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Comparison of connectivity inference algorithms for classification of neuronal cultures using graph kernels

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Abstract. Neurons derived from human induced pluripotent stem cells (iPSCs), provide new means to study aspects of severe neurological diseases in vitro. Network features extracted from electrophysiological recordings of iPSC-derived neurons could be useful to better understand and study disease phenotypes. However, up to this date, there is no fully-validated method to infer connectivity between neurons when using spike trains as input. In this study, we compare two types of human iPSC-derived dopaminergic neurons: wild type cells and cells with a genetic mutation associated with Parkinson's disease. Moreover, we use graph kernels to train a classifier on the inferred functional networks and probe which connectivity inference parameters generate networks with more discriminative features.

Keywords: Connectivity estimation · classification · graph kernels

1 Introduction

Several electrophysiological studies have demonstrated that network-level features of in vitro developing human iPSC-derived neurons, such as their topology and network dynamics, are informative features to study disease-relevant phenotypes in vitro [1]. However, it is not well understood which inference technique is most appropriate to derive these features and existing inference methods have varying stances in selecting parameters, such as the delay and embedding window, to infer functional connectivity from pairwise spike trains [2]. In this work we compare three different connectivity inference methods [3–5]. Our goal is to better understand the relation between connectivity method and classification accuracy when having to distinguish between two types of cells: wild type (WT) and A53T mutant (Parkinson's disease). Additionally, we study the effect of the embedding parameter on functional connectivity and classification accuracy.

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2 Methods

In the following, we provide an overview of our workflow (Figure 1). All data analyzed in this study was in-house recorded, along with the analysis pipeline and experimental protocol.

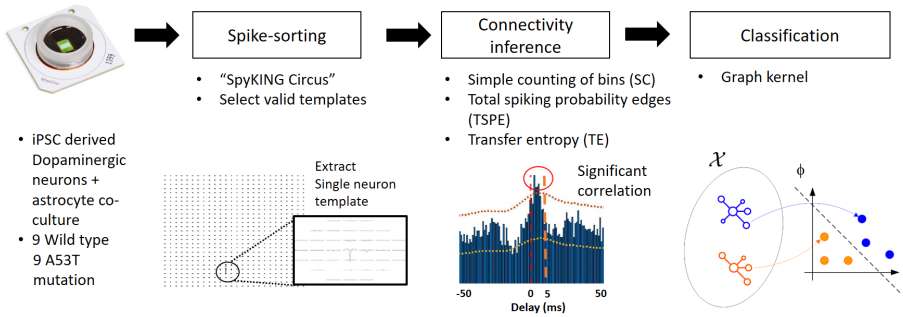


Fig. 1. Workflow of our analysis to compare connectivity inference algorithms.

2.1 Cell culture

Electrophysiological recordings were obtained using high-density microelectrode arrays (HD-MEA)[6] developed in our group and commercialized by MaxWell Biosystems [7]. The HD-MEA can record from up to 1024 electrodes simultaneously at a sampling rate of 20 kHz. Commercially available (FUJIFILM Cellular Dynamics [8]) dopaminergic (DA) neurons (100.000) and astrocytes (20.000) were seeded and co-cultured on HD-MEA for over 4 weeks. We followed the standard culturing protocol developed in the group. Recordings from week 2 were used for this analysis (n = 18, 9 WT and 9 A53T).

2.2 Spike sorting and preprocessing of HD-MEA data

Recordings were spike-sorted using SpyKING Circus 0.8.5 [9] and the sorted templates were screened based on firing rate and refractory period violation, following the standard protocol developed in the group. The output of this step were binary spike trains (bin size = 1 ms) of the full-length spike-sorted recordings (15 min). These spike trains were used to infer connectivity between neurons, as detailed below.

2.3 Inference and validation of functional/effective connectivity

We focus on methods that can deal with HD-MEA recordings with a size of >800 neurons per recording and a duration of 15 min of length. Specifically, we

compared three methods in order of increasing complexity: i) simple counting (SC) of cross-correlogram bins within mono-synaptic delay inspired by [4], ii) total spiking probability edges (TSPE) [3] and iii) delayed / higher-order transfer entropy (TE) [5]. Additionally, we set out to study the differential effect on phenotype classification accuracy as we increase the embedding (SC, TSPE $<$ TE) and delay window between neuron pairs (SC $<$ TSPE, TE). As shown in the transfer entropy equation 1, embedding is the past time bins that are assumed to have an effect onto the future state of the target neuron.

$$TE_{J\to I}(k,l) = \sum_{i\in I, j\in J} p(i_{t+d}, j_t^l, i_t^k) \log \frac{p(i_{t+d} \mid j_t^l, i_t^k)}{p(i_{t+d} \mid i_t^k)} \tag{1}$$

where k and l are embedding lengths for target and source neurons, respectively (e.g., $k, l = 2$ and $[i_1, i_2, j_1, j_2] = [0, 1, 1, 0]$). Depending on the choice of d, different delay lengths can be computed.

We used default parameter settings for TSPE (details can be found in [3]). To compute the counts in SC, we used 5 bins $(1, 2, \ldots, 5$ ms.) and averaged them. For TE we compared different embedding value pairs (k, l) where $k = l$ and $k, l \in \{1, 2, 3, 4\}$ and 20 ms. delay. In order to facilitate the comparability between computed connectivity and subsequent results from our graph kernels analysis, we proportionally thresholded the inferred networks to have graphs with the same number of nodes (N=480), to have undirected edges and a comparable density (5%).

2.4 Classification using graph kernels

Graph kernels measure similarity between graphs and are used in learning tasks consisting of graph-structured data [10]. For our analysis, we computed different graph kernels on the connectivity networks inferred by the methods listed above. The Weisfeiler-Lehman (WL) kernel [11] with $h = 3$ performed best in the training set and was thus used throughout the analyses. The kernel matrices obtained via the WL kernel constituted the input to the kernel machines: SVM for classification and kernel PCA for visualization.

Classification with SVM required an external 3-fold cross-validation with an additional internal 2-fold cross-validation to optimize the parameter C of the SVM. The cross-validation schemes were stratified to maintain balanced datasets. The kernel matrix K was computed once for all data points. The cross-validation required the partitioning of K to guarantee that no graph in the test set was used during training or for parameter optimization.

Implementation The graph kernels were obtained using the Python package `graphkernels` [12]. The SVM classifier and kernel PCA were implemented with `Scikit-learn` [13]. Proportional thresholding of the inferred networks was done with the `NCCT` toolbox [14].

3 Results

We performed classification of the electrophysiological recordings in our balanced dataset ($n_1 = 9$ WT samples and $n_2 = 9$ A53T samples). For each sample, we derived a network using the three network inference algorithms described in Section 2.3 (SC, TSPE and TE). For a given method, 18 networks were obtained and the WL graph kernel with $h = 3$ iterations was used to compute a kernel matrix K. The kernel matrix was fed to an SVM classifier, which computed an average accuracy and standard deviation. The results are shown in Table 1. The last row in the table shows the classification effect of the TE method upon extending the embedding to 4 ms. For TE we see a monotonic increase in classification performance as the embedding goes from 1 to 4 ms. The discriminative power of the computed graph kernels, in terms of average accuracy, is larger than when using TE with the minimum embedding of 1 ms.

Table 1. Classification performance of SVM with precomputed kernels obtained from the WL graph kernel ($h = 3$)

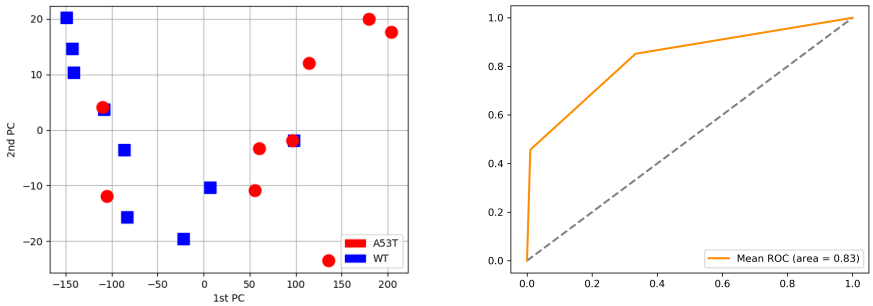
Network inference method	Avg. accuracy	Std. dev.
SC	0.611	0.079
TSPE	0.444	0.157
TE with embed=1 ms.	0.722	0.157
TE with embed=2 ms.	0.722	0.157
TE with embed=3 ms.	0.778	0.208
TE with embed=4 ms.	**0.833**	**0.136**

Using graph kernels as proxy, we can reduce the dimensionality of HD-MEA network data and obtain a plot of the recordings using the first two principal components of a kernel PCA [15] (Figure 2.a). The mean roc curve of the SVM classifier based on the networks inferred by TE with an embedding of 4 ms is shown in Figure 2.b

4 Discussion

Our results confirm that network-level features inferred from HD-MEA recordings of iPSC-derived DA neurons allow us to effectively classify WT and A53T cultures. Moreover, we found that using longer embedding windows for functional connectivity inference resulted in inferred networks with better discriminative power; accounting for longer histories (embeddings) enables us to derive more discriminative networks for the phenotypes of interest.

Graph kernels are an ideal tool for settings with small sample size, like ours. Because the number of nodes in all networks are similar and nodes/edges are unlabeled, other graph kernels based on histograms of nodes/edges do not perform well (data not shown). The WL graph kernel aggregates neighborhood information and, as a result, detects differences in neighborhood structures between



(a) **(b)**

Fig. 2. For networks derived from TE with embed=4 and WL kernel ($h = 3$), (a) samples plotted with the first 2 principal components of kernel PCA and (b) Mean ROC curve of SVM

WT and A53T. Despite this positive result, the small sample size of our study raises caution about any final conclusions that can be derived. It is important to note that the embedding lengths yielding more discriminative inferred networks do not guarantee the best detection of actual synaptic connections. Our future work will focus on i) increasing the number of recordings and ii) studying the mechanism on how this longer embedding amplifies differential features between networks.

5 Acknowledgements and funding information

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References

1. Kirwan, P., Turner-Bridger, B., Peter, M., Momoh, A., Arambepola, D., Robinson, H.P., Livesey, F.J.: Development and function of human cerebral cortex neural networks from pluripotent stem cells in vitro. Development 142(18), 3178–3187 (2015)
2. De Abril, I.M., Yoshimoto, J., Doya, K.: Connectivity inference from neural recording data: Challenges, mathematical bases and research directions. Neural Networks 102, 120–137 (2018)
3. De Blasi, S., Ciba, M., Bahmer, A., Thielemann, C.: Total spiking probability edges: A cross-correlation based method for effective connectivity estimation of cortical spiking neurons. Journal of Neuroscience Methods 312(November 2018), 169–181 (2019), https://doi.org/10.1016/j.jneumeth.2018.11.013

4. English, D.F., McKenzie, S., Evans, T., Kim, K., Yoon, E., Buzsáki, G.: Pyramidal cell-interneuron circuit architecture and dynamics in hippocampal networks. Neuron 96(2), 505–520 (2017)
5. Ito, S., Hansen, M.E., Heiland, R., Lumsdaine, A., Litke, A.M., Beggs, J.M.: Extending transfer entropy improves identification of effective connectivity in a spiking cortical network model. PLoS ONE 6(11) (2011)
6. Müller, J., Ballini, M., Livi, P., Chen, Y., Radivojevic, M., Shadmani, A., Viswam, V., Jones, I.L., Fiscella, M., Diggelmann, R., Stettler, A., Frey, U., Bakkum, D.J., Hierlemann, A.: High-resolution CMOS MEA platform to study neurons at subcellular, cellular, and network levels. Lab on a Chip 15(13), 2767–2780 (2015)
7. MaxWell Biosystems, https://www.mxwbio.com
8. FUJIFILM Cellular Dynamics, https://fujifilmcdi.com
9. Yger, P., Spampinato, G.L., Esposito, E., Lefebvre, B., Deny, S., Gardella, C., Stimberg, M., Jetter, F., Zeck, G., Picaud, S., et al.: A spike sorting toolbox for up to thousands of electrodes validated with ground truth recordings in vitro and in vivo. Elife 7, e34518 (2018)
10. Vishwanathan, S.V.N., Schraudolph, N.N., Kondor, R., Borgwardt, K.M.: Graph kernels. The Journal of Machine Learning Research 11, 1201–1242 (2010)
11. Shervashidze, N., Borgwardt, K.: Fast subtree kernels on graphs. In: Advances in neural information processing systems. pp. 1660–1668 (2009)
12. Sugiyama, M., Ghisu, M.E., Llinares-López, F., Borgwardt, K.: graphkernels: R and python packages for graph comparison. Bioinformatics 34(3), 530–532 (2018)
13. Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., et al.: Scikit-learn: Machine learning in python. The Journal of Machine Learning Research 12, 2825–2830 (2011)
14. Marshall, N., Timme, N.M., Bennett, N., Ripp, M., Lautzenhiser, E., Beggs, J.M.: Analysis of Power Laws, Shape Collapses, and Neural Complexity: New Techniques and MATLAB Support via the NCC Toolbox. Frontiers in Physiology 7(June), 1–18 (2016)
15. Schölkopf, B., Smola, A., Müller, K.R.: Nonlinear component analysis as a kernel eigenvalue problem. Neural computation 10(5), 1299–1319 (1998)