computing" will be shown in the file viewer box.
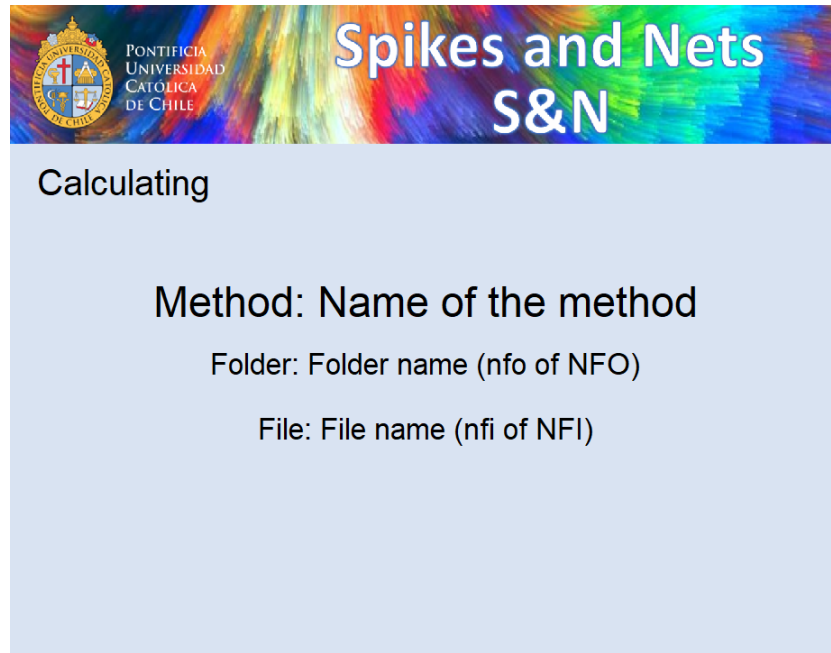
Figure 1.10: **Format of the fourth tab.**

1.2 S&N output files

S&N generates a multiple sets of output files for every selected analysis method, presenting them according to the previously defined groups. In a more global context the toolbox S&N generates: Data files (.txt), Matlab Figures (.fig) and reports (.pdf) which are included into new folders for each defined group.

Due to the large number of methods and files generated, S&N uses abbreviations for all of their results (Table 1.2).

1.2.1 Data files and Matlab figures

S&N build names for results files consisting on three parts: Name, method of analysis and file format, with the form

$$“file\_name” + “Method of analysis” + “.file\_format”$$

i.e., a neuronal file called "Cell1.txt" analyzed for AC method will generate 2 files: "Cell1AC.txt" and "cell1AC.fig". The first file contains the raw data of the plot (which can be read by any statistical program for post-processing purposes) and the second one, corresponds to the Matlab figure (which can open using the "figure reader button" in the first window).

Table 1.3: **Abbreviations for all the analysis methods use in S&N**

Method Name	abbreviations
Raster plot	RP
Auto-correlation	AC
Auto-Spectral	AS
Inter spike interval	ISI
Poincaré	PO
Phase	PA
Fractal	FA
Burst	BU
Cross-correlation	CC
Joint Interspike Scatter Diagram	JISD

All S&N analysis methods can generate a data and a fig file, except for Burst Analysis (BA) and Raster plot (RP) that only generate a figure file.

Data files (.txt)

Some considerations should be taken into account to use the S&N result data file in another software.

1. Auto-Correlation: To plot the "name_AC.txt" files, is necessary a histogram plot of the data.

2. Auto-Spectral analysis: S&N uses an average moving average filter with a size of 5. The data saved in "name_AS.txt" is not filtered. To obtain the same result in another software, you must first filter the data.

3. Inter spike interval: To plot the "name_IS.txt" files, is necessary a histogram plot of the data.

4. Poincaré and Phase: The way in which the data is saved in the "name_PO.txt" and "name_PHA.txt" is exactly the same, the difference lies in how each method is plotted. These files contain 2 vectors: one for X positions and the other for Y positions. These vector shape a map of points. For Poincaré one point must be plotted and a line generated from that point to the second one, then a line from the second one to the third one and so on. For Phases just to a scatter plot with the positions (X,Y).

5. Fractal: To plot the "name_FA.txt" files, is necessary a normal plot of the data.

6. Burst: This method only generated a Matlab figure file that can be open using the "figure reader button" in the first window of S&N.

7. Cross-Correlation: To plot the "name_CC.txt" files, is necessary a histogram plot of the data.

8. JISD: This method generated 3D plots. To plot the "name_JISD.txt" files you must do a 3D histogram plot. This data file contains 2 vectors: one for X positions and the other for Y position.

Matlab figure files (.fig)

All the figures created by S&N are in invisible mode. You can open this figures using the "figure button reader" in the first button or with the following code on Matlab:

```
openfig('MySavedPlot.fig','visible').
```

Matlab provides a set of tools (Zoom in, Zoom out, pan, rotate, etc) that can be used (even with the compile version) that will be discussed later.

1.2.2 Segmentation

As previously mentioned segmentation's divide the data into parts of equal time length. S&N can divide the data up to 9 segments for any of the methods mentioned before. All of this new fractions are an independent figure or plot, that have their own data files and figures.

If segmentation number (SS) is 1 it will generate: "Name_Method.txt" and "Name_Method.fig", but if $SS > 1$ it will create a new set of data files equal to the SS and a new figure that contains the SS plots. The data files will be saved in the format presented in Table 1.4, that can be used for any method.

For figures files, the software generates just one figure that incorporates the SS plots. The format of the name includes a label "time" for the figure of the segmented parts. For example, as shown in Table 1.4, it will also generate a fig file with the name "Cell_AS_time.fig" indicating that this figure has the segmentation plots.

1.2.3 Reports (.pdf)

In a single group of files, there can be a huge number of data files that need to be processed in the methods previously mentioned. To make it easier, S&N generates two .pdf files: **Report files** and **Result files.** .

Table 1.4: .
In this case, a file with spike train data called "Cell.txt" was processed for Auto-Spectral analysis. The first file corresponds to the file without segmentation. The following ones correspond to the segmentation. This files contains the data for SS plots and are labeled with a number that goes up to SS. The Last one correspond to the Matlab figure file that have all of the plots on the same figure.

Cell_AS.txt
Cell_AS**1**.txt
Cell_AS**2**.txt
⋮
Cell_AS**SS**.txt
Cell_AS_time.fig

1. **Report files**: This file contains the name of all the spike train data used in a particular group. When is used for single neuron analysis it just generated a list with the spike train data utilized in the analysis. On the other hand, when is used for CC or JISD it contains all the combinations of two and three elements respectively.

2. **Result files**: Due to the large number of figure files (especially in methods like CC or JISD), S&N append all of this files into this pdf file, with a great image quality.

The format name for this two .pdf files varies between the principal methods: For single neuron analysis is "Report Files Single_*FolderName*" and "Result Files Single_*FolderName*", for Cross-correlation is "Report Files CC_*FolderName*" and "Result Files CC_*FolderName*", for JISD is "Report Files JISD_*FolderName*" and "Result Files JISD_*FolderName*".

Where *FolderName* correspond to assign name of the second window, for any group. By default, the Folder name is given by "Group *C*", where C is a number between 1 and 112.

1.2.4 Folder

Each group of files is saved in a different folder with their respective Folder Name. The files that where process, will have beginning label "DP" ,"CC" or "JISD" , follow by the folder name, i.e "DP*FolderName*" , "CC*FolderName*" or "JISD*FolderName*". This will be apply to each group of files.

Method	Counts of Calculation	Neuronal Files	Count of ISIs	Parallel computing time (sec)	No Parallel computing time(sec)
Discharge Pattern	300	300	5000	375	2242
Cross-Correlation	300	25	5000	86	370
JISD	286	13	5000	301	2000

Table 1.5: S&N processing runtimes for neuronal files with 5000 spikes each, time segmented in 4, for the discharge pattern analysis, i.e., Raster Plot, Interspike Intervals, Multiparametric Burst, Auto-Correlation, Auto-Spectral, Fractal, Poincaré, Phase plots, and cross-correlation and joint impulse scatter diagram for all possible neuronal combinations. Runtimes include the generation of the pdf report and all vector figures.

1.3 Computational Time

S&N computational time depends mostly on the number of neuronal files, the methods used, and whether the parallel computing toolbox is used. The toolbox allow to S&N to completely use all the cores of the machine, speeding the process linearly as more cores the machine have. The parallel computer capabilities are available for the 3 methods, Discharge Patterns, Connectivity and JISD analysis, and the software will automatically use it always when the toolbox is present in the machine or with the use of the standalone version of S&N. All the cores will be used for the analyzes.

1.4 Data segmentation

This option divides the neuronal file data into a user-defined equal duration number of files to perform selected analysis and it aims to detect time-related discharge and/or neuronal connectivity changes in one or more of the resulting segments.

1.4.1 Data segmentation in discharge patterns

For single neuron analysis, the neuronal files are split into SS (segmentation number) parts, of the same time length, that not depend on the number of spikes of the data but in the recording times.

1.4.2 Data segmentation in Cross correlation

For CC analysis, the split of the data depends on two files. Given that the division of the data is done in relation to the time, is chosen the file with the shortest time, and then

the other file is cropped to the same time. The segmentation is now done in the same way that single neuron analysis. For example, if there are two files, the first of 6 minutes and the second one of 8 minutes, the split of the data will be done in relation to the first file (the shortest one). The 2 minutes leftovers of the second file will not be analyzed, this file will be cut to 6 minutes.

1.4.3 Data segmentation in JISD

For JISD analysis, the split of the data depends on three files. Similar to the slip of the data in CC, the file with the shortest recorded time is chosen and the other two are cropped to that time. The segmentation is now done in the same way that single neuron analysis. For example, with three files, the first one of 8 minutes, the second one of 9 minutes and the third one of 7 minutes, the split of the data will be done in relation to the third file (the shortest one). The 1 minute and 2 minutes leftovers of the first and the second file respectively, will not take part in the analysis, and both will be cropped to the time length of the third one.

1.5 Setting up a Problem

1.5.1 Step 1: Selecting code or stand-alone application

Both S&N versions (code or compiled) have the same features. The first one makes use of Matlab 2017a software and the second one depends on the freely available runtime application provides for MathWorks. For advances Matlab users, the code version allows modifying the code to add new features, change some saving options, make use only of the analysis functions and so on, who also may have the parallel computing toolbox.

For code version use make sure that the parallel computing toolbox is installed, otherwise, it will miss this feature of S&N. On the other hand, the compiled application has built it this toolbox.

For this example, the stand-alone application will be our choice in a Windows 10 environment.

1.5.2 Considerations in the opening the Stand-alone application

Once you download the software from `http://marodriguezf.sitios.ing.uc.cl/software.html`, you must open the "S&N.exe" file. This process takes a certain amount of time due to Matlab itself and the type of storage device (hard drive or solid state drive (SSD)). Depending on the specifications of your computer it can go up to 1 minute. Even do an SSD disk is not required, we recommend the use of it.

1.5.3 Step 2: File selection

To select files, you must click in the "Select Files" button. This will open a new window in which you must select the files that you want. The selected files must be all from the same folder and need to be selected at the same time, as shown in the Figure 1.11.
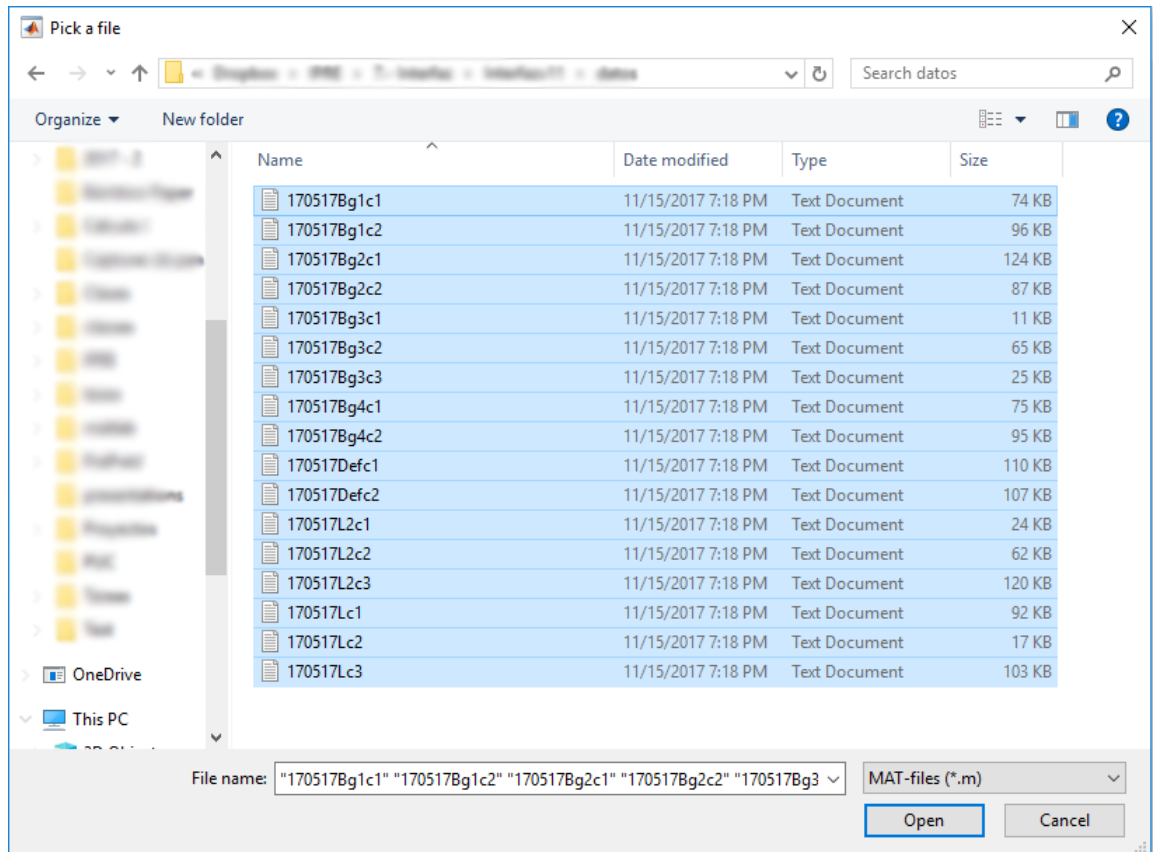
Figure 1.11: **Selection of files**.

Once you are ready, you must click in open and the files will be loaded to S&N as shown in Figure 1.12.

1.5.4 Step 3: Group generation

In the first step, all of the files have number group of 1, although the groups are not generated. To generate the group you must press the "generate group" button, this leads to a single group contented of all the files as shown in Figure 1.13.

Even do S&N can handle 17 files in the same group, for our purposes we will divide the files in groups in accordance to their name. The first six numbers correspond to the date
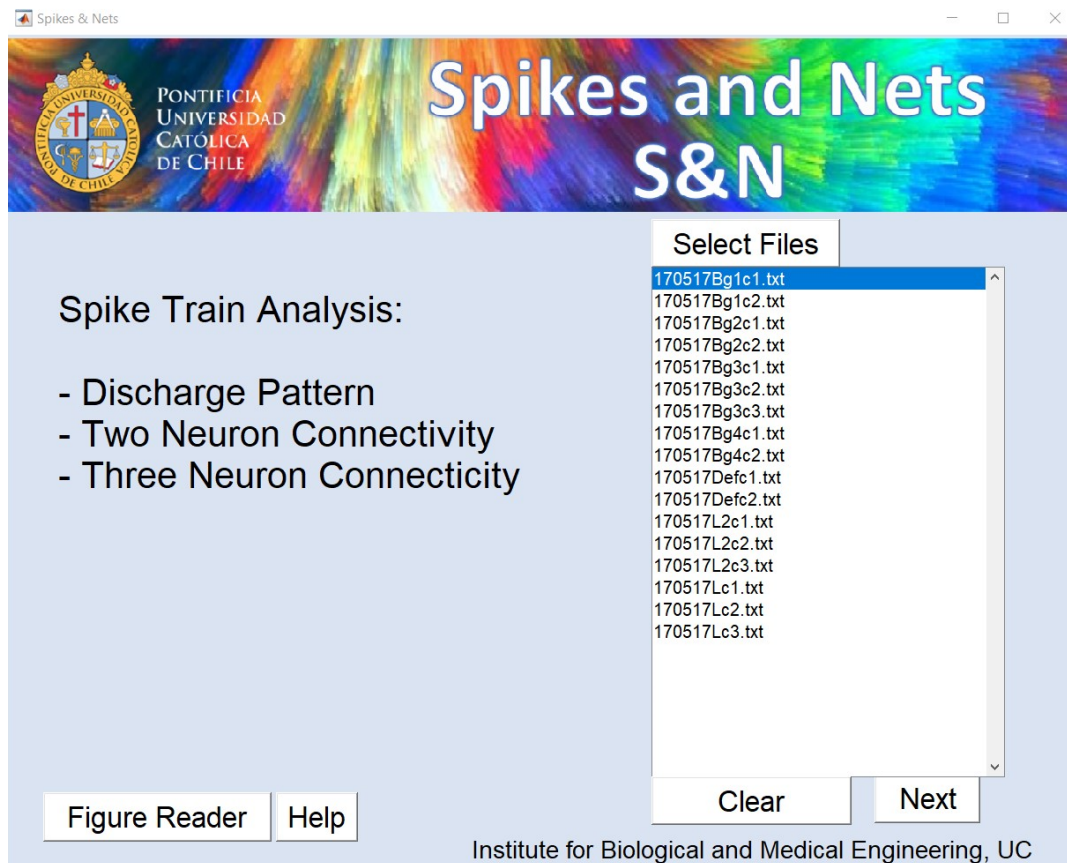
Figure 1.12: **Files load in S&N**. As previously mentioned, this software does not load the file, it saves the file name and the folder to load them up when needed.

of the experiment, while Bg, Def and L correspond to the type of experiment. Also next to the type of experiment you will find the number of the experiment plus the number of the cell. In this case, we want to divide the data into groups of the same type of experiments and the same number of experiment and to make the result easier to read, every group has an identification name. Above mentioned lead to the Figure 1.14, in which is possible to observe that there are 7 groups with their respective files.

1.5.5 Step 4: Parameters modification and run

For this files, I will select all possible methods of analysis provides by S&N include Phase and raster plot. To get a better view of the evolution of the spike trains in relation to time I will select a segmentation number 9, i.e. it will divide the data by time in 9 parts. This configuration is presented in Figure 1.15. The next step is to select the folder in which you want your result files to be saved. For this example, I saved the files in a folder called "results".
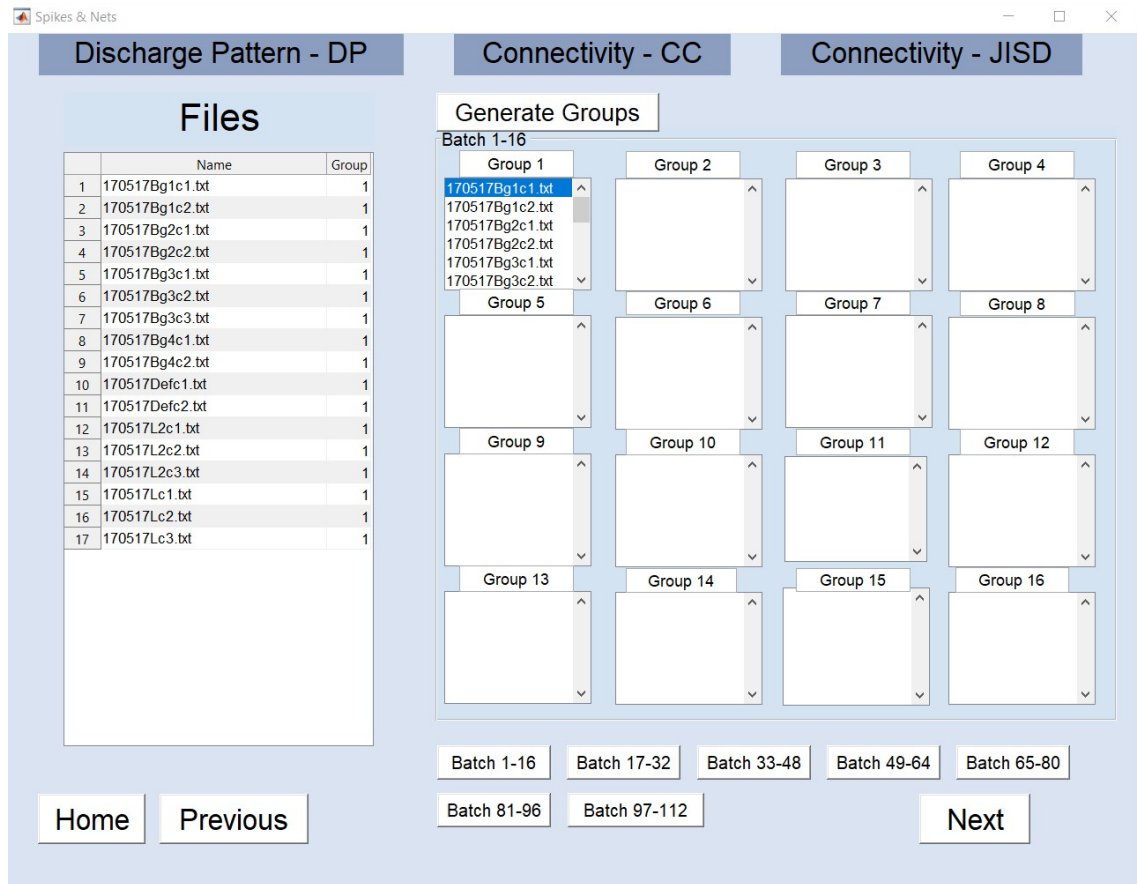
Figure 1.13: **Groups generated**.All files were assign to the first group called "group 1"

When you are ready, next step is to press run. When the processing ends you will have in your results folder, 21 new folders for the 3 principal methods, as shown in Figure 1.16.
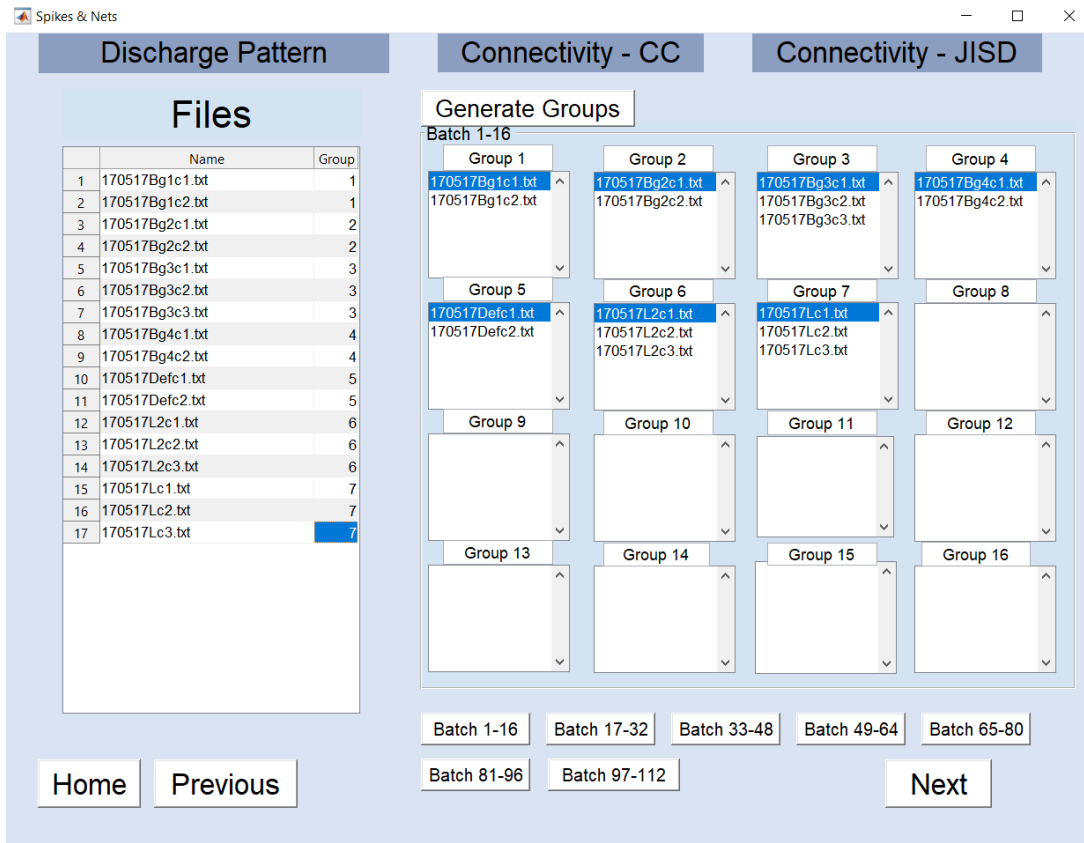
Figure 1.14: **New groups generated**.There are 7 groups with their respective name with up to 3 files per group.
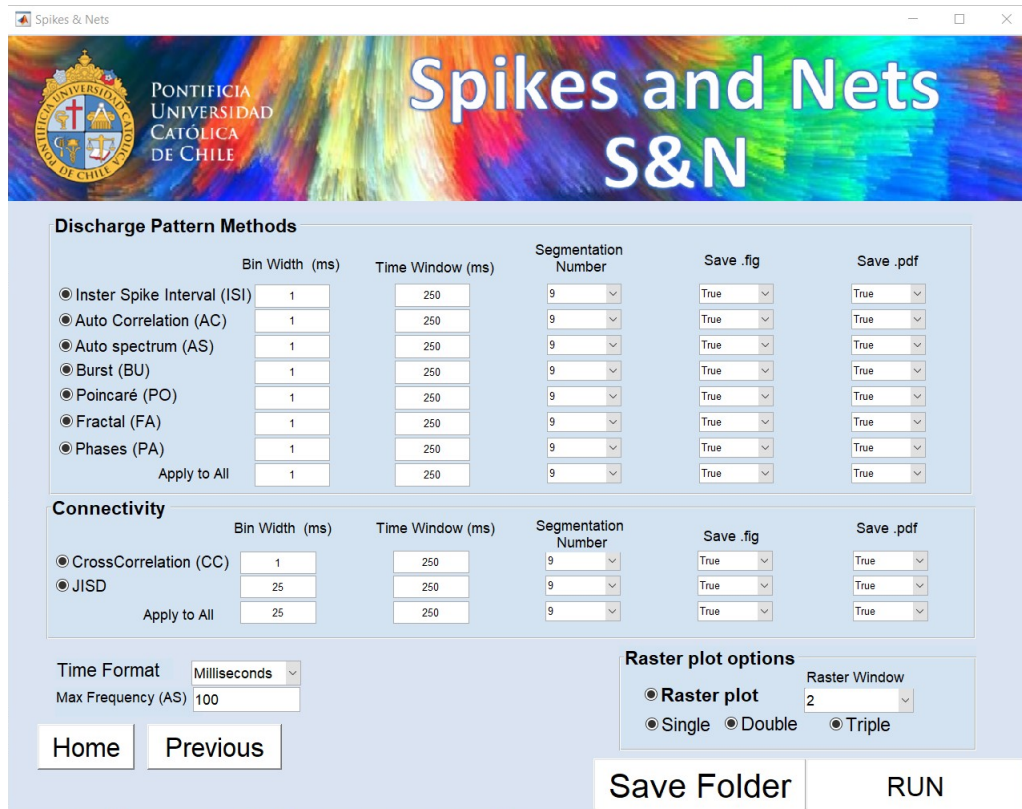
Figure 1.15: **Configuration of parameters**.For this example all methods will be selected

Figure 1.16: **Results Folder.**

2 Methodology

When a neuron receives a supra threshold stimulus a series of action potentials (spikes) is generated. The spike shape does not show significant changes thus the main information is carried by the spike time points when they occur and its sequence. S&N is able to process multiple spike trains (tested up to 100) automatically for 9 different analyses and to detect time-related changes through the file segmentation option in both discharge pattern but also for neuronal connectivity.

2.1 Discharge Pattern Analysis

This is a set of methods aimed to analyze neuronal discharge patterns from different points of view. It integrate 7 different methods e.g., **Raster single and multi neuronal plot Inter Spike Interval**, **Auto-correlation**, **Auto-Spectral**, **Poincaré / Phases**, **Fractal** and **Burst** analysis. Additionally, the segmentation option allows to detect time-related changes in the discharge patterns. The mathematical definitions implemented in S&N have been previously described in detail by

1. **Spike train**: Neuronal discharges, i.e. series of action potentials are represented by

$$f(t) = \sum_{k=1}^{N} \delta(t - t_k) \tag{2.1}$$

   where the occurrence time of neuronal spikes is t_k and the description of the spatial density of a physical magnitude located at that time is a Dirac delta function where $\delta(t) = 1$ if $t = 0$ else $\delta(t) = 0$.

   S&N implements first order interspike interval (ISI) calculations represented by

$$x_k^{n-1} = t_{k+1} - t_k \tag{2.2}$$

   Higher order ISI calculations which are used due to the assumptions that they are less affected by Gaussian noise and thus given better signal-to-noise ratio, are possible in S&N by modifying how ISIs are calculated in the Matlab code but not in the compiled version.

2. **Interspike Interval Histogram**: defined by

$$F(t) = \int_a^t dt = P(T \leq t) \tag{2.3}$$

estimated by:

$$F_j = \sum_{k=1}^{n} f_k \tag{2.4}$$

This method analyzes analytic and graphically the ISI distribution of the spike train independently of the ISI sequence using a 1 ms bin width. ISI distribution is a relevant tool for neuronal discharge pattern characterization and recognition. It can also detects temporal coding e.g., discharge frequency (applying $f = \frac{1}{T}$), kind of distribution (Gaussian or exponential using alpha value from gamma distributions), as well as other mathematical characterizations of the ISI.

3. **Auto-correlation function**: is defined by

$$h(t) = \sum_{k=1}^{n} f_k(t) \tag{2.5}$$

This method estimates the probability of a neuron to discharge again a spike as a function of time after a previous spike and correspond to a renewal density function, showing high sensitivity to detect discharge rhythms as well as long-term neuronal discharge modulation due to n order ISI calculations.

4. **Auto-Spectral Analysis**: defined by

$$I_{f_i} = \frac{n}{2}(a_i^2 + b_i^2) \tag{2.6}$$

$$a_i = \frac{2}{n} \sum_{k=1}^{n} Z_k \cos(2\pi f_i k) \tag{2.7}$$

$$b_i = \frac{2}{n} \sum_{k=1}^{n} Z_t \sin(2\pi f_i k) \tag{2.8}$$

The auto-spectral analysis uses auto-correlation (equidistant values) of the spike train to estimate the Fourier transform thus overcoming the difficulties to estimate the spectral density directly from a point process (non-equidistant values). Spectrum's are estimated with a 0.2 Hz resolution and smoothed by 5 terms moving average method. Spectral peaks significance is tested by:

- **Fisher kappa test for significant Auto-Spectral peaks**:

$$\kappa = \frac{maxI_{fi}}{\sum_{j=i}^{n} I_{fj}} \tag{2.9}$$

- **A standard deviation over the mean as a detector cut off point:**

$$cut = \bar{X} + \sigma \tag{2.10}$$

5. **Burst analysis**: Three cut-off time criteria denoted as burst time windows (BTW) for burst detection were used, the intrinsic value from the spike train i.e., the ISI mean and two often used values 10 ms and 50 ms, ISIs greater than these values were considered as inter-burst ISI (long) and all the small ones as intra-burst ISI (short) . These three BTW offer the possibility to select the one that fix most like to the neuronal discharge frequency but also to the intra- and inter-burst frequencies. The burst ratio was estimated by:

$$BurstRatio = \frac{nshortISI}{nlongISI} \tag{2.11}$$

where n is the number of corresponding ISI. Burst intensity is calculated by the mean value of intra-burst (short) ISIs:

$$BurstIntensity = \frac{1}{t_i} \int_{t_i} \sum_{k=1}^{n_{ti}} \delta(t - t_k)dt = \frac{n_{ti}}{t_i} \tag{2.12}$$

where t_i is the time duration of the burst (see previous definition of cut-off time criteria for burst detection) and n_{ti} is the number of intraburst spikes.

6. **Fractal analysis:** Complexity of a neuronal discharge depends on many effective synaptic inputs which in turn trigger action potentials. When the heterogeneity of effective synaptic sources is large, a more complex discharge pattern will result from processes such as integration and summation. Fractal analysis is a method to measure the complexity of the spike sequence thus, revealing novel neuronal coding strategies and responses . Fractal dimension D The spike train is represented as the natural logarithm of the duration (ms) of every ISI vs its sequential order (position within the spike train) whose fractal dimension D is calculated by:

$$D = \frac{log(n)}{log(n) + log(\frac{d}{L})} \tag{2.13}$$

where n is the number of ISI, L and d are the total length and the planar extent of the curve respectively.

7. **Poincaré Plot:** Sequential or recursive ISI patterns as a component of the complexity of the neuronal discharge can be evaluated by the Poincaré plot, by plotting cartesian x,y coordinates obtained for each ISI by:

$$x_n = (n+1) - n, y_n = (n+2) - (n+1) \tag{2.14}$$

8. **Phase Plot:** corresponds to the sequential vectorization of the Poincaré plot allowing easy visual detection of repetitive sequential discharge patterns . Both methods, i.e., Poincaré and Phase plots are able to detect recursive sequence of ISI, e.g., long- followed by a longer- followed by a shorter ISIs over the recording time, given proof for auto similarity, i.e., multiscale pattern repetition, which is a main characteristic of fractal behavior not detected by the fractal dimension calculation.

2.2 Functional neuronal connectivity analysis

1. **Cross-correlation function**: defined by

$$\zeta_{AB}(t) = \sum_{k=1}^{NA} \sum_{j=1}^{NB} \delta(t_k - t_j - t) \frac{1}{2\Delta tT} \tag{2.15}$$

Causally related neuronal discharges within a network can be detected and characterized by this method estimating the probability as a function of time to discharge of a neuron after or before the other neuron discharge. These probabilities are presented in a graph with a time lag at the x-axis with zero lag at the center and $\pm$ 500 ms time window. Excitatory or inhibitory effect strength can be estimated by the ratio between peak or through value against the mean value of the background.

2. **Joint Impulse Scatter Diagram**: defined by

$$x = \frac{(tC_i - tA_j) - (tB_k - tC_i)}{\sqrt{3}}, y = tB_k - tA_j \tag{2.16}$$

This connectivity analysis method based on cross-correlation allows to evaluate 3 simultaneously recorded spike trains by estimation of time occurrence differences and plotting them on a plane with three axes each one set at 120 degrees from the others i.e., one for every neuronal pair interaction. Excitatory or inhibitory effects and strength are expressed by an increased or decreased point density bands respectively parallel to the corresponding neuronal pair axis. S&N presents the three axial graph with a color intensity coded bins.

2.3 Segmentation of the recording time

Time-dependent changes in discharge patterns and/or neuronal connectivity could be masked within long recording times thus, they can be carefully addressed on S&N by segmentation of the recording time and by analysis of feature changes in every one of those segments. The total recorded time of every neuronal data file can be divided by a user-defined number of sequential files with the same time length, i.e., if a spike train file has 6 minutes of recording time, the segmentation option assigned to 4 will split it into 4 sequential files with the same duration each (in this case, 90 seconds each). Time-related changes can be detected by differences on one or more of the segmented files results. Note that file segmentation is based on the recording time and not on time events. Furthermore, the two or three simultaneously analyzed neurons are performed only within the two or three common recorded times. For CC and JISD analyze, segmented graph are reported with the percentage of total number (without segmentation) spikes evaluated during each segment.

The time-segmentation option implemented in S&N is intended to detect time-depend changes in neuronal discharge patterns and/or connectivity during resting state data or steady recording conditions. For datasets that include trials with timed interventions (pharmacological, stimulations, etc.) the time flags or markers inserted at the raw file by the recording software should be used to separate trials which can be easily analyzed by grouping in S&N.

3 S&N Screenshots
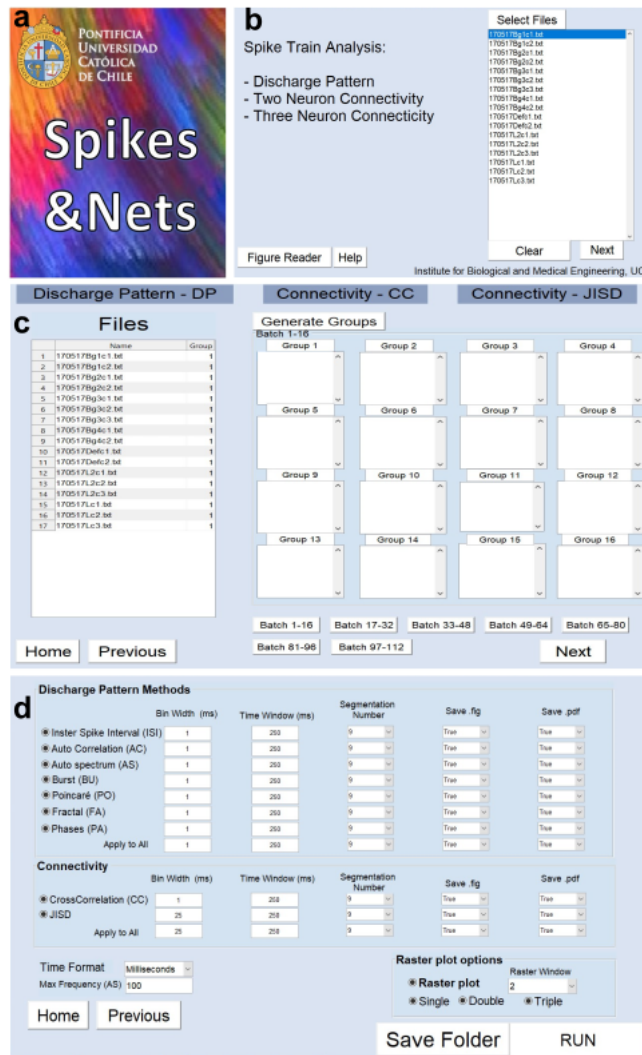
Figure 3.1: **Principal windows of S&N.** S&N welcome window (upper left); Selection of neuronal discharge pattern or neuronal connectivity analysis and neuronal data files to be analyzed (upper right); Neuronal data file grouping according to the type of experiment, epoch or trial (lower left); and selection of specific analysis methods and their parameters (lower right)
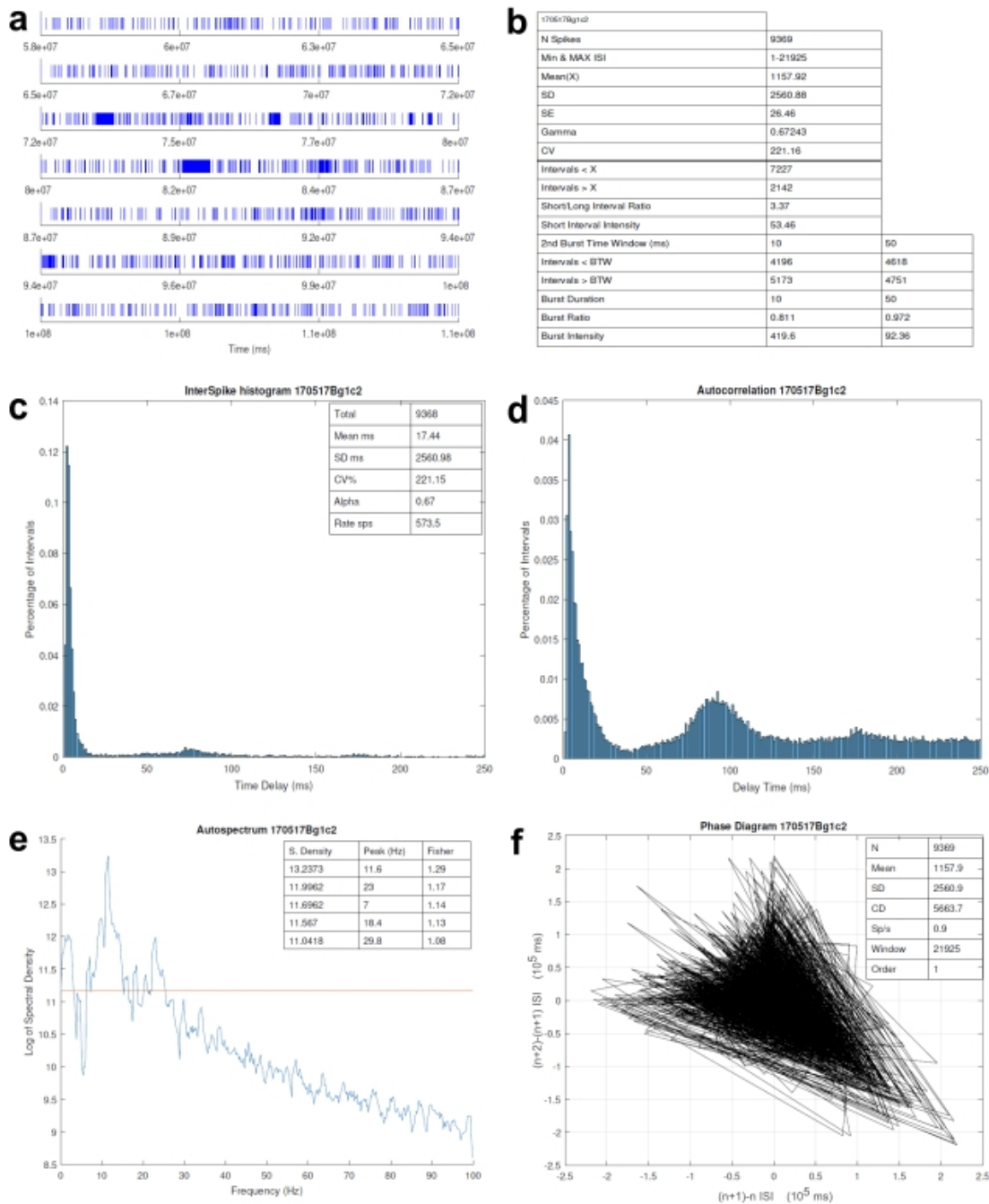
Figure 3.2: **Discharge pattern analysis of a GI neuron from TAG.** Multiparametric burst analysis (upper left) based on ISI statistics and three different burst time windows (BTW) i.e., ISI mean, 10ms and 50 ms suggesting burst discharges which are also detected by the ISI distribution (upper right) showing a bimodal shape, the first one (short ISI) for intra-burst and the second (long ISI) for inter-burst ISI. Autocorrelation function of this neuron (lower left) shows an initial zero bin expression of the refractory period and an increased probability to fire after about 3-4 ms and after 90 ms. Spectral analysis detected significant rhythmic discharges at 2, 10 and 22 Hz (lower right)
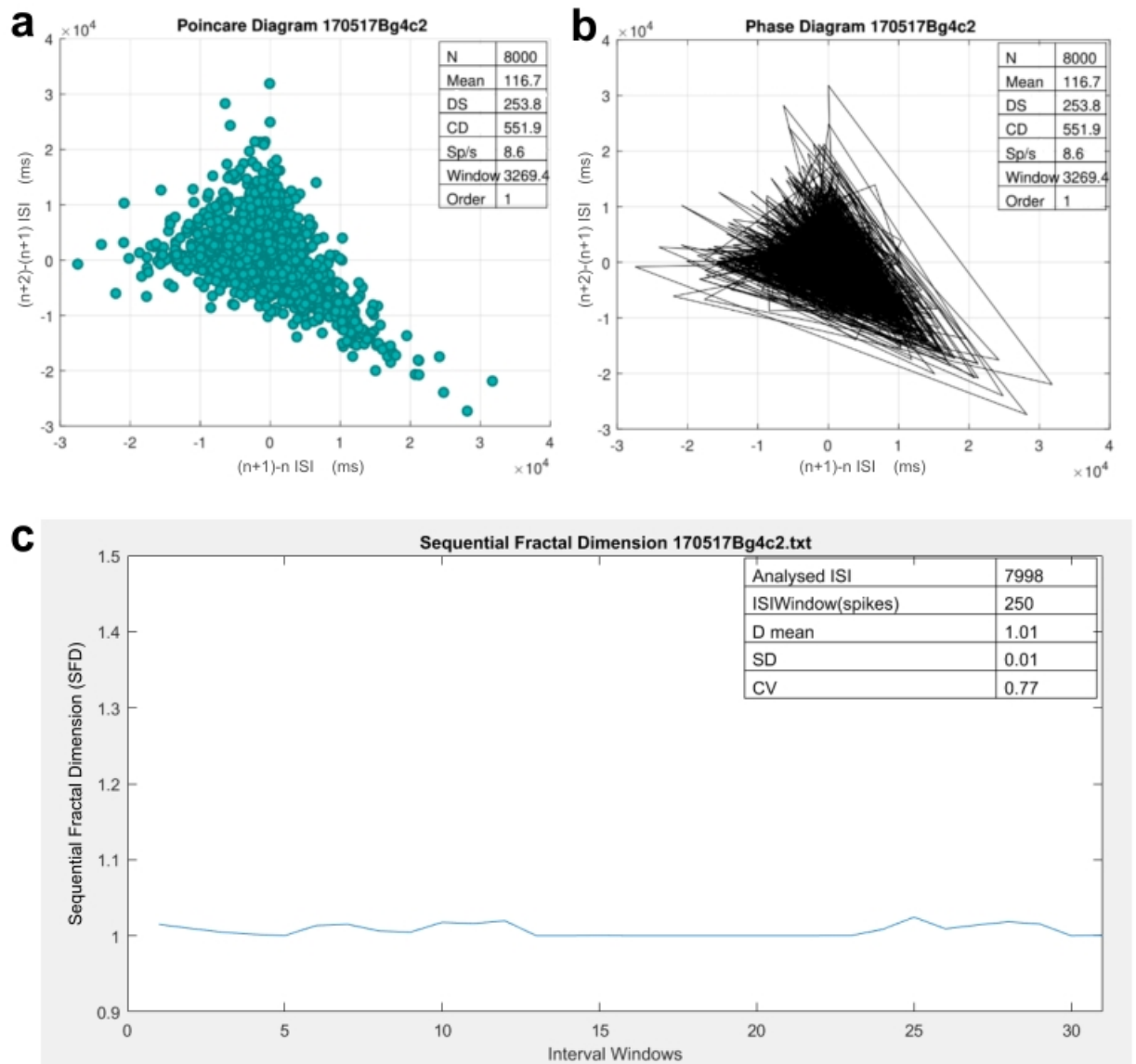
Figure 3.3: Poincaré (a) and Phase (b) diagrams from a cricket TAG GI neuron showing a ISI sequence with a triangular pattern suggesting a recursive sequence of short-to-long-to-equal durations in the ISIs. Table with values of total ISIs evaluated, mean duration (ms), standard deviation (SD), Coefficient of Variation (CV), Spikes per second (Sp/s), Graph window (ms) and order of the ISIs. Sequential fractal dimension graph shows changes in the complexity of the neuronal discharge throughout more than 30 blocks of 250 spikes each (c), values of analyzed spikes, spikes per block or window, D mean, standard deviation (SD) and coefficient of variation (CV) of the fractal dimension D are calculated.
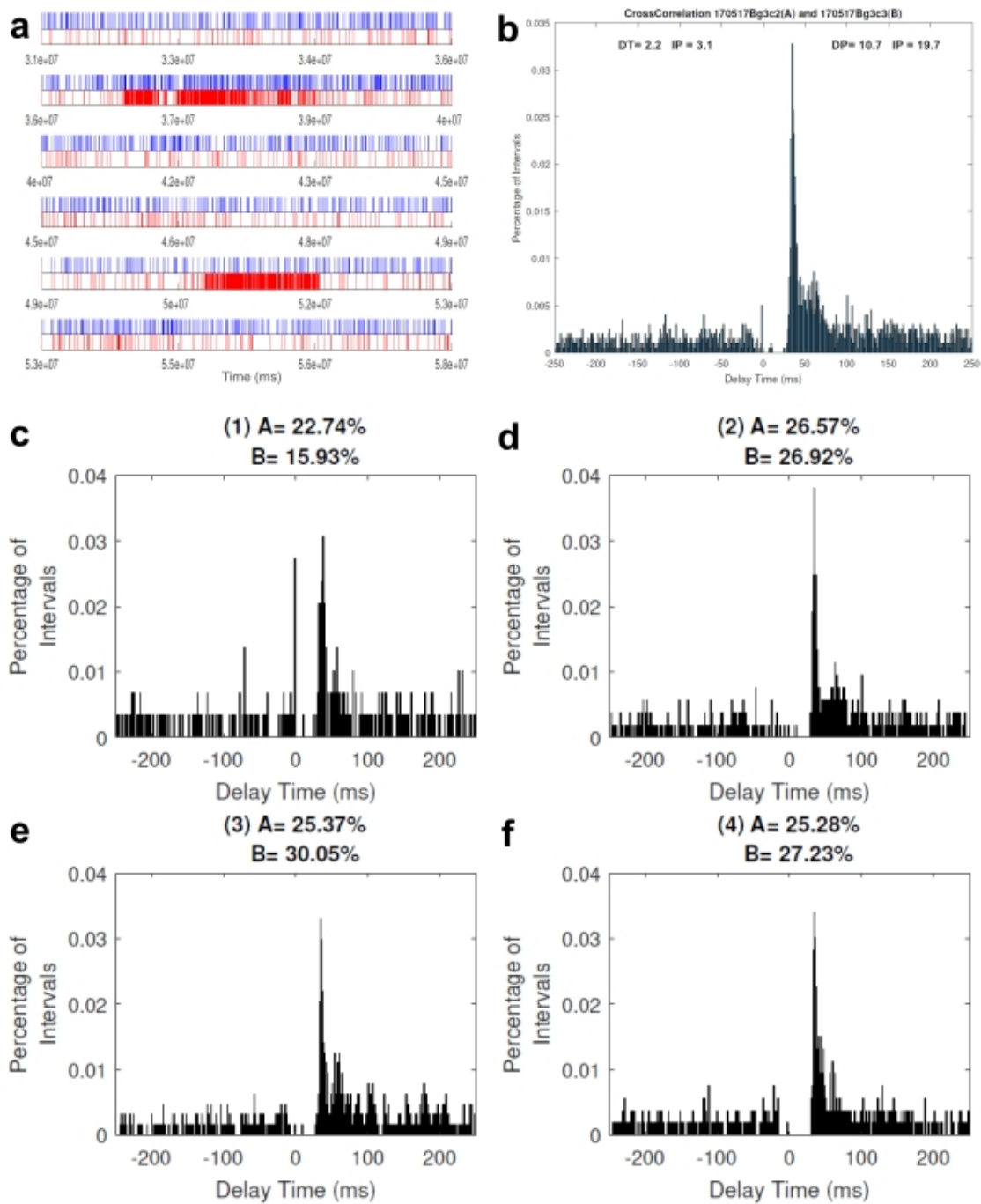
Figure 3.4: Cross-correlations between C1 and C2 GIs (a) showing a trough at ±10 ms suggesting a desynchronization input to both cells; C1 and C3 (b) with an asymmetrical trough at ±10 ms and an excitation from C1 to C3; Interaction between C2 and C3 denotes also desynchronization input to both cells but with lower intensity (c). CC features, i.e., peaks and troughs, were tested by the detectability index (DP and DT) and the intensity index (IP and IT, see text for details); Neuronal data file segmentations (n=4) presented in (c to f) are evidence of time-related changes in neuronal connectivity, mostly quantitative. Percentages of total spikes from each neuron included in every CC time segmented graph (4 segments) are presented at the top of each one.
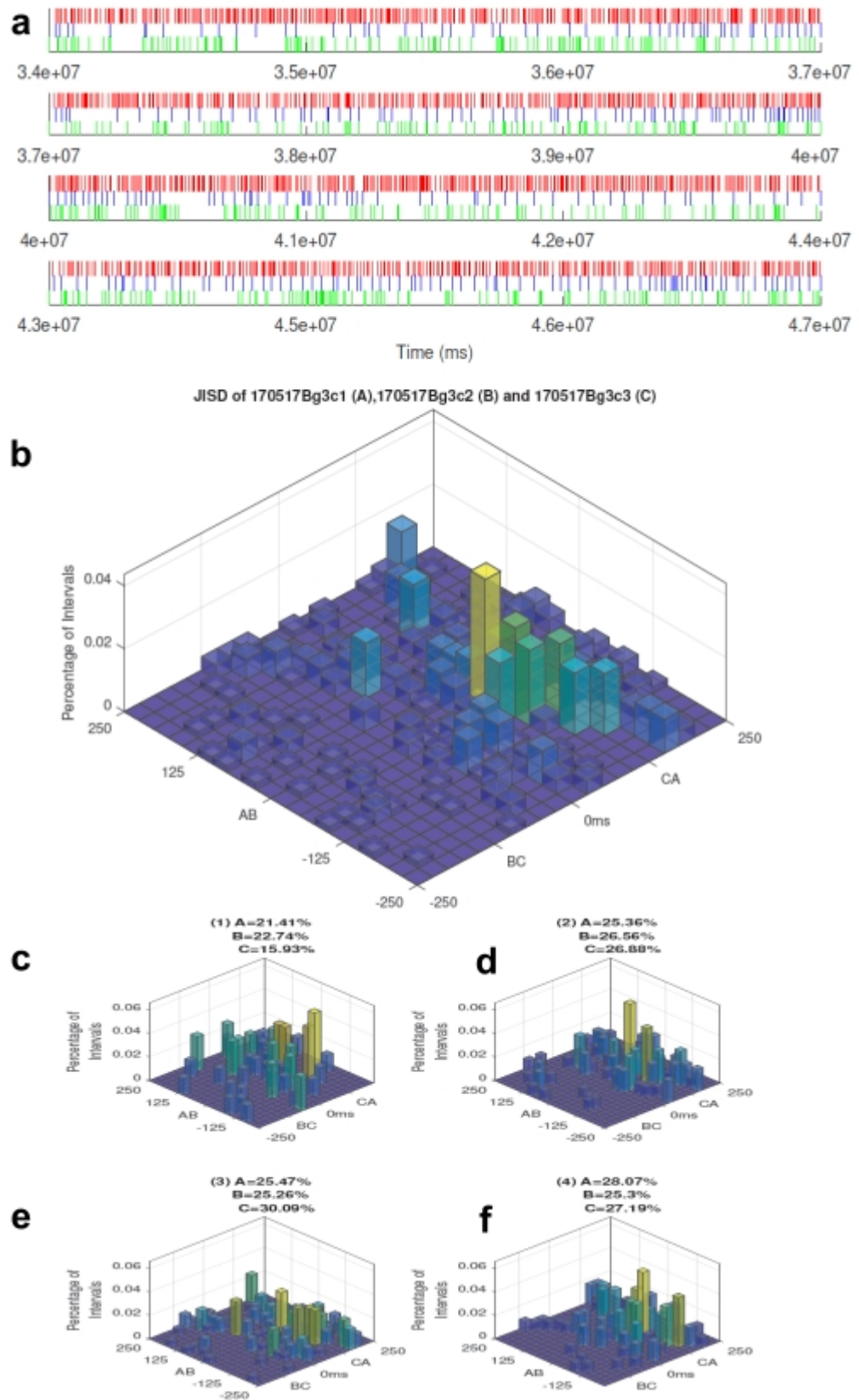
32

Figure 3.5: Raster plot from three simultaneously recorded GI neurons from the TAG (a); Neuronal connectivity among these cells is 3D displayed by JISD (b); The time-related changes in the strength and delays of these connections are seen by neuronal file segmentation (n=4) in (c to f). Percentages of total spikes from each neuron included in every JISD are presented at the top of each one.

c — ECG Rs

ECG Rs		
N Spikes	82	
Min & MAX ISI	800-1040	
Mean(X)	910.85	
SD	115.37	
SE	12.74	
Gamma	2.8099	
CV	12.67	
Intervals < X	43	
Intervals > X	39	
Short/Long Interval Ratio	1.1	
Short Interval Intensity	0.05	
2nd Burst Time Window (ms)	10	50
Intervals < BTW	1	1
Intervals > BTW	81	81
Burst Duration	10	50
Burst Ratio	0.012	0.012
Burst Intensity	0.1	0.02

d — InterSpike histogram ECG Rs

Total	81
Mean ms	910.25
SD ms	54.56
CV%	5.92
Alpha	4.11
Rate sps	10.99

e — Poincare Diagram RRs

N	3447
Mean	1040
SD	905.7
CD	786.2
Sp/s	1
Window	39390
Order	1

f — Autospectrum ECG Rs

S. Density	Peak (Hz)	Fisher
4.8672	0.8	2.59
3.3474	2.8	1.78
2.6164	4	1.39
2.1741	5	1.16
2.1481	5.4	1.14

Figure 3.6: Raw ECG D2 signal displaying the classical P-QRS-T waves (a); Raster plot of R waves after threshold detection (b); R-R intervals analysis (c) including burst analysis based on R-R statistics, i.e., R-R mean of 910.85 ms, suggesting no burst pattern which agrees to the R-R distribution histogram (d) showing a gaussian shape; Poincaré diagram shows for a specific segment of the recording, a low difference among RR interval sequence by the over plot centered at the origin of the cartesian (e); The autospectral analysis detected significant spectral peak at 0.8 Hz (f).

4 About

Please cite the present document/paper/software as: Spikes and Nets (S&N) a new fast, parallel computing, point process software for multineuronal discharge and connectivity analysis. Valle C, Rodriguez-Fernandez M and Eblen-Zajjur A. Neural Processing Letters, 2019; XX: XXX-XXX.

Visit http://marodriguezf.sitios.ing.uc.cl/SN.html for updated information.

5 References

1. Boyan GS (1988) Presynaptic inhibition of identified wind-sensitive afferents in the cercal system of the locust. The Journal of Neuroscience 8: 2748–2757.

2. Cajigas I, Malik WQ, Brown EN (2012) NSTAT: Open-source neural spike train analysis toolbox for Matlab. Journal of Neuroscience Methods 211:245–264.

3. Chiappalone M, Novellino A, Vajda I, Vato A, Martinoia S, van Pelt J (2005) Burst detection algorithms for the analysis of spatio-temporal patterns in cortical networks of neurons. Neurocomputing 65–66: 653–662.

4. Chiba A, Kamper G, Murphey RK (1992) Response properties of interneurons of the cricket cercal sensory system are conserved in spite of changes in peripheral receptors during maturation. Journal of Experimental Biology 164: 205–226.

5. Da Costa JJ, Villanueva C, Eblen-Zajjur A (2004) Microcontroller-based double window sorter for biosignals. Acta Cientifica Venezolana 55:91–96.

6. Eblen-Zajjur A (1995) Analysis of single and multiple neuronal discharges as point processes: a computer program set. Acta Científica Venezolana 46:34–40.

7. Eblen-Zajjur A, Salas R, Vanegas H (1996) Fractal analysis of spinal dorsal horn neuron discharges by means of sequential fractal dimension D. Computers in Biology and Medicine 26:87–95.

8. Eblen-Zajjur A, Sandkühler J (1997) Synchronicity of nociceptive and non-nociceptive adjacent neurons in the spinal dorsal horn of the rat: stimulus-induced plasticity. Neuroscience 76, 39-54.

9. Eblen-Zajjur A, Salas R, Vanegas H (1999) Fractal analysis of spinal nociceptive neuronal responses to receptive field stimulation and to heterotopic noxious stimulation in the rat. Neuroscience Research Communications 25(1): 51-60.

10. French AS, Holden AV (1971) Alias-free sampling of neuronal spike trains. Kybernetik 8(5): 165-171.

11. Garcia S (2009) OpenElectrophy: an electrophysiological data and analysis sharing framework. Frontiers in Neuroinformatics 3:1–10.

12. Goldberg DH, Victor JD, Gardner EP, Gardner D (2009) Spike train analysis toolkit: Enabling wider application of information-theoretic techniques to neurophysiology. Neuroinformatics 7:165–178.

13. Heitler WJ (2007) DataView: A Tutorial Tool for Data Analysis. Template-based Spike Sorting and Frequency Analysis. Journal of Undergraduate Neuroscience Education 6:A1-7.

14. Kwon KY, Eldawlatly S, Oweiss K (2012) NeuroQuest: A comprehensive analysis tool for extracellular neural ensemble recordings. Journal of Neuroscience Methods 204:189–201.

5 References

15. Lewicki M (1998) A review of methods for spike sorting: the detection and classification of neural action potentials. Network Computation in Neural Systems 9:R53–R78.

16. Lidierth M (2009) sigTOOL: A MATLAB-based environment for sharing laboratory-developed software to analyze biological signals. Journal of Neuroscience Methods 178:188–196.

17. Magri C, Whittingstall K, Singh V, Logothetis NK, Panzeri S (2009) A toolbox for the fast information analysis of multiple-site LFP, EEG and spike train recordings. BMC Neuroscience 10:81.

18. Mahmud M, Bertoldo A, Girardi S, Maschietto M, Vassanelli S (2012) SigMate: A Matlab-based automated tool for extracellular neuronal signal processing and analysis. Journal of Neuroscience Methods 207:97–112.

19. Mahmud M, Vassanelli S (2016) Processing and analysis of multichannel extracellular neuronal signals: State-of-the-art and challenges. Frontiers in Neuroscience 10:1–12.

20. Mazzoni A, Broccard FD, Garcia-Perez E, Bonifazi P, Ruaro ME, Torre V (2007). On the dynamics of the spontaneous activity in neuronal networks. PLoS ONE 2:e439 10.1371/journal.pone.0000439.

21. Nick C, Goldhammer M, Bestel R, Steger F, Thielemann C (2013) DrCell – A Software Tool for the Analysis of Cell Signals. Signal Processing: An International Journal 7(2): 96.

22. Oliynyk A, Bonifazzi C, Montani F, Fadiga L (2012) Automatic online spike sorting with singular value decomposition and fuzzy C-mean clustering. BMC Neuroscience 13: 96.

23. Pastore VP, Godjoski A, Martinoia S, Massobrio P (2017) A toolbox for dynamic and connectivity analysis of neuronal spike trains data, 8th International IEEE/EMBS Conference on Neural Engineering (NER), Shanghai, 580-583.

24. Perkel DH, Gerstein GL, Moore GP (1967) Neuronal Spike Trains and Stochastic Point Processes: I. The Single Spike Train. Biophysical Journal 7:391–418.

25. Perkel DH, Gerstein GL, Moore GP (1967) Neuronal Spike Trains and Stochastic Point Processes: II. Simultaneous Spike Trains. Biophysical Journal 7:419–440.

26. Perkel DH, Gerstein GL, Smith MS, Tatton WG (1975) Nerve-impulse patterns: a quantitative display technique for three neurons. Brain Research 100(2): 271-296.

27. Pröpper R, Obermayer K (2013) Spyke Viewer: a flexible and extensible platform for electrophysiological data analysis. Frontiers in Neuroinformatics 7:26.

28. Quevedo C, Eblen-Zajjur A (2013) Effect of voltage dependent calcium channels block on mechanoreceptive neuronal discharges. Salus 17(3): 62-70.

29. Sandkühler J, Eblen-Zajjur A (1994) Identification and characterization of rhythmic nociceptive and non-nociceptive spinal dorsal horn neurons in the rat. Neuroscience 61: 991-1006.

30. Sandkühler J, Eblen-Zajjur A, Liu X-G (1994) Differential effects of skin inflammation, extrasynaptic substance P, and noxious skin heating on rhythmicity, synchrony, and nonlinear dynamics in rat spinal dorsal horn neurons. In GF Gebhart, DL Hammond, TS Jensen (Ed.) Progress in Pain Research and Management (2: 347-358). Seattle: IASP Press.

31. Segers LS, Nuding SC, Dick TE, Shannon R, Baekey DM, Solomon IC, Morris KF, Lindsey BG (2008). Functional connectivity in the pontomedullary respiratory network. Journal of Neurophysiology 100(4): 1749-1769.

32. Seth AK (2010) A MATLAB toolbox for Granger causal connectivity analysis. Journal of Neuroscience Methods 186(2): 262-273.

33. Shi X, Lu Q (2009). Burst synchronization of electrically and chemically coupled map-based neurons. Physica A: Statistical Mechanics and its Applications 388(12): 2410-2419.

34. Tam DC (1999). A spike train analysis for detecting temporal integration in neurons. Neuro-computing 26: 1055-1060.

35. Torre E, Canova C, Denker M, Gerstein G, Helias M, Grün S (2016) ASSET: Analysis of Sequences of Synchronous Events in Massively Parallel Spike Trains. PLoS Computational Biology 12:1–34.

6 Social Contract

S&N Spikes and Nets Social Contract. Version 1.2; 2019.

The producers of the SN system, have created the SN Social Contract. The SN Free Software Guidelines (S&NFSG) part of the contract, initially designed as a set of commitments that we agree to abide by, has been adopted by the free software community as the basis of the Open Source Definition. Social Contract with the Free Software Community. S&N will remain 100% free We provide the guidelines that we use to determine if a work is free in the document entitled The S&N Free Software Guidelines. We promise that the SN system and all its components will be free according to these guidelines. We will support people who create or use both free and non-free works on SN. We will never make the system require the use of a non-free component. We will give back to the free software community When we write new components of the S&N system, we will license them in a manner consistent with the S&N Free Software Guidelines. We will make the best system we can, so that free works will be widely distributed and used. We will communicate things such as bug fixes, improvements and user requests to the upstream authors of works included in our system. We will not hide problems We will keep our entire bug report database open for public view at all times. Reports that people file online will promptly become visible to others. Our priorities are our users and free software We will be guided by the needs of our users and the free software community. We will place their interests first in our priorities. We will support the needs of our users for operation in many different kinds of computing environments. We will not object to non-free works that are intended to be used on S&N systems, or attempt to charge a fee to people who create or use such works. We will allow others to create distributions containing both the S&N system and other works, without any fee from us. In furtherance of these goals, we will provide an integrated system of high-quality materials with no legal restrictions that would prevent such uses of the system. Works that do not meet our free software standards We acknowledge that some of our users require the use of works that do not conform to the S&N Free Software Guidelines. We have created contributions and non-free areas in our archive for these works. The packages in these areas are not part of the SN system, although they have been configured for use with SN. We encourage CD manufacturers to read the licenses of the packages in these areas and determine if they can distribute the packages on their CDs. Thus, although non-free works are not a part of S&N, we support their use and provide infrastructure for non-free packages (such as our bug tracking system and mailing lists). The S&N Free Software Guidelines (S&NFSG) Free Redistribution The license of a S&N component may not restrict any party from selling or giving away the software as a component of an aggregate software distribution containing programs from several different sources. The license may not require a royalty or other fee for such sale. Source Code The program must include source code, and must allow distribution in source code as well as compiled form. Derived Works The license must allow modifications and derived works, and must allow them to be distributed under the same terms as the license of the original software. Integrity of The Author's Source Code The license may restrict source-code from being distributed in modified form only if the license allows the distribution of patch files with the source code for the purpose of modifying the program at build time. The license must explicitly permit distribution of software built from modified source code. The license may require derived works to carry a different name or version number from the

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