A program for analysing single neuron activity by methods based on estimation of a change-point

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A program for analysing the sequences of discharge of a single neuron is presented. This program performs the following tasks: (1) it tests if there is a neuronal response after a stimulus, (2) it estimates the response latency and, in the case of an experiment with a conditioned movement, (3) it estimates the onset of the movement and (4) gives indications about the functional role of the neuron by analysing the variances of the stimulus–response and response–movement times. The methods used have been developed by the authors and are based on the estimation of a change-point. The basic methods are published elsewhere but some novelties are presented here, in particular a robust estimator of a variance ratio.

Neuronal response  Change-point  Variance ratio  Robust estimators

1. Introduction

One of the most widely used approaches for studying the functional role of different structures in the central nervous system consists of recording and analysing the electrical activity of single neurons located in a defined structure. This activity appears as a sequence of action potentials known as a spike train. Most spike trains have a stochastic character and for this reason we call them spike processes.

In most experiments (see for instance [1]) a stimulus is presented to an animal and the first question which arises is whether there is a change in the activity of the neuron after the stimulus.

The question which follows is to estimate the mean latency time between the onset of the stimulus and the onset of the response of the neuron.

Often in this type of experiment, the animal is conditioned to make a movement after the stimulus. In this case, we can imagine that there is a chain of central nervous events leading from the perception of the stimulus to the execution of the movement [2] and one may ask whether the recorded neuron can be characterized as having a functional role at the beginning or at the end of the chain.

We have developed statistical methods [3,4] for answering these three questions and we propose here a program for the application of these methods to real data. Before presenting the methods and the program, let us describe precisely the typical experiment and our general approach. (For more details, see [4].)

The experiment consists in repeating a certain number of times (generally 10–50) the elementary experiment which is called a trial. For each trial, the spike train for one neuron is recorded during a time $t_f$, a stimulus is presented at time $t_s$ after the beginning of the recording period (in our experiments $t_f = 2000$ ms and $t_s = 500$ ms). If this is an experiment with a conditioned movement, a spatial measurement is also taken on the part of the
body which the animal has to move.

Our main hypothesis (see [4]) is that the spike process is an Erlang process, that is, a stochastic point process with independent intervals having gamma distributions of probability density functions (p.d.f.):

\[ f(x; \alpha; \gamma) = \Gamma(\gamma)^{-1} \cdot \alpha^\gamma \cdot \exp(-\alpha \cdot x) \cdot x^{\gamma-1} \]

(1)

The shape parameter \( \gamma \) is considered as being constant while the scale parameter \( \alpha \) can change either abruptly or progressively. Abrupt changes are indicative of a specific intervention of the neuron in the information processing while progressive changes are due to fluctuations in the overall state of the animal. We consider that if the neuron is involved in the information processing following the stimulus, there is an abrupt change in the spike process and, after a certain time of excited or inhibited activity, the neuron reverts to its previous state.

In contrast to the conventional methods, which try to interpret the data on the basis of the peri-stimulus time (PST) histogram [5], which is the histogram of the times of occurrence of the spikes for all the trials, our approach consists in estimating the location of the change, trial by trial, and using this set of estimated values to make an inference about the whole experiment.

The different methods we have developed are described in Section 2 and 3, and the program in Section 4.

2. Methods

2.1. Estimation of the change in each trial

All the following methods are based on the possibility of estimating the change for each trial. The original problem is to estimate the location of a change in an Erlang process, but we have simplified this problem by considering the sequence of intervals between spikes. We have developed a method for estimating the index of change, \( r \), in a sequence of random variables, and the time of change, \( t \), is estimated by summing the first \( r \) variables: we have proved that the estimator \( \hat{r} \) thus obtained has the same consistency property as the maximum likelihood estimator (this is not consistency in the ordinary sense).

Most previous work (e.g. [6]) has concentrated on problems with one point of change. This is why we have used an original method which is designed to be robust when there is an unknown number of changes: in this case, the most clear-cut change is given. The method is based on the two period circular sequence (TPCS) model and is as follows: the p.d.f. of the variable \( i \) is \( f(x, \alpha, \gamma) \) with:

\[ \alpha = a_0 \quad \text{for} \quad i \in R_0 \]
\[ \alpha = a_1 \quad \text{for} \quad i \in R_1 \]

with \( R_0 = \{ i : 1 \leq i \leq r_1 \text{ or } r_2 < i \leq n \} \)

and \( R_1 = \{ i : r_1 < i \leq r_2 \} \)

(2)

The estimated indexes of change are the values of \( r_1 \) and \( r_2 \) which maximise:

\[ L(r_1, r_2) = \bar{x}_{[R_0]} \cdot \bar{x}_{[R_1]} \]

(3)

where \( |R_0| = n - r_1 + r_2 \)

and

\[ |R_1| = r_2 - r_1 \]

with \( 1 \leq r_1 < r_2 \leq n \)

(4)

and \( \bar{x}_0 \) and \( \bar{x}_1 \) are the empirical means of the intervals for the sets \( R_0 \) and \( R_1 \).

The latency time can be estimated by:

\[ \hat{t} = t_{h+1} - t_s \]

(5)

where \( t_i \) is the time occurrence of the \( i \)th spike.

As a by-product, the duration of the neuronal response can be estimated by:

\[ \hat{D} = t_{h+1} - t_{h+1} \]

(6)
2.2 Estimation of the mean latency time

For a more precise estimation in each trial, certain constraints are added. We can specify whether we are looking for an excitation or an inhibition. In case of an excitation we must have: \( \bar{x}_t < \bar{x}_0 \).

The interesting change must occur after the stimulus, thus: \( \hat{t} > 0 \). It is easy to maximize \( L(r_1, r_2) \) with these constraints.

If there is no maximum which satisfies the constraints, the trial is eliminated. We obtain a set of estimated values \( \hat{t}_i \), \( i = 1, \ldots, m \). The mean latency time can be estimated by the mean of \( \hat{t}_i \). In order to diminish the influence of outliers, we use a trimmed mean:

\[
\hat{t} = (m - 2p)^{-1} \sum_{j=p+1}^{m-p} \hat{t}_{(j)}
\]

(7)

where \( \hat{t}_{(j)} \) \( (j = 1, \ldots, m) \) are the order statistics. A confidence interval can be obtained ([7], p. 349).

2.3. Testing if there is a response of the neuron in the experiment

The question we want to answer is the following: Is the neuron involved in one of the stages of the experimental process? For instance, in an experiment with a conditioned movement there are grossly three stages: perception of the stimulus, decision of action, execution of the movement. If the neuron is involved in one of these stages it will exhibit a change in activity after a certain time, \( t \), following the stimulus. This time is random with a relatively small variance.

The test is based on the idea that if the neuron is not involved in the experiment at hand, the estimated value of the change occurs anywhere in the recording period with uniform probability.

More precisely, we estimate the time of change in each trial by an estimator \( \hat{t}_i \), slightly different from \( \hat{t} \), which is as follows. Choose a time \( t_0 \) which is such that the probabilities of the two following events are small: (1) the response occurs after \( t_0 \), (2) there is no spike after \( t_0 \) (if (2) occurs, the trial is eliminated). Then we define:

\[
\hat{t}_i = \begin{cases} 
\hat{t}_{i+1} & \text{if } \hat{t}_{i+1} \leq t_0 \\
\hat{t}_1 & \text{if } \hat{t}_{i+1} = n \\
\text{undefined otherwise}
\end{cases}
\]

(8)

We have proved that under the null hypothesis (neuron not involved in the experiment) \( \hat{t}_i \) has an approximately uniform distribution on \([0, t_0]\). On the basis of \( \hat{t}_{ij} \) \( (j = 1, \ldots, m) \) we can test this hypothesis by using the Kolmogorov test; we prefer, however, to use a more specific test statistic which is the conventional estimator of the variance:

\[
Q = (m - 1)^{-1} \sum (\hat{t}_{ij} - \hat{t}_i)^2
\]

(9)

where

\[
\hat{t}_i = m^{-1} \sum_{j=1}^{m} \hat{t}_{ij}
\]

We have previously shown that \( Q/a \) has approximately a \( \chi^2 \) distribution with \( d \) degrees of freedom where:

\[
a = \frac{2m + 3}{60m(m - 1)} t_0^2
\]

and

\[
d = \frac{5m(m - 1)}{2m + 3}
\]

(10)

\( H_0 \) is rejected for low values of \( Q/a \).

2.4. Inference on the variance ratio in experiments with a conditioned movement: a test

Let \( X \) be the time elapsed from the stimulus to the onset of the neuronal response and \( Y \) the time elapsed from the onset of the response to the onset of the movement.

The properties of the random variables \( X \) and \( Y \) can be used to throw some light on the functional role of the neuron.

We argued in [4] that if two events are linked by a causal relationship, the time elapsed between them often has a small variance. In the neurophysiological context, we can imagine that there is a chain of elementary neuronal processing stages leading from the stimulus to the movement.

In order to determine whether the neuron is involved at the beginning or at the end of the chain it is possible to compare the variance of \( X \)
to that of Y. If, for instance, the variance of Y is significantly smaller than that of X we may conclude that the recorded neuron is involved in a stage which is nearer to the movement than to the stimulus. Consideration of the expectations does not lead necessarily to the same conclusions. (Consider for example a neuron which exhibits a response to the stimulus, but does not belong to the stimulus–movement chain.)

Morgan [8] proposed a test for comparing two variances in a sample from a bivariate population. The idea is that the variables $Z = X + Y$ and $W = X - Y$ are uncorrelated if and only if $\text{var} \ Y = \text{var} \ X$. Thus a test can be obtained by considering the correlation coefficient between $Z$ and $W$. Kepner and Randless [9] extended this idea to obtain a distribution-free test based on the use of Kendall’s correlation coefficient ($\tau$). Our program performs this test, using a statistic which has an approximately normal distribution for moderately large sample size ($m \geq 15$). In fact, we are not interested in knowing whether the null hypothesis is true or false but rather which of the two alternative hypotheses $\text{var} \ X < \text{var} \ Y$ or $\text{var} \ Y < \text{var} \ X$ is true.

2.5. Inference on the variance ratio: a robust estimator

The comparison of variances is chiefly interesting in clear-cut cases and may serve to discriminate between neurons which have direct links to motor output and those which are linked to sensory input. However, outside the primary motor and receiving areas, the relationship of a neural response to the stimulus which precedes it or the motor act that follows it is not evident. If the model of successive elementary stages leading from the stimulus to the movement is to be accepted, a continuous measure of the location of the neuron within this chain will seem more appealing. Such a measure can be given by the variance ratio $\Delta^2 = \frac{\text{var} \ Y}{\text{var} \ X}$.

We use an original robust estimator of the variance ratio. It is based on Morgan’s idea that there exists a relationship between $\Delta^2$ and the correlation coefficient of $Z$ and $W$, $R$. $R$ can be expressed as a function of the variance ratio ($\Delta^2$) and the correlation coefficient ($r$) of $X$ and $Y$. This formula can be inverted and we obtain:

$$\Delta^2 = \frac{(R \sqrt{1 - r^2} - \sqrt{1 - R^2 \cdot r^2})^2}{1 - R^2} \quad (11)$$

An estimator of $\Delta^2$ can be obtained by replacing $R$ and $r$ by their estimators. If we choose the conventional ML estimators for normal distributions (Pearson’s correlation coefficients) we also obtained for $\Delta^2$ the ML estimator, which is the ratio of the conventional ML estimators of the variances. For each robust estimator of the correlation coefficient, this formula generates a robust estimator of the variance ratio by replacing $r$ and $R$ by their estimators.

Our program uses Spearman’s rank correlation coefficient, which gives a distribution-free measure of a distance between the two variances. Simulations (not presented here) have shown that the statistic thus obtained accurately estimates the variance ratio and is much less sensitive to outlier values than the conventional estimator.

In our application of this estimator to the neurophysiological experiment we must recall that we have to work with estimated values, $X'$ and $Y'$, of $X$ and $Y$ and we have $X' = X + \epsilon$ and $Y' = Y - \epsilon$.

Thus, rather than $\Delta$, we estimate $\Delta^2 = (\text{var} \ Y + \text{var} \ \epsilon)/(\text{var} \ X + \text{var} \ \epsilon)$ and we have:

$$|\Delta^2 - 1| < |\Delta^2 - 1| \quad (12)$$

Because of this, our estimator is shrunk towards 1. We must also be conscious that the value of $Y$ can be affected by the uncertainty on the onset of the movement. Thus, $\Delta^2$ is positively biased and only high values (say $> 2$) should be interpreted (if $\Delta' > 1$).

3. Some empirical solutions

3.1. Estimation of the properties of the estimator of the change-point $t$

It is very difficult to investigate the properties of $\hat{t}$ analytically. It is possible to do it by stimulation using, as parameter values, the trimmed means of
\( \hat{i} \) and \( \hat{D} \) for the times of change and, for the parameters of the gamma distributions \( \tilde{\alpha}_0, \tilde{\alpha}_1 \) and \( \tilde{\gamma} \), which are computed as follows. We have \( 2m \) samples of variables having gamma distributions with parameters \( (\alpha'_0, \gamma) \) and \( (\alpha'_1, \gamma) \), \( i = 1, \ldots, m \).

We estimate the ML estimators \( \hat{\alpha}'_0, \hat{\alpha}'_1 \) and \( \hat{\gamma} \) (this requires a non-linear search for \( \hat{\gamma} \)) and we define \( \hat{\alpha}_0 \) and \( \hat{\alpha}_1 \) as the trimmed means of \( \hat{\alpha}'_0 \) and \( \hat{\alpha}'_1 \). As it is easier to generate gamma variables with \( \gamma \) an integer parameter, we use for the simulation \( \tilde{\gamma} \), the integer nearest to \( \hat{\gamma} \) and \( \tilde{\alpha}_0 \) and \( \tilde{\alpha}_1 \), which are such that the expectations of the variables are not modified: \( \tilde{\alpha}_j \cdot \tilde{\gamma} = \hat{\alpha}_j \cdot \hat{\gamma}, \ j = 0, \ldots, 1 \).

In the program we generate 100 trials according to these values and can thus estimate the bias and variance of \( \hat{\gamma} \) for a typical trial of the experiment at hand. As the operation is time-consuming, it is optional. From our experience we can say that the bias of \( \hat{\gamma} \) is generally very small. The variance of \( \hat{\gamma} \) is an estimate of var \( \epsilon \) and it may be used for a correction of \( \Delta^2 \). Such a method is in development.

### 3.2. Estimation of the onset of the movement

In an experiment with a conditioned movement, a spatial measurement, usually of the limb displacement about an axis, is taken throughout the recording period. The measurement first oscillates around an initial value and then suddenly increases or decreases. The problem of estimating the time at which this occurs can itself be put into the framework of a change-point estimation problem. Indeed, if the speed of displacement varies only slightly, the derivative of the measurements (or the difference between two consecutive measurements) is approximately constant: the mean of the derivative is zero when there is no movement and becomes suddenly equal to a certain constant when there is a movement.

It is possible to use the TPCS method and Eq. 3 to estimate the point of change in the derivative of the movement: however, some preliminary operations have to be made.

First, the values have to be recoded because Eq. 3 is valid for positive variables only. The following code has been chosen: 1 for a negative difference, 2 for a positive one.

Second, in many cases there are in fact three different regimes (instead of two for the TPCS model) because, after having performed the expected movement, the limb returns to its initial position (see Fig. 1). In order to safely apply the TPCS model we have to truncate this part of the sequence. Let \( X_0, X_M, X_m \) be the initial, the maximum and the minimum value of the measurements.

If \( |X_0 - X_M| < |X_0 - X_m| \), \( X_M \) is the value reached at the end of the movement, and this is \( X_m \) in the opposite case. The sequence is truncated at the time of occurrence of \( X_M \) (or \( X_m \)) and partitioned into two regimes by the analysis based on the TPCS model. The regime corresponding to the absence of movement has a mean value near 1.5 since positive and negative values occur with equal probabilities. Thus the beginning of the other regime estimates the onset of the movement.

### 4. Program structure

A flowchart of the program is given in Fig. 2.
Fig. 2. Flowchart of the program.
4.1. Input

4.1.1. Data recording during the experiment
These are stored on a file and read at the beginning of the program and are as follows:
(1) Name of the cell under study: array TITLE (character).
(2) Number of trials (NBE) for this cell.
For each trial:
(3) Measurements for the movement (in case of an experiment with a conditioned movement the measurements are taken every \( IT \) ms during the recording period). They are stored in the array MOUVMT (real).
(4) Number of spikes recorded (N).
(5) Values of the interspike intervals stored in the array INTER (real). INTER (1) is the occurrence of the first spike).

4.1.2. Parameters fixed in the program by data declaration
These parameters can be modified in order to adapt the program to a particular experiment and are:
TS: time of presentation of stimulus (in our experiment \( TS = 500 \)).
TO: time used for testing if the neuron is experiment-related (\( TO = 1500 \) ms in our experiment for which the recording period is \( t_f = 2000 \) ms).
IM: Indicator of the movement: IM = 0, no movement; IM = 1, presence of a movement (in our experiment, IM = 1).
IT: Sampling period (ms) of the movement (in our experiment \( IT = 8 \)).

4.1.3. Parameter in an input mode
ICHOIX: specifies if an excitation or an inhibition is sought: ICHOIX = 1, excitation; ICHOIX = 2, inhibition.
IHIS: ‘yes’: computation and edition of the peri-stimulus histogram and, if IM = 1, the peri-response histogram.
ISIM: ‘yes’: stimulation of 100 trials and estimation of the properties of the estimator.

4.2. Output
An example of output is shown in Figs. 3 and 4. The following information is printed:
(1) The result of the test of the hypothesis \( H_0 \) “the neuron is not experiment-related”. The value of \( Q \), its expectation under \( H_0 \), the values of \( a \) and \( d \), and the \( p \)-value are printed; if \( p > 0.05 \), \( H_0 \) cannot be rejected;
(2) The conventional and trimmed means, with their 95% confidence intervals, of the estimates of the following times:
X: Time between stimulus and the first change of the spike process (called \( t \) in section 2.1).
Y: Time between the first change of the spike process and the movement.
Z: Time between the stimulus and the movement.
V: Time between the stimulus and the second change of the spike process.
W: Time between the second change and the movement.
D: Time between the two changes (duration of the neuronal response).
(3) Result of the Kepner and Randles test and the robust variance ratio (under the null hypothesis, \( p \) is a normal deviate).
(4) Histograms (if IHIS = ‘yes’), for each histogram number of spike in each bin and drawing of the histogram.
(5) Simulation (if ISIM = ‘yes’):
(a) Parameters of the simulation: value of \( X \) and \( D \) and parameters of the gamma distributions for periods one, two and three.
(b) Result of the stimulation: mean, variance and standard deviation of the estimators of \( X \) and \( D \).

4.3. Subroutines
The main subroutines are:
MAXEX : Estimation of a change-point without constraint except the type of response (excitation or inhibition).
EXCITATION HAS BEEN SOUGHT

IS THE NEURON RELATED TO THE EXPERIMENT

---

**H0**: UNIFORM DISTRIBUTION OF THE CHANGE POINTS TESTED ON 48 TRIALS

\[ Q = 79451.2 \quad \text{E}(Q) = 187500 \]  
\[ Q/1045.61 = 79.4152 \]  
\[ \text{q} = 0.0000 \]

---

**49 TRIALS**  
**TRIMMED VALUES (35 TRIALS)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Trimmed Mean</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>224.1</td>
<td>133.6</td>
<td>(117.7, 149.5)</td>
</tr>
<tr>
<td>Y</td>
<td>4.5</td>
<td>95.2</td>
<td>(73.9, 116.5)</td>
</tr>
<tr>
<td>Z</td>
<td>228.6</td>
<td>256.5</td>
<td>(245.3, 267.6)</td>
</tr>
<tr>
<td>V</td>
<td>955.3</td>
<td>1004.6</td>
<td>(960.4, 1048.8)</td>
</tr>
<tr>
<td>W</td>
<td>-736.7</td>
<td>-770.7</td>
<td>(-816.8, -724.7)</td>
</tr>
<tr>
<td>D</td>
<td>741.2</td>
<td>742.5</td>
<td>(694.1, 790.9)</td>
</tr>
</tbody>
</table>

**KEPNER & RANDLES TEST**

**FOR THE SAMPLES** (X,Y), P : -3.9118

**FOR THE SAMPLES** (V,W), P : -2.2642

**ROBUST VARIANCE RATIO**

\[ \text{var}_Y / \text{var}_X : 2.07 \]  
\[ \text{var}_W / \text{var}_V : 1.21 \]

---

Fig. 3. Output of the program for an experiment with a conditioned movement: first page.

**MAXMOU** : Estimation of the onset of the movement.  
**HISTOG** : Computation of the histograms.  
**MAXMOD** : Estimation of a change-point with the constraint that \( t_{r+1} > t_s \) and the constraint of the type of response.  
**NCHYPO** : Computations for testing whether the neuron is experiment-related.  
**PARAGA** : Estimation of the parameters (\( \gamma, \hat{a}_j \)) of the set of trials.  
**SIMUGA** : Simulation of the trials and estimation of the mean, variance and standard deviation of X and D.  
**SPVARO** : Computation of the robust variance ratio estimator.  
**DUMSTO** : Comparison of variances of paired samples (X, Y) using the Kepner and Randles test.  
**HIST** : Drawing of histogram.
Excitation has been sought

Simulation

Parameters:

\[
\begin{align*}
X &= 134, \\
\theta &= 743, \\
\text{Period 1} &= (a_1; \gamma) = (0.074; 2) \\
\text{Period 2} &= (a_2; \gamma) = (0.181; 2) \\
\text{Period 3} &= (a_1; \gamma) = (0.074; 2)
\end{align*}
\]

Results:

<table>
<thead>
<tr>
<th>Estimator of X</th>
<th>Estimator of ( \theta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean: 138.8</td>
<td>Mean: 753.5</td>
</tr>
<tr>
<td>Variance: 4867.7</td>
<td>Variance: 8118.9</td>
</tr>
<tr>
<td>Standard Deviation: 69.8</td>
<td>Standard Deviation: 90.1</td>
</tr>
</tbody>
</table>

Fig. 4. Output of the program: second page. Mean and variance of the estimator of the change obtained by simulation for a standard trial of the experiment are optional.

Other subroutines are:

**ORDR** : Ranking of values in increasing order.

**MOYCEA** : Mean and standard deviation.

**CORREL** : Mean, variance and correlation coefficient.

**CORSPI** : Correlation coefficient of Spearman.

**SEARCH** : Minimization of a non-linear function with one parameter.

**AIGAMM** : ML function of gamma sequences.

**VALFOB** : Computation of minus the ML function.

**DLGAM** : Computation of the gamma function.

**FDCHI** : Computation of the distribution function of a \( \chi^2 \) variable.

**FDNORM** : Computation of the distribution function for a normal reduced law.

**RVGAMA** : Generation of a gamma random variable.

**BGOS** : Generation of a normal random variable.

**URAND** : Generation of an uniform random variable. This subroutine uses an integer on 32 bits. According to the computer used, this subroutine, as well as RVGAMA and BGOS, may have to be modified. The algorithm is taken from [10] (p. 240).

5. Hardware and software specifications

The program presented here is written in FORTRAN 77 and is currently running on a Mini 6/92. The main source program requires less than 30 Kbytes and the compiled program less than 100 Kbytes core memory. The amount of computer time depends on the number of spike events per trial, the number of trials, the duration of each trial, whether or not a movement is also studied and on the choice of options. For example, the program takes about 4 minutes to analyze 49 trials of duration 2 s and a mean of 100 spikes events per trial with a movement and the options for histogram and the simulation.

6. Availability

A listing of the FORTRAN Source code of the program is available from D. Commenges.

References


[8] W.A. Morgan, A test for the significance of the difference between two variances in a sample from a normal bivariate population, Biometrika 13 (1939) 13–19.