In-vitro neuronal networks: evidence for synaptic plasticity

Anat Elhalal, David Horn
School of Physics and Astronomy, Tel Aviv University Tel Aviv, 69978, Israel

Abstract

In vitro neuronal networks are known to fire in Synchronized Bursting Events (SBEs), with characteristic temporal width of 100 ms. We treat these events as the principal data atoms of the network. Applying SVD (or PCA) to the spatial information, i.e. activity of neurons per burst, we demonstrate characteristic changes that take place over time scales of hours. We consider this as evidence for synaptic plasticity. We discover clusters of SBEs in the reduced SVD space, representing behaviour of the experiments at different times. We find two interesting characteristics of SVD analysis of these data which may be helpful to future users of SVD and PCA.

***Key words*:** SVD, clustering, synchronized bursting events, neuronal networks

1 Introduction

We present new findings regarding neuronal network recordings utilizing SVD. *In vitro* neuronal networks were found to fire in Synchronized Bursting Events (SBE). Segev et al [2] found clusters of SBE, based on cross-correlations between their spatio-temporal patterns. We find new attributes of the SBEs utilizing SVD.

Singular value decomposition (SVD) has become a common method for analyzing biological data [1,3,5]. In this work, the usage of SVD is identical to Principal Component Analysis (PCA). We apply it to data of several experiments carried out in the laboratory of E. Ben Jacob at Tel Aviv University.

Recently, work by Segev [4] enabled long-term measurements of living *in-vitro* neuronal networks. The networks exhibit SBE, a period of about 100 ms in which the entire network fires rapidly. Between SBEs neurons tend to fire little and sporadically. Therefore, the SBE is considered as the principal data-atom of the network.
These networks are grown on a Multi-Electrode-Array (MEA) from cells dissociated from a one day-old rat brain. The electrical activity is then transferred through amplifiers to spike-sorting software. The resulting recordings are from specific neurons, with a time-resolution of 12,000 bins per second. The Bursts are extracted from the data, and presented as matrices of Neurons × time bins ($\boldsymbol{N}\times \boldsymbol{t}$), see figure 1.

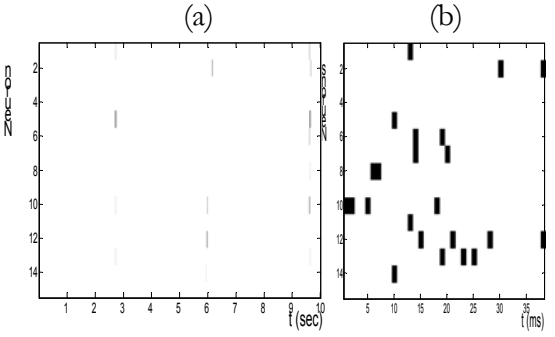


Figure 1 Example of SBEs from an experiment in which 15 neurons were recorded. The SBE lasts about 50 ms. **(a)** 3 different SBEs during 10 sec **(b)** A single SBE, time scale of ~50 ms.
(Data derived from an experiment whose recordings started on 13/03/00)

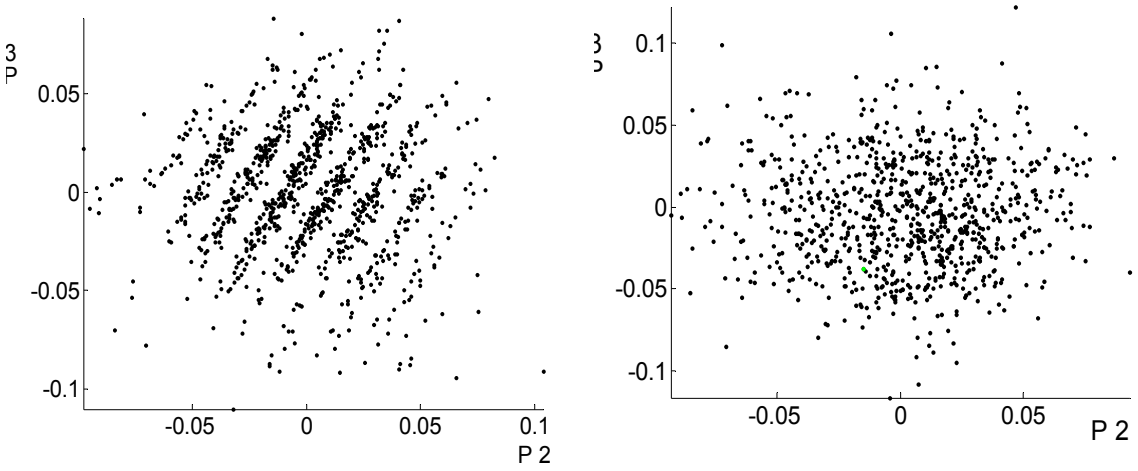


Figure 2 The distribution observed in the space spanned by SVD components 2 and 3, of spatial information extracted from data that was recorded starting on 13/03/00. **(a)** SBEs from the first few hours of the experiment **(b)** SBEs from later hours. Stripes are visible in **(a)** but not in **(b)**.

As biological phenomena, SBE have variable durations and noisy temporal start points. In order to apply SVD, the data should be arranged in vectors of constant length, whose entries have the same functional meaning. Therefore, it is impossible to apply SVD to SBEs as they are, and some standardization process is needed. We chose to use the spatial information of the SBE, by summing them over the temporal dimension. We apply SVD to a matrix composed of such representations of all SBEs: [number of firings per neuron per SBE] x [neurons]. Our goal is finding some structure within the ensemble of SBEs. Therefore, we inspect the first principal components of SVD. These components are responsible for most of the variance in the SBE space, and therefore looking at them can reveal the main structures in the data, structures that in the original space might have been hidden in noise.

2 New observations regarding the experimental data

Analyzing the ensembles of SBEs with SVD, we discovered evidence for development with time. This development is of special interest, as a clue to the plasticity of *in-vitro* networks.

2.1 SVD reveals network development

Analyzing the data with SVD has revealed a pattern of stripes in the projection plot for SBEs from the first few hours of an experiment, but not from a later time (see figure 2, we will discuss the reasons for appearance of stripes in SVD projection plots in section 3). Clearly, the system has gone through some change. In order to investigate the nature of this change we created simulated data with the same distribution as the original data, but with no correlations between neurons. The distributions were calculated separately for the two data-sets, of earlier and later SBEs. Stripes appeared in all visualizations of SVD of the simulated data, meaning that it was not the difference in the distributions but the correlations in the data, which were responsible for appearance of stripes. Removing from the data some of the most active neurons has also made the stripes disappear.

The direction perpendicular to the stripes can be expressed as a vector in the space of neural activities. Looking at the component values we realize that the significant ones emphasize the difference between two key neurons. We hypothesized that stripes disappear when correlation evolves between these key neurons. Calculation of the Pearson correlation between these neurons in the earlier and later data-sets strengthens this hypothesis. Very low correlations were found in the earlier SBEs (and the simulated data), and higher magnitude correlations in the later SBEs. It can be claimed that these correlations can be found directly, with no need of the analysis with SVD. It is however the latter that pointed us to the right place to seek these correlations.

2.2 New clusters are found with the use of SVD

New clusters of SBEs were found using SVD, analysing the spatial information of a few experiments. Example of a projection plot that exhibits such clusters can be seen in figure 3a.

It is now possible to go back to the biological data and investigate what are the differences between SBEs that belong to different clusters. The three clusters in figure 3a (as well as clusters found for data from other experiments) were different in the mean firing rates of the neurons, the standard deviations of the firing rates and the mean SBE (see figure 3c). The most interesting difference, however, is the tendency of SBEs from different clusters to appear in different temporal experimental periods. The clusters allow segmentation of the experiment into three different periods (see figure 3b).

We have demonstrated that the system changes with time in two independent methods, based on clustering, and on appearance of special patterns in SVD. It is hard to measure directly synaptic plasticity at a network level, but our findings clearly demonstrate it.

3 New observations regarding the SVD method

During our research we have reached some new understandings of the nature of SVD that may be of use to future users. The first is the appearance of a special pattern of stripes under certain conditions, and the second is the appearance of axis-like formations.

3.1 Appearance of stripes in SVD visualization

As stated in the former section, stripes appeared in SVD projection plots of data with low correlations, and a few key neurons (whose firing rate distributions had higher magnitude mean and standard deviation). In this section we will elaborate on the mathematical reasons for appearance of such stripes.

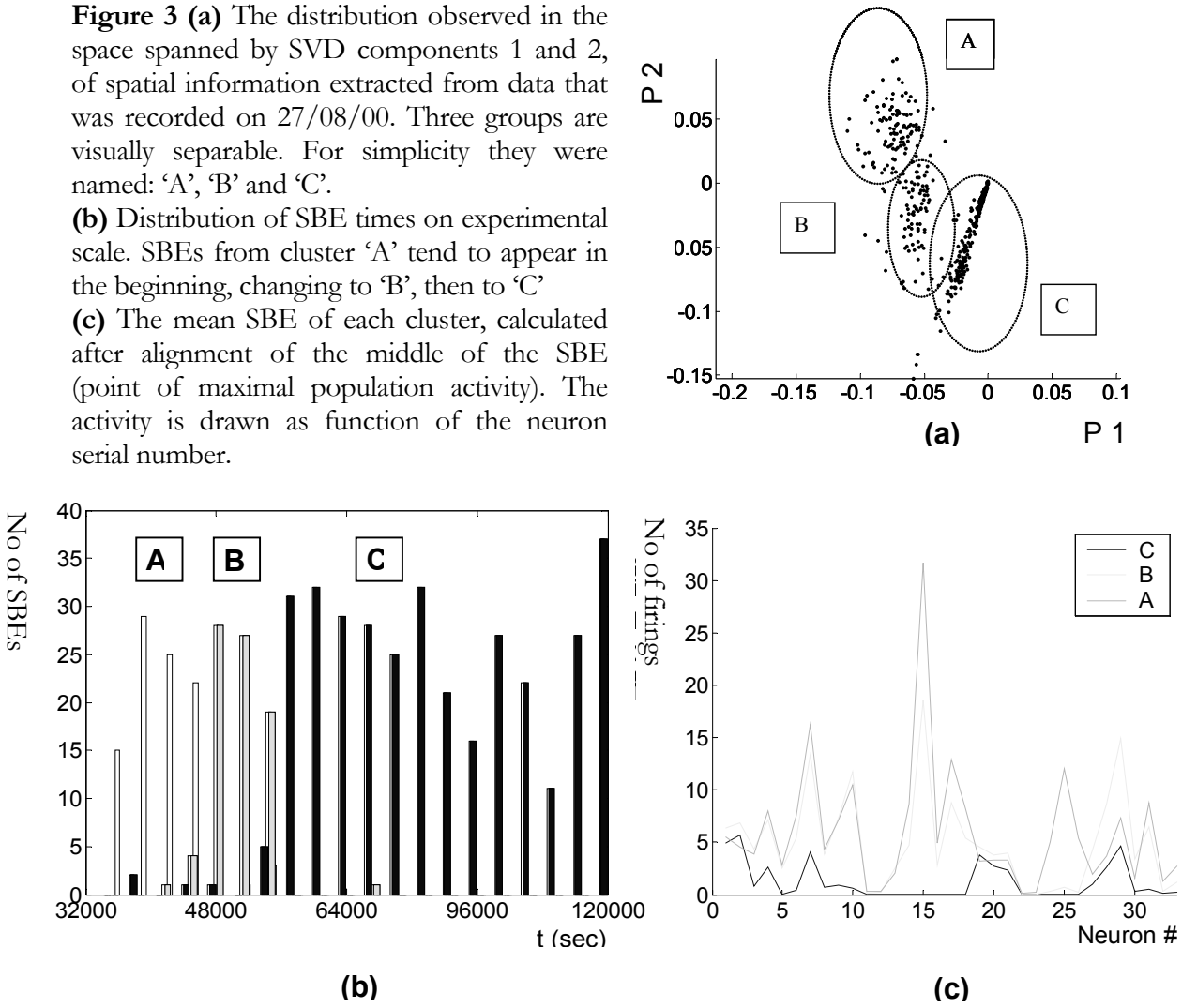
SVD chooses eigenvectors (principal components) according to their eigenvalues. Once there are active neurons with low correlations between them, SVD may be expected to choose their difference as one of its leading components:

Consider C = <(nα-nβ)^2>, which is the correlation that would correspond to a variable nα-nβ. (The eigenvalue corresponding to C would be $s = \sqrt{m * C}$, where m is the number of data points.)

C = <nα^2> + <nβ^2> - 2 * <nα•nβ>

If the first two terms are large, and the third term is small, C will have a large numerical value. Hence nα-nβ has high chance of being one of the leading components, or playing a

Figure 3 (a) The distribution observed in the space spanned by SVD components 1 and 2, of spatial information extracted from data that was recorded on 27/08/00. Three groups are visually separable. For simplicity they were named: 'A', 'B' and 'C'.
(b) Distribution of SBE times on experimental scale. SBEs from cluster 'A' tend to appear in the beginning, changing to 'B', then to 'C'
(c) The mean SBE of each cluster, calculated after alignment of the middle of the SBE (point of maximal population activity). The activity is drawn as function of the neuron serial number.



prominent role in some combination of the leading components. Since the first principal component has usually coefficients of the same sign, such a difference may be expected to appear in principal components 2 and 3. In our case this difference was a good approximation to a linear combination of principal components 2 and 3, hence stripes were visible in the plane spanned by these two components. The stripes reflect quantization of the values of $n\alpha$-$n\beta$, which is natural if the two variables have integer values. The fact that the stripes have some thickness is due to the contamination by small components of other neurons in the linear combination of principal components 2 and 3.

We have verified that the stripes are due to the principal component selection of SVD using the following simple exercise. Using the two data sets of figure 1, we have run separate SVD analysis on each, and then used the coordinates of the early data to draw the latter data and vice versa. It turned out that the first case was one that showed stripes and the latter case did not. Hence we proved that it was the selection of principal components by

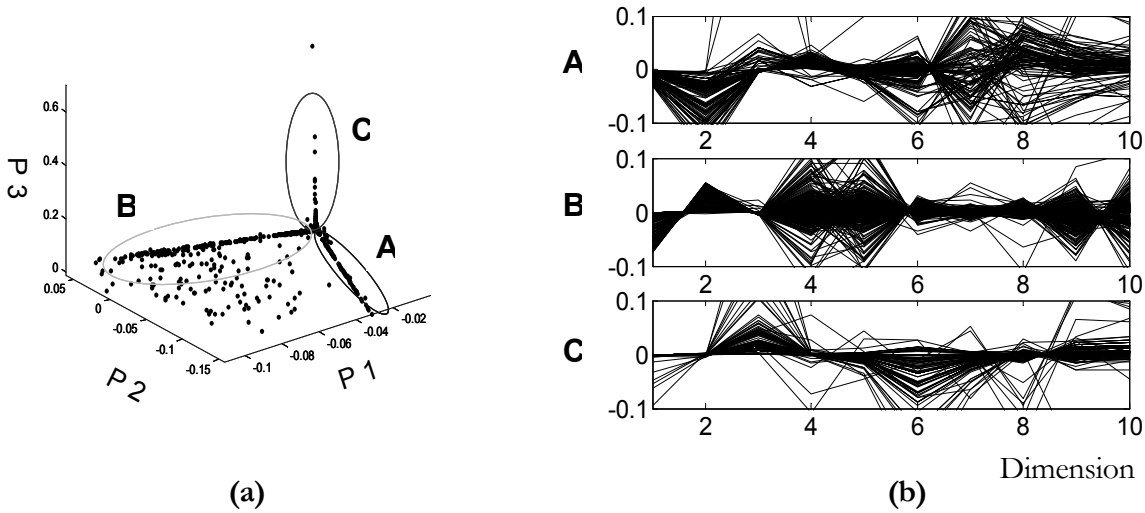


Figure 4 (a) Axis like formation in SVD 3-D projection plot **(b)** Drawing of the data in parallel coordinates: the values of the first 10 principal components for each SBE are plotted for each cluster separately. Each point of **(a)** is described by a polygon in **(b)**. (This analysis was performed on data derived from an experiment whose recordings started on 3/10/00.)

the early set of data that is responsible for stripe formation. Once again this fits the understanding that in the early data there was no correlation between the two key neurons, hence their difference turns out to be an almost pure linear combination of principal components 2 and 3.

3.2 Appearance of an axis like formation in SVD visualization

In some of our analysis, axis like formations appeared in SVD projection plots (for example see figure 5a). SBEs that lie on the different axes can be analysed separately, in order to understand the biological meaning of such patterns. Doing so, we found out that the three clusters of SBEs are orthogonal to each other, in the sense that in each group the SBEs are composed of firings of different neurons.

Drawing the data in parallel coordinates [2] demonstrates that SBEs from different groups tend to express themselves in different principal components. The three clusters have therefore other dimensions that express their variance, but they appear as almost linear (and orthogonal) when plotted in the first 3 principal components.

4 Summary

We have demonstrated how SVD analysis of SBEs can lead to biological insights even when the analysis is based only on spike counts of neurons within the SBEs. This is sufficient to discover clusters of SBEs. Usually these clusters reflect grouping of SBEs according to the time periods in the experiment. Each such group may contain events produced during several hours of an experiment.

Obviously changes in time should reflect underlying dynamic plasticity. It is only natural to assume that this plasticity occurs at synaptic levels, modifying the connectivity between neurons. In one experiment we were able to demonstrate this modification by comparing correlations between two neurons during different periods. The fact that these correlations should be investigated came about through the observation of stripes in the SVD analysis.

This phenomenon of stripe formation is an epiphenomenon, since, while giving an impression of clustering, it does not reflect clustering. Yet it played an important role in leading us to the conclusion that correlations should be looked at.

We have emphasized two curious phenomena that we have encountered in SVD visualization. One is stripe formation, that we understand as due to the occurrence of strongly active neurons that are uncorrelated. Such neurons influence the choice of principal components in SVD, leading to stripes that are not clusters although they may look as such. The second is formation of linear clusters that look like orthogonal structures. Apparent orthogonality is due to true orthogonality: these clusters are characterized by activities of different neurons in each cluster. The difference in identity of neurons leads to the expression of SBEs of different clusters by different principal components and, when viewed in a selected three-dimensional representation of all clusters, results in the apparent axis-like structure.

All in all, SVD seems to be a promising method for analysis of this kind of data.

Acknowledgements

This research was partly supported by the Israel National Science Foundation.
We wish to thank Eshel Ben-Jacob, Ronen Segev and Itay Baruchi for fruitful discussions and for sharing their data.

Bibliography

[1] O. Alter, P. O. Brown and D. Botstein, Singular Value Decomposition for Genome-Wide Expression Data Processing and Modeling, Proceedings of the National Academy of Sciences 18 (2000) 10101–10106
[2] A. Inselberg, B. Dimsdale, Parallel coordinates – a tool for visualizing multidimensional geometry, Proceedings of the first IEEE conference on visualization – visualization 90 (1990) 261-378
[3] O. Kliper, D. Horn, B. Quenet and G. Dror, Analysis of Spatiotemporal Patterns in a Model of Olfaction, presented at CNS03.
[4] R. Segev, Self-organization of in-vitro neuronal networks, PhD thesis, Tel Aviv university, Israel, 2002.
[5] M. E. Wall, A. Rechtsteiner, L. M. Rocha, Singular value decomposition and principal component analysis, to appear in: A practical Approach to Microarray Data Analysis. D. P. Berrar, W. Dubitzky, M. Granzow, eds. LANL LA-UR-02-4001.