Phase Lock Analysis of Signals from Neuronal Networks on Multi-well Microelectrode Array with a Common Group/Reference*

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***Abstract*— Here, we investigate phase lock value (PLV) analysis of six-well microelectrode array (MEA) data from neuronal cell cultures. These MEAs have a common ground and reference electrode (CGRE) for all microelectrodes (MEs) in all the separate wells on one MEA plate. We computed PLVs over all channel pairs in a single well and over all channels on one MEA plate with regard to one channel, to demonstrate the applicability and a pitfall of the PLV analysis. PLV may sometimes be strong even between signals measured from different wells; this indicates that a common signal, although not easily otherwise observable, may be present in those signals.**

I. Introduction

MEAs [1] are employed to record electrical field potentials generated by neuronal cells and populations, in general, neuronal cell network. To investigate the properties of neuronal networks, it is traditional to observe the action potential spikes and compute characteristic figures, such as average number of spikes per minute or spike burst duration. To gain information on the networks, information from several channels should be jointly analyzed. In this paper, we investigate PLV [2] as a network connectivity measure.

II. Methods

A. MEA, Recordings, and Cell Cultures

Six-well MEAs (Multi Channel Systems MCS, GmbH, Reutlingen, Germany, ME ø30 µm, inter-ME distance 200 µm, 9 MEs and a CGRE in each well) consist of six separate cell culture wells, with a large CGRE in each. The CGREs on a MEA plate are connected together, and the MEA amplifiers utilize only one reference. In contrast, in one often employed MEA type there are 59 MEs in one well and one reference.

MEA data was recorded at 20 kHz sampling rate using the MC_Rack software (MCS) and MCS hardware (MEA1060-INV-BC) with the passband of 1 Hz – 8 kHz.

The cell cultures consisted of embryonic stem cell derived neuronal cells treated with bicuculline, originally for another study.

B. PLV

Given two signals measured concurrently via different channels and their instantaneous phases Φ_1 and Φ_2, complete phase locking is observed as $\Delta\Phi = \Phi_1 - \Phi_2 = c$, with c a constant. For complete phase locking PLV is unity, and for total asynchrony zero. Here PLVs were calculated between all channels in one well, and between one channel and all channels on one MEA plate, i.e., also in different wells.

III. Results

A PLV analysis example for one well is shown in Table I indicating that in this case signals measured via electrodes e_4 and e_7 are highly phase locked, and most PLVs are above significance threshold.

TABLE I. PLVS FOR EACH SIGNAL PAIR IN ONE WELL. BOLD INDICATES SIGNIFICANT PLV.

	e_2	e_3	e_4	e_5	e_6	e_7	e_8	e_9
e_1	0.1541	**0.2089**	**0.5455**	**0.1430**	**0.1615**	**0.3053**	**0.3767**	**0.1542**
e_2		**0.3108**	**0.1592**	**0.1849**	**0.2177**	**0.2603**	**0.1849**	**0.2405**
e_3			**0.1575**	**0.3729**	**0.4165**	**0.1836**	**0.2290**	**0.4632**
e_4				**0.1060**	**0.1127**	**0.7372**	0.1669	**0.1154**
e_5					**0.2967**	**0.1260**	**0.1772**	**0.2903**
e_6						**0.1367**	**0.1791**	**0.3197**
e_7							0.2091	**0.1436**
e_8								**0.1600**

PLVs between signals from all MEs on one 6-well MEA plate and one channel (C4) are shown in Fig. 1. From Fig. 1 it can be seen that the C4 signal is to some extent phase locked with one other channel in well C (the orange bar), but also phase locked with three channels in well B, which should not be the case for independent wells.

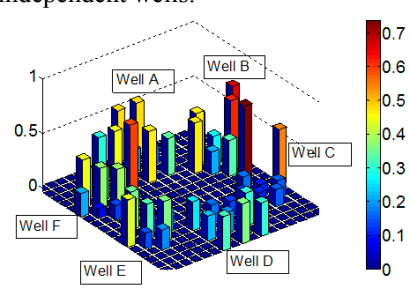

Figure 1. PLVs for all channels of a 6-well MEA with reference to the channel C4.

IV. Conclusions

PLV may be a useful measure of network connectivity, but due attention must be paid on the signals, analysis and the results, especially when analyzing signals from systems with a CGRE for otherwise independent measurements.

References

[1] M. Taketani and M. Baudry (Eds.), *Advances in Network Electrophysiology*. New York: Springer, 2006.

[2] F. Mormann, K. Lehnertz, P. David, and C. E. Elger, "Mean phase coherence as a measure for phase synchronization and its application to the EEG of epilepsy patients," *Physica D: Nonlinear Phenomena*, vol.144, pp.358-369, Oct. 2000.

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