

SOME DETAILS ON THE METHOD FOR MEASURING ACTIVITY CHANGES OF NEURONAL POPULATIONS

A. KALAUZI¹, SLAĐANA SPASIĆ¹ and MILKA ČULIĆ²

¹Center for Multidisciplinary Studies,

²Institute for Biological Research "Siniša Stanković",
11000 Belgrade, Serbia and Montenegro

Abstract – Simple, complex action potentials (AP) and interspike recorded background activity (RBA) were detected and extracted from 12 rat cerebellar cortical recordings. For each recording, an averaged simple action potential (SAP) was formed. Fourier amplitude spectra of SAP and RBA were similar in all recordings. For one of the recordings, a series of RBA simulations (SBA) was generated, superimposing random SAPs, varying the superposition frequency (f_{sup}). Average Fourier amplitude, $Amp(SBA)$, was calculated, in order to use $Amp(SBA) = f(f_{sup})$ as a calibration line for reading f_{sup} of RBA, which is a measure of the population activity. A probability distribution for SAP intensities was derived and a family of calibration lines constructed. As all lines were linear and parallel (slope S) in log-log plots, a population activity change could be calculated as $f_{sup2} / f_{sup1} = [Amp2(RBA) / Amp1(RBA)]^{1/S}$.

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INTRODUCTION

Measuring the activity of neuronal populations plays a significant role in contemporary neurophysiology. Classical approach is based on equipment with a considerable number of microelectrodes, where each of them is placed near one of the neurons from the population. By counting all action potentials registered within a short time interval Δt , one can extract information about the number of active neurons (n_{act}). If we denote the total number of population neurons with N , activity of the population is usually defined (Gerstner and Kistler 2002) as

$$A(t, t+\Delta t) = \lim_{\Delta t \rightarrow 0} \frac{1}{\Delta t} \frac{n_{act}(t, t+\Delta t)}{N}$$

However, the background activity was treated as a noise (Gerstner and Kistler 2002). A few interesting questions are still not completely answered: could a change in the neuronal population activity be detected by single channel recordings (Kalauzi *et al.* 2003) and could any valuable information be contained in signal intervals between complex and simple spikes of cerebellar Purkinje neurons (Šaponjić *et al.* 2001).

We recently proposed a procedure for estimating population activity changes in rat cerebellar cortex (Kalauzi and Spasić 2003). In this work we describe some new details (concerning the dimensionality of neuronal arrangements around the electrode tip) of modeling the probability distribution of spike intensities contributing to the background activity (interspike segments).

MATERIALS AND METHODS

Simulation of the RBA signal using uniform probability distribution for SAP intensities

Simple and complex action potentials (originating from rat Purkinje neurons) as well as the interspike background activity (RBA) were recorded extracellularly by a single channel electrode system (more details in: Šaponjić *et al.* 2001). A method for separating and extracting these components was described in our previous work (Janaković *et al.* 2001). Owing to their mutual similarity, it is possible to average all recorded simple (as well as complex) action potentials originating from one recording. The waveforms obtained in such a way we named averaged simple (SAP) and complex (CAP) action potentials. Their Fourier amplitude spectra are shown in Fig. 1.

It could be seen (Fig. 1.), that the SAP spectrum is characterized by one maximum, positioned approximately at 500 Hz, while in the CAP spectrum three maximums appear: at around 200, 800 and 1500 Hz. On the other hand, normalized mean Fourier amplitude spectra of the interspike recorded background activity, from 12 recordings, are presented in Fig. 2. All 12 mean RBA spectra had shapes similar to a typical SAP spectrum, with one maximum positioned at around 500 Hz. Mean RBA spectra, however, possess some features not present in the SAP spectrum: a wide band noise and a low frequency (<100 Hz) activity. In addition, amplitude scaling of all spectra was different.

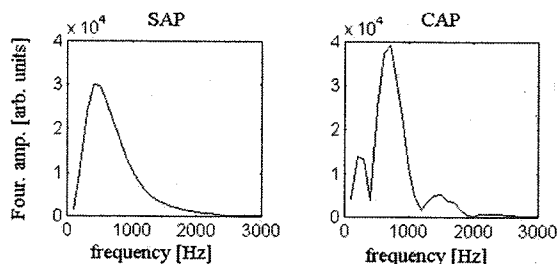


Fig. 1. Typical Fourier amplitude spectra of averaged simple (SAP) and complex (CAP) action potentials, obtained from one of the 12 rat cerebellar cortex recordings.

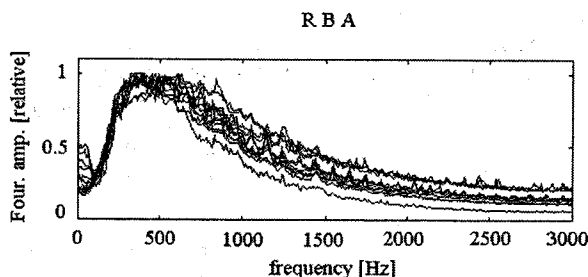


Fig. 2. Normalized mean Fourier amplitude spectra of interspike recorded background activity (RBA), calculated from 12 recordings, 120 s each.

Similarity between RBA and SAP Fourier amplitude spectra shapes indicated that it could be possible to simulate RBA by superimposing a certain number of elementary SAP waveforms. During this process, each individual SAP should be attributed with a random intensity (amplitude) and position within the simulated background activity (SBA). As the resulting SBA amplitude spectrum shape depends neither on intensities nor on positions of individual SAP waveforms, it should be expected that SBA and SAP spectral shapes do not differ significantly, as well. This was confirmed in case of all 12 RBA simulations, presented in Fig. 3.

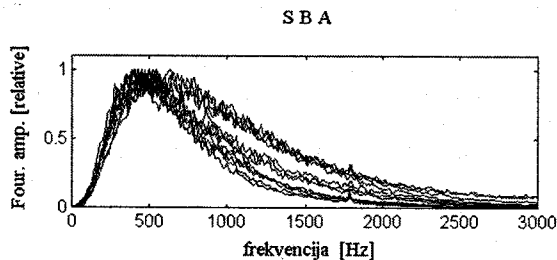


Fig. 3. Normalized mean Fourier amplitude spectra of SBA, simulations of the 12 RBA signals.

If one regards the low frequency components as negligible, and if the wide band noise is assumed to be additive, relation between mean spectra of the recorded,

Amp(RBA), and simulated, Amp(SBA), background activities could be approximately expressed as $\text{Amp(RBA)} = C \cdot \text{Amp(SBA)} + N$, where C stands for the scaling factor and N for the mean noise amplitude. This linear relation was tested and a significant correlation between all 12 mean RBA and SBA spectra was found (Kalauzi *et al.* 2003).

As the number of superimposed SAP increases, so do all amplitudes of the mean SBA spectra, Amp(SBA), without altering the spectral shape. In order to establish a relationship between the mean amplitude of the Amp(SBA) spectrum, $\overline{\text{Amp(SBA)}}$, and the superposition frequency, f_{sup} (which is ratio of the number of SAP waveforms superimposed and duration of the SBA, $T = 4$ s), a series of 8 SBA signals was generated, where f_{sup} was varied: 25, 50, 125, 250, 1250, 2500, 5000 and 12500 SAP/s. (Fig. 4.). In this series of simulations, uniform probability distribution for SAP intensities and positions was adopted as the simplest one.

In each of the 12 recordings, SAP waveform and the corresponding RBA signal were extracted from the same signal, recorded by one amplification system. Consequently, there was no need for an independent calibration of the system. On all plots, Fourier amplitudes were expressed in arbitrary units, rather than μV .

The simulations we performed, as well as RBA/SBA spectral similarities, allow us to assume that an RBA signal could also be characterized by a superposition frequency, originating from spikes of neurons positioned around the electrode. The superposition frequency of surrounding neuron spikes may then serve as a measure of the population activity. The function $\overline{\text{Amp(SBA)}} = f(f_{sup})$, from Fig. 4., could be used as a kind of a calibration line for reading these natural f_{sup} values, if the corresponding value $\overline{\text{Amp(RBA)}}$ had been previously measured. However, the use of this particular calibration line resulted in systematic reading of low values for f_{sup} . As an example, from one of the recordings, the measured mean amplitude of the background activity was $\overline{\text{Amp(RBA)}} = 712.28$, which led to reading the value of $f_{sup} = 21$ spikes/s, from the line presented in Fig. 4. (the corresponding point could not even be drawn on the plot, since it would be positioned in the extreme left lower corner of the picture). By observing the corresponding SBA signal in time domain, a conclusion could be derived that this value for f_{sup} is an underestimation. Spikes in SBA appear rarely, individual SAP waveforms are visible as distinct phenomena, which is not the case in any of the RBA recordings. Visually, RBA and SBA start to resemble at $f_{sup} > 4000 - 5000$

SAP/s. The described underestimation could only be due to the uniform probability distribution for SAP intensities, used for SBA generation. Obviously, uniform distribution does not match the real one, acting when the surrounding neurons AP are being recorded with a microelectrode in the rat cerebellum. Further, the direction of this systematic error indicates that, with a real distribution, there are more spikes with small intensities, than in the case of the uniform one. A need arose, therefore, to derive a more realistic distribution function for probabilities that an AP, generated by a neuron surrounding the electrode, is being recorded as an RBA component of intensity i . In order to be more accurate, the new calibration lines should lie below the one obtained with uniform distribution.

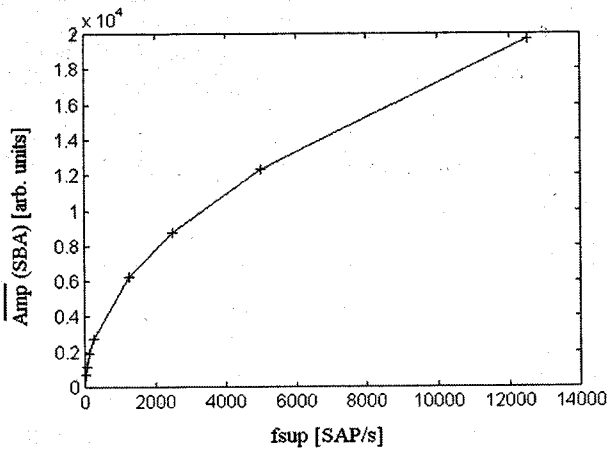


Fig. 4. Dependence of $\overline{\text{Amp}}(\text{SBA})$, average Fourier amplitude of the simulated background activity SBA (computed from mean spectra; range: 10-3000 Hz, resolution 10 Hz), on f_{sup} , superposition frequency of averaged simple action potential (SAP) waveforms.

Derivation of the probability distribution that an AP, as part of the RBA signal, is recorded with intensity i

Suppose that there is an even, planar, arrangement of neurons around the electrode tip (which approximately corresponds to the situation in a cortical layer), with surface density η [cells/m²]. let us observe a differential ring of width dr , centered around the electrode tip, the integration limits being $r_0 < r < r_m$ (r_0 distance from the nearest, r_m from the most distant neuron). if we denote the ring surface as dS , the number of neurons in the ring is

$$dN = \eta dS = \eta 2\pi r dr.$$

The number of neurons in the whole field of integration is then

$$N_t = \int_{r_0}^{r_m} dN = \int_{r_0}^{r_m} \eta 2\pi r dr = \eta 2\pi \frac{r_m^2 - r_0^2}{2} = \eta \pi (r_m^2 - r_0^2).$$

Probability density function that a neuron is found at a distance r could be calculated as

$$Pr = \frac{dN}{N_t dr} = \frac{2\pi \eta r}{\pi \eta (r_m^2 - r_0^2)} = \frac{2r}{r_m^2 - r_0^2}$$

Spike generation frequency varies among neurons. Let us observe a cell ensemble, containing N_e neurons, and let $ns(j)$ be the number of spikes generated, during a time interval T , by the j -th cell. The average number of spikes generated by an ensemble cell, during T , would then be

$$\overline{ns} = \frac{1}{N_e} \sum_{j=1}^{N_e} ns(j).$$

It may further be assumed that \overline{ns} does not depend on the choice of ensemble, if its neurons are contained within an independently formed geometric structure, such as a differential ring. Therefore, \overline{ns} does not depend on r - neuron-electrode distance. Number of spikes, recorded from the whole integration area during T , could then be expressed as

$$nt = \overline{ns} N_t,$$

therefore

$$d(nt) = \overline{ns} d(N).$$

Probability density function that a spike is generated from a distance r is then the same as that a neuron is located at a distance r :

$$P_{sr} = \frac{d(nt)}{(nt) dr} = \frac{\overline{ns} dN}{\overline{ns} N_t dr} = \frac{2r}{r_m^2 - r_0^2},$$

while the corresponding probability distribution functions would be

$$Pr = P_{sr} = \int_{r_0}^r \frac{2r}{r_m^2 - r_0^2} dr = \frac{r^2 - r_0^2}{r_m^2 - r_0^2}. \quad (1)$$

From this point on, in order to derive the distribution for the probability that a spike of intensity i is recorded by the electrode, a spike attenuation law (relating i with r , and modeling this process in the extracellular environment) is required. For this purpose we adopted the power function (although other analytical expressions could equally be tested):

$$i(r) = \frac{C}{r^k},$$

where k is the attenuation exponent, C - attenuation constant. If i_0 and i_m are spike intensities recorded from the nearest neuron and the most distant one, respectively, and r_0 and r_m the corresponding distances, then

$$i_0(r) = \frac{C}{r_0^k}, \quad i_m(r) = \frac{C}{r_m^k}.$$

By differentiating these two relations, and substituting into (1), one obtains:

$$P_i = \left(\frac{i_0}{i} \right)^{2/k} \frac{i^{2/k} - i_m^{2/k}}{i_0^{2/k} - i_m^{2/k}}.$$

Finally, if relative intensities are introduced:

$$i_r = \frac{i}{i_0}, \quad i_{rm} = \frac{i_m}{i_0},$$

the required distribution could be written as

$$P_{ir} = \frac{1 - i_{rm}^{2/k} i_r^{-2/k}}{1 - i_{rm}^{2/k}}. \quad (2)$$

In this work we explored how dimensionality of the arrangement of neurons around the electrode influences the distribution (2). Instead of a planar ring, we observed a 3-dimensional cell arrangement, (spherical shell centered around the electrode tip). Applying the same mathematical procedure as described for the 2-dimensional case, an isomorphic distribution has been obtained

$$P_{ir} = \frac{1 - i_{rm}^{3/k} i_r^{-3/k}}{1 - i_{rm}^{3/k}}. \quad (3)$$

RESULTS AND DISCUSSION

Using distributions (2) and (3), a new series of SBA signals was generated, and the corresponding fam-

ily of calibration lines was constructed, with parameter values $k = 1; 2; 3; 4$, and $i_{rm} = 0.01; 0.001$ (Fig. 5., only lines obtained with $i_{rm} = 0.01$ are shown). The 3-dimensional calibration line was obtained by setting $k = 1, i_{rm} = 0.01$. On log-log plots, all calibration lines were highly linear, with slopes: $S = 0.5655 \pm 0.0443$, for 2-dimensional cell arrangement, 8 lines, 0.5126 for the 3-dimensional case, 0.5310 for uniform distribution. Based on this, a method for measuring activity changes of neuronal populations has been suggested (K a l a u z i and S p a s i ć 2003). If we analytically express the family of lines from Fig. 5. as

$$\log[\overline{Amp}(SBA)] = S * \log(fs_{sup}) + P,$$

where S stands for the slope, P for the y-intercept, and if $\overline{Amp1}(RBA)$ and $\overline{Amp2}(RBA)$ are two measured RBA average Fourier amplitude values under different experimental conditions, such as before and after intensifying excitatory or inhibitory inputs (by electrical stimulation or pharmacological treatment), the following formula is valid

$$fs_{sup2} / fs_{sup1} = [\overline{Amp2}(RBA) / \overline{Amp1}(RBA)]^{1/S},$$

defining ratio between two values of the population activity.

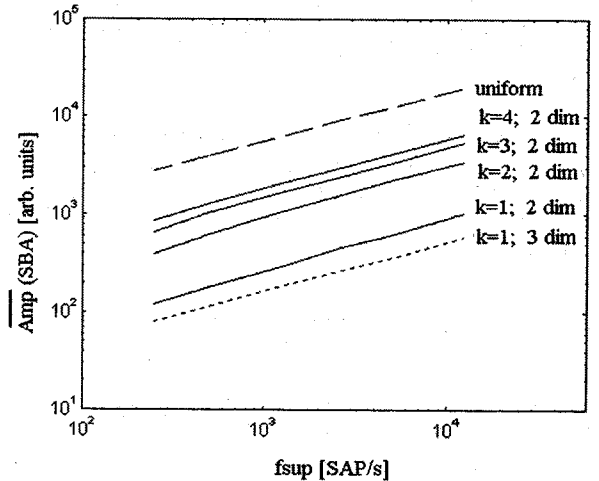


Fig. 5. Calibration lines, constructed using uniform (long dashed), and the new distributions, defined with formulas (2) and (3) (solid line for 2-dim.; short dashed for 3-dim. neuron arrangements). New lines lie below the uniform one, confirming the greater accuracy of the distributions defined by (2) and (3).

Quantitative comparison of individual neuronal behavior with the populations they are part of, still remains a significant problem in neurophysiology. With single channel recordings, this could be achieved by counting individual spikes of the nearest neuron and

comparing the result with population activity changes, obtained by the method described in our work.

Single channel approach is complementary to the application of multichannel measuring technique of neuronal population activity. Multichannel recording technique allows detection of spike sequences and field potentials of certain number of neurons but not of large population (in hippocampus, Nadasdy 1998), since the number of neurons to be recorded is limited by the number of channels. However, it is possible to measure absolute values of the population activity, rather than their changes, but not to follow activities of two or more populations simultaneously using one set of electrodes.

Single channel approach, presented in our study, allows measuring changes, but not absolute values of a population activity. We found that calibration line slopes were similar for the two tested distributions (uniform and the one defined by expression (2)), as well as for the two dimensionally different cell arrangements. Whether this indicates that slopes may be generally independent on these factors, remains to be explored. In addition, error of this method, based on calibration lines slopes, still has to be estimated.

We need to be cautious about the transition from the present, relative, to an absolute method. At this point, we are still not able to select only one from the family of calibration lines. This goal could perhaps be achieved by modeling a more accurate distribution for spike intensities, determining parameter values of the new distribution and accounting for exact dimensionality of neuronal arrangements in different brain structures.

Advantages of this method include the possibility to track activities of large neuronal populations. Namely, it was found that SBA/RBA resemblance could be achieved for $fsup > 4000 - 5000$ spikes/s. Although the average firing rate per cell, \bar{n}_S , has not been measured for our populations, we were probably dealing with populations larger ($N_e = fsup / \bar{n}_S$) than the number of neurons that had been followed with a typical multichannel equipment. Also, by placing more microelec-

trodes in different brain sites, a new possibility arises: to follow and correlate activities of more than one populations simultaneously.

In future, it may be possible to obtain an activity waveform of a neuronal population, continuously in time, by introducing a moving Fourier window, and calculating $Amp(RBA)(t)$.

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НЕКИ ДЕТАЉИ МЕТОДЕ ЗА МЕРЕЊЕ ПРОМЕНА АКТИВНОСТИ НЕУРОНСКИХ ПОПУЛАЦИЈА

А. КАЛАУЗИ¹, СЛАЂАНА СПАСИЋ¹ и МИЛКА ЂУЛИЋ²

¹Центар за мултидисциплинарне студије, Универзитет у Београду,

²Институт за биолошка истраживања "Синиша Спанковић",

11000 Београд, Србија и Црна Гора

Једноставни, сложени акциони потенцијали (АР) и међуспајковна основна активност (МОА) детектовани су и издвојени из 12 регистрованих сигнала коре малог мозга пацова. За сваку регистрацију, формиран је усредњени једноставни АР (ЈАР). Спектри Фуријеових амплитуда ЈАР и МОА сигнала били су слични код свих регистрација. За једну од регистрација, суперпозирањем случајних усредњених једноставних акционих потенцијала формиран је низ симулација међуспајковне основне активности (SOA), при чему је варирана фреквенција суперпозиције

(*f_{sup}*). Израчунавана је средња Фуријеова амплитуда, $Amp(SOA)$, у циљу коришћења $Amp(SOA) = f(f_{sup})$ као калибрационе линије за читавање *f_{sup}* од МОА, што је мера за активност популације. Изведена је расподела вероватноћа за интензитете ЈАР, помоћу које је конструисана фамилија калибрационих линија. Како су све линије линеарне и паралелне у лог-лог дијаграму, са нагибом *S*, промена активности популације може се израчунати као

$$f_{sup2} / f_{sup1} = [Amp2(RBA) / Amp1(RBA)]^{1/S}$$