

Evaluation of the effective and functional connectivity estimators for microelectrode array recordings during *in vitro* neuronal network maturation

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Abstract—During maturation, neurons and neuronal ensembles interact and build connections. Changes in the network structure have effects on the overall electrophysiological activity, and consequently on the observable connectivity. In this paper, we assessed effective and functional connectivities during neuronal network development by means of directed connectivity and synchronization, respectively.

For that, we analyzed *in vitro* dissociated mouse cortical neuronal networks during four weeks using microelectrode arrays. Functional and effective connectivities were estimated with CorSE and transfer entropy (TE), respectively. Here, we describe the advantages of the methods relative to each other.

We observed that the functional connectivity analysis may provide networking information in earlier phases of network development than effective connectivity. On the other hand, effective connectivity analysis provides information on the sources and targets of information flows. By corroborative analysis using CorSE and TE, one can investigate possible effects of early synchronizations on information transfer during the later stages of network development.

In conclusion, using effective and functional connectivity assessments jointly provides for enhanced analysis of the development of information transfer during the structural development of a neuronal network.

Keywords—functional connectivity, CorSE, effective connectivity, transfer entropy, microelectrode array.

I. INTRODUCTION

Functioning neuronal cells have a need to structurally connect to one another and form neuronal populations during their maturation [1]: Originally distinct neuronal populations wire together, communicate with each other, and form larger networks. It is well known that during this process, although the number of interacting populations can vary highly due to neuronal plasticity, the overall number of interactions is expected increase [2,3]. Neuronal network relations have been quantified and analyzed widely by means of functional and effective connectivity measures [4,5,6,7]. In this paper, we use the term “functional connectivity” for non-directed connectivity, and “effective connectivity” for directed connectivity given, as in [8,9], here, quantified by correlated spectral entropies (CorSE), and transfer entropy, respectively.

Functional connectivity is commonly analyzed by means of neuronal synchrony by evaluating simultaneous occurrences of detected action potentials, i.e., spikes [10,11], or neuronal bursts [12,13]. Also, quantifying frequency/phase couplings to assess the synchrony between signals in predefined frequency bands, e.g., brain oscillation frequencies seen in electroencephalograms, is also commonly used in functional connectivity analysis [14].

Effective connectivity provides information on the direction of the information transferred, resulting in pathway specific information. Salinas and Sejnowski [15] discussed that the presence of correlations between the activities of pairs of neurons, or synchrony per se, may arise from common inputs or synaptic interactions, or from overlapping perceptive fields, respectively; however, changes in the correlation structure of a neuronal network reflect changes in functional connectivity. The pattern of synchronization also determines the pattern of neuronal interactions, and information transfer efficiency is modulated by synchrony [16,17]. Thus, we here propose that analyzing functional connectivity together with effective connectivity is essential for the assessment of developing neuronal networks, which are highly plastic.

In this work, we evaluated the functional connectivity by means of CorSE, and calculated detailed information pathways with a well-known effective connectivity estimator, transfer entropy (TE), for a developing network of mouse cortical neurons whose electrical activity was measured with a microelectrode array (MEA).

II. METHODS

A. Analysis Overview

Measurements were conducted once on each measurement day. Functional and effective connectivities were calculated between all electrodes for each measurement. Interactions between neuronal populations were analyzed considering each MEA electrode a network node location. The existence of links between nodes was determined according a connectivity strength threshold. For effective connectivity analysis, we revealed information flows by revealing information

sources and targets. The results are given as functions of days *in vitro* (DIV) after plating the cells on MEAs.

B. MEA Measurements

The *in vitro* experiments were performed with commercial dissociated mouse cortical cells (A15586, Gibco, Thermo Fisher) plated on MEAs (Multi Channel Systems MCS GmbH, Reutlingen, Germany). The MEAs were coated with poly-L-lysine and laminin, and cells were seeded as droplets on the MEA plates with 60 TiN electrodes of 30 μm in diameter and 200 μm interelectrode distances on an 8x8 rectangular grid with corner electrodes missing. MEA data were recorded three times a week starting from four DIV until 29 DIV (12 measurements in total).

C. Functional connectivity calculation: CorSE

The functional connectivity of the *in vitro* data used in this paper has previously been estimated by the correlated spectral entropy (CorSE) method [6]. CorSE calculates time variant changes in spectral uniformity distribution and estimates the correlations of these changes between different signal pairs. Here, the same parameters were used; for CorSE calculations we use the same parameters as in [6,18].

Briefly, CorSE was calculated between all signal pairs measured from MEA different MEA channels for each measurement day. Connectivities were decided by thresholding the magnitudes of the correlations at $\text{CorSE} > 0.5$, and connectivity maps were drawn accordingly [6].

D. Effective connectivity calculation: TE

TE from signal y to x , $TE_{y \rightarrow x}$ measures the increase in the predictability of the future and the past of x , once y is known [19]. Thus, TE provides also information on directionality, i.e., information flow. In this paper, we calculated TE in 1 ms bins for delays up to ten bins, and the maximum value of TE was considered as in [5].

Here, connectivity was calculated between all signal pairs measured from different MEA electrodes for each measurement day, and directed connectivity maps were formed according to arbitrarily chosen criterion $TE > 0.1$ after normalization. Normalization was done with respect to the maximum TE value over all 12 measurements.

We also observed network behaviour at each channel by calculating the ratio $r = N_t + N_s / N_t - N_s$, where N_t is the number of connections with the channel in question as the target, and N_s is the number of connections for which the channel is the source. For a purely target channel $r = 1$, for a purely source $r = -1$, and $-1 \leq r \leq 1$.

III. RESULTS

A. Functional Connectivity

The functional connectivity maps of the networks between the 13th and 29th DIV are presented in Fig. 1A. The first connections were seen on the 13th DIV, and thereafter the network gradually expanded while the average connectivity strength was also increasing. The strongest network synchronizations were seen on the 22nd DIV. The results show that

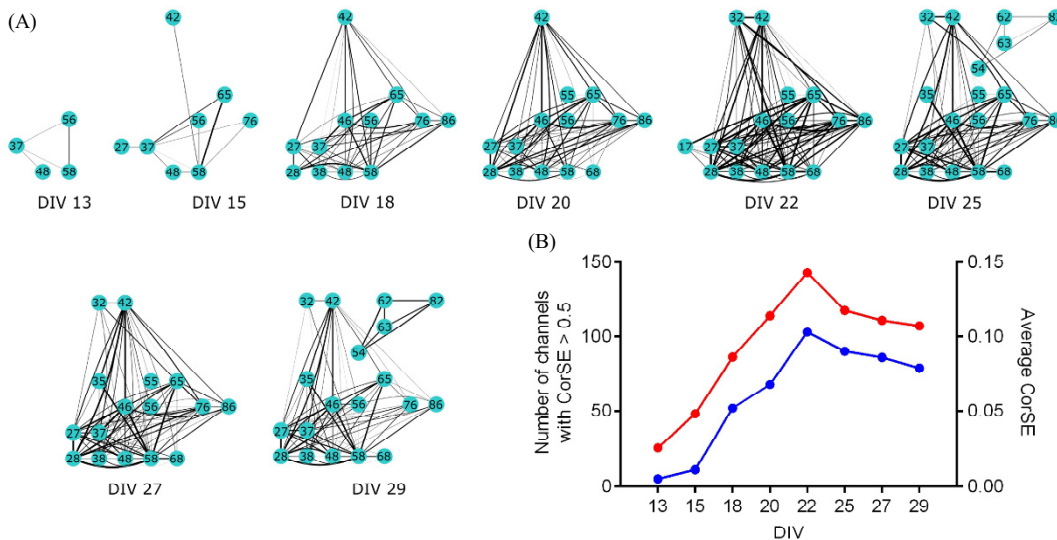


Fig. 1 (A) The functional connectivity maps of the networks between the 13th and 29th DIV. (B) Number of channels with $\text{CorSE} > 0.5$ (blue) and the average connectivity strength (red).

the average connectivity strength was also correlated with the number of connected channel pairs (Fig. 1B).

B. Effective Connectivity

The effective connectivity maps between the 18th and 29th DIV are shown in Fig. 2. The first directed connections were seen on the 18th DIV. Thereafter, the network gradually expanded, and the average connectivity strengths got stronger. The maximum number of connections was seen on the 27th DIV (Table 1).

The results of the source-target analysis are presented in Fig. 3 giving the r values for each channel on the different measurement days. A noteworthy behavior can be observed on the 27th DIV, when the maximum number of channels with $TE > 0.1$ was observed and more channels than before were acting as only sources or targets, i.e., having $r = -1$ or $r = 1$, respectively. The ratio maps (Fig. 3) also show that the channels acting more like sources were more localized, particularly on 27th DIV.

Table 1 presents the source and target behavior of active channels on the measurement days. The total number of connections for some channels are seen to be higher than the median of this network, 4.5. This hub-like behavior (channels that have $N_s + N_t \geq 5$ on at least one of the measurement days) was observed for not only the channels acting as both a source and target simultaneously (e.g., channel 27) but also

for the channels acting almost merely as sources (e.g., channel 58) or targets (e.g., channel 48).

IV. DISCUSSION AND CONCLUSIONS

Results from functional and effective connectivity analysis showed consistent network development during the 4 weeks of culturing. CorSE was advantageous for revealing network synchrony starting from 13th DIV, when no directional information could yet be obtained by effective connectivity analysis. The synchronized channels revealed earlier by CorSE were also later found directionally connected and by TE. A possible cause for this would be the nature of these algorithms: CorSE considers not only detected EAPs but also local field potentials since the algorithm works on complete recordings, whereas TE only considers detected EAPs (see [6] for more information). TE also provides information on whether the local network acts as a source and/or target. Such information is invaluable for locating and analyzing information sources and pathways.

We also observed that some of the channels were functionally connected, i.e., synchronized (e.g., channel 32 on the 22nd DIV) before they could be considered directly connected (on the 27th DIV). This would be the case where the pattern

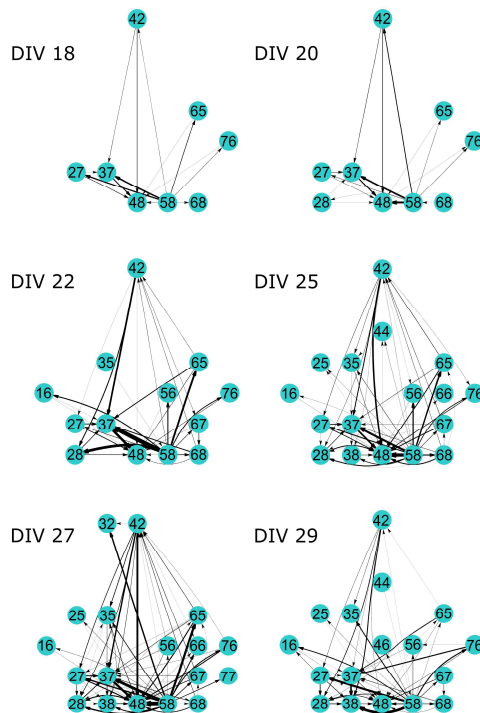


Fig. 2 The effective connectivity maps between the 18th and 29th DIV.

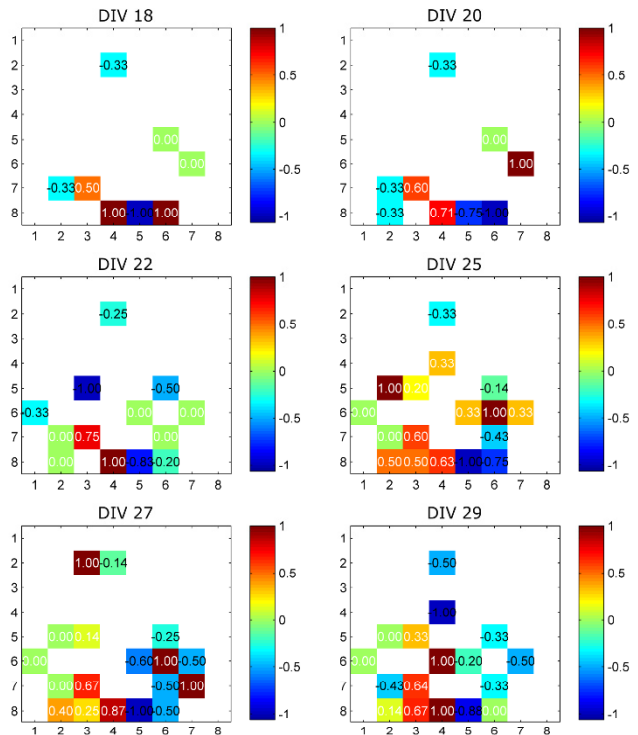


Fig. 3 The r values calculated for each channel of the effective connectivity maps between the 18th and 29th DIV. Channels are placed to coordinates according to their numbers as in MEA layout, e.g., Channel 32 at (3,2).

of synchronization also determined the pattern of neuronal interactions, and the efficiency of transferred information was modulated by synchrony as discussed in [16,17].

In conclusion, the directional information obtained by TE and functional connectivity information obtained by CorSE provided corroborative results in this study. Such multifaceted assessments of neuronal networks is promising for the evaluation of not only *in vitro* network development but also during the assessment of plasticity in the brain where neurons are forming new circuitry to contribute to learning and memory formation.

Table 1 Network behavior at the active channels on the measurement days given as N_s/N_t , with N_s and N_t the numbers of connections for which the channel acted as a source or a target, respectively. Hub-like channels, with $N_s + N_t \geq 5$ on at least one measurement day are shown in bold.

Channel	DIV 18	DIV 20	DIV 22	DIV 25	DIV 27	DIV 29
16	-	-	2/1	1/1	1/1	2/2
25	-	-	-	0/2	1/1	1/1
27	2/1	2/1	2/2	3/3	5/5	5/2
28	-	2/1	2/2	2/6	3/7	3/4
32	-	-	-	-	0/2	-
35	-	-	0/1	2/3	3/4	1/2
37	1/3	1/4	1/7	2/8	2/10	2/9
38	-	-	-	1/3	3/5	1/5
42	2/1	2/1	5/3	8/4	8/6	6/2
44	-	-	-	1/2	-	1/0
46	-	-	-	-	-	0/1
48	0/6	1/6	0/11	3/13	1/14	0/14
56	-	-	1/1	1/2	4/1	3/2
58	7/0	6/1	11/1	15/0	17/0	15/1
65	1/1	1/1	2/1	4/3	5/3	4/2
66	-	-	-	0/1	0/3	-
67	-	-	2/2	5/2	6/2	2/1
68	0/1	1/0	3/2	7/1	6/2	2/2
76	1/1	0/2	1/1	1/2	3/1	3/1
77	-	-	-	-	0/1	-

ACKNOWLEDGMENT

The work of F.E.K. has been supported by the Academy of Finland under the project Bio-integrated Software Development for Adaptive Sensor Networks, project number 278882, by Human Spare Parts Project funded by Tekes – the Finnish Funding Agency for Innovation, and by Ella and Georg Ehrnrooth Foundation, Finland. The work of J.M.A.T. has been supported by Jane and Aatos Erkko Foundation, Finland, under the project Biological Neuronal Communications and Computing with ICT.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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