Functional Connectivity Analysis of Stem Cell Cultures with Parkinson's Disease

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Abstract: We studied the activity and functional network characteristics of neurons derived from human induced pluripotent stem cells (iPSCs) grown in culture. The cells were obtained from healthy and Parkinson's–affected patients. Using a network perspective, different magnitudes were analyzed to demonstrate connectivity and activity differences between healthy controls, diseased Parkinson's cultures, and genetically cured ones. We unveiled an excess of integration and a loss of both modularity and small–worldness for Parkinson's diseased cultures. The study may help the medical society to better understand the disease and develop efficient therapies.

I. INTRODUCTION

Parkinson's disease (PD) is one of the most common neurodegenerative disorders present in society. It is caused by the loss of a particular neuronal type called *dopaminergic neurons*, which play an important role in balancing brain dynamics and making it stable. Dopaminergic neurons inhibit or stop too much 'traffic', keeping everything smooth. Their loss is therefore associated with an increase of information flow (or integration) across neuronal circuits [1]. *Integration* is related to the capacity of the brain to operate as a global system, whereas *segregation* gives a measure of the specialization of the network since it is associated with the capacity to form separated circuits and share specialized information [6]. For example, upon excessive integration caused by Parkinson's, a patient gives the order to move an arm and the whole body shakes: information goes everywhere.

The brain operates dynamically by keeping a coexistence of inhibitory and excitatory neurons. A fortunate analogy to explain this is that one can think of the inhibitory neurons as the red light in a traffic light signal, whereas excitatory neurons are the green light. There is a need for balance for a city to be functional. In Parkinson's disease, the inhibitory neurons die, so there is an excess of 'green lights', and equilibrium is lost.

Here we investigated PD networks grown *in vitro*, and compared the differences between healthy control cultures and those affected by PD. To test therapeutic treatments, another dataset was analyzed: genetically–corrected isogenic cultures (isoPD), which are cultures affected by the disease that were genetically modified to correct for the malfunctioning genes associated with the disease and that, in principle, shaped healthy controls.

II. EXPERIMENTS AND DATA ANALYSIS

Experiments were carried with human induced pluripotent stem cells (iPSCs), i.e. human cell lines that are differentiated into neurons and grown *in vitro* (Fig. 1). The iPSCs cultures used in this work were extracted from patients at IDIBELL, the Biomedical Research Institute of the Bellvitge Hospital. Cultures were grown at IDIBELL, but recorded and analyzed in Dr Soriano's laboratory. Neuronal cultures are small networks grown in 13 mm diameter coverslips and monitored using Calcium imaging. Here, a fluorescence dye (named Fluo–4) binds to Ca^{2+} ions upon neuronal activity, changes its conformation and emits fluorescence, which is detected by a camera. A total of 3 cultures for each condition (control, PD, and isoPD) were studied.

Fluorescence traces of neuronal activity were extracted through the program NETCAL, run on Matlab and provided by Dr. Soriano. These traces were then inspected to detect sharp peaks that reveal activity. These peaks were next set as '1' for the presence of an activation, and '0' for absence. This allowed us to build raster plots of activity, as shown in Fig. 2, top.

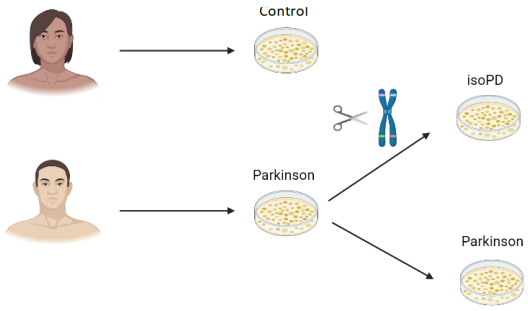

FIG. 1: Sketch of the different cultures used.

The data in the raster plots was analyzed with different MATLAB programmes, all of them provided by Dr. Jordi Soriano. The first one, "Visualize Front", studies the collective activity and allows us to identify groups of neurons that tend to fire synchronously. As shown in Fig. 2, bottom, sharp peaks indicate synchronous activity. One typically retains those peaks with at least 5% of the network participating, so that random activations can be discarded. The second program, "Main GTE" performs an analysis of functional connectivity. For simplicity, we used the simplest option of the program and took Pearson's correlation as a way to reveal the tendency of a

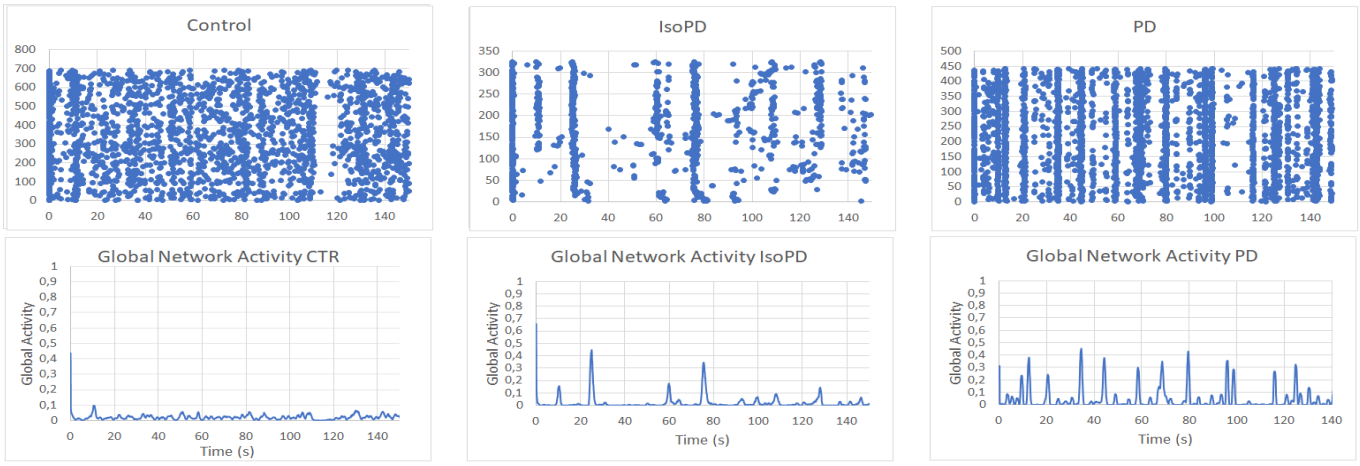

FIG. 2: Raster plots (top) and ' Global Network Activity' for representative cultures (bottom).

neuron to communicate with any other.

III. METHODS

To test whether PD cultures are more integrated than control or isoPD cultures, we needed first to evaluate the degree of interaction between neurons. For that we used the Pearson's correlation coefficient, defined as:

$$r_{ij} = \frac{\sum_t (x_i(t) - \bar{x_i})(x_j(t) - \bar{x_j})}{\sqrt{\sum_t (x_i(t) - \bar{x_i})^2 \sum_t (x_j(t) - \bar{x_j})^2}}, \qquad (1)$$

where x_i and x_j are the neuronal spikes, which are binary signals representing '1' for the presence of a spike and '0' for the absence [6] for the i and j neurons (Fig. 3), and $\bar{x_i}$, $\bar{x_i}$ are their time–averaged values. When $r_{ij} = 1$, it means that there is a perfect correlation (identical sets of data), whereas when $r_{ij} = 0$ there is no correlation at all.

To assess whether communication between neurons is significant, i.e. that interaction cannot occur by chance, one can randomize the raster plot and calculate the corresponding Pearson coefficient, r^R_{ij}. This allows us to set the significant connections as those that verify $r_{ij} > \chi$, where χ represents a certain cutoff value because the data sets are not infinite and thus $r^R_{ij} > 0$.

The program already evaluates this threshold χ above which correlations are significant. In detail, the program does the following. First, calculates the Pearson coefficient and creates the connectivity matrix. Second, it randomizes the raster plot, but maintaining the activity of the neuron (it keeps constant the number of '1' and '0' but mixes them up). In the sketch of Fig. 3 this means to maintain the black dots. Then, the Pearson's correlation is recalculated again, providing a mean value $\langle r^R_{ij} \rangle$ and its standard deviation SD^R. The threshold χ is then set as $\chi = \langle r^R_{ij} \rangle + 2\,\mathrm{SD}^R$, and typically $\chi \simeq 0.2$. And third, values are binarized as: every $r_{ij} < \chi \to 0$, while $r_{ij} > \chi \to 1$. Therefore, the connectivity matrix will be binarized as well (in black and white) where each dot is a value of the functional connectivity: if the dot is white, two neurons do not exchange information, whereas if it is black, the two neurons are exchanging information.

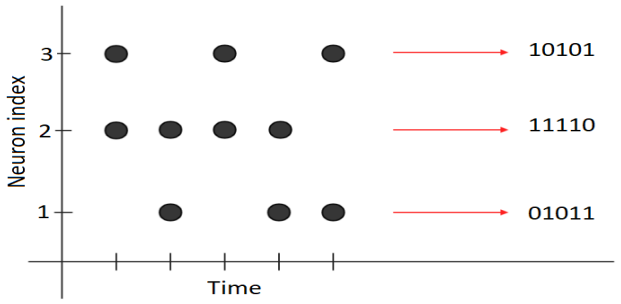

FIG. 3: Sketch of a raster plot. The vertical axis shows the neuron index, and the horizontal one provides time. For each time window, if there is an activation ('1'), a black dot appears for that neuron, otherwise ('0') the plot is blank. Synchronous activity can be spotted by analyzing the raster plot in vertical: if there is a steady line, a lot of neurons have fired together, whether if dots are separated, there is no such synchrony. Collective behaviour is considered if at least 5% of the network fires simultaneously.

In addition to functional connectivity, a magnitude to be analyzed is the Global Network Activity (GNA). It is defined as the fraction of neurons that fire jointly in a time window of 1 second, i.e. the size of coactivation events or percentage of the network that fires simultaneously. It can be considered as the sum of a column in the raster plot. A sketch is provided in Fig. 3, and the results for actual data were shown previously in Fig. 2.

Another magnitude of interest is the Global Efficiency G_{eff}, which provides a measure of how integrated is the network.It is defined as

$$G_{\mathrm{eff}} = \frac{1}{N(N-1)} \sum_{i \neq j} \frac{1}{d_{ij}}, \qquad (2)$$

where N represents the total number of neurons in the culture and d_{ij} the shortest topological path length between neurons i and j. A path is any unique sequence of links that connects a neuron with another, and its length is given by the number of steps or the sum of the edge lengths [3]. If the degree of integrability of the culture is very high, the path needed for the neurons i,j to interact is shorter, and therefore G_{eff} is higher. Note that for disconnected pairs of neurons, $d_{ij} \to \infty$. A sketch illustrating G_{eff} is shown in Fig. 4.

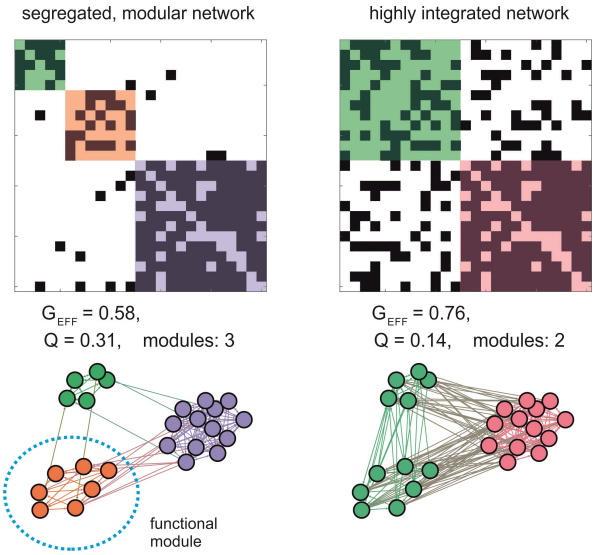

FIG. 4: Sketch comparing two contrasting networks. On the left, G_{eff} is small, and on the right G_{eff} is high. The sketch also shows the relation between Q and the number of communities. The higher the Q, the stronger the tendency of the culture to form communities and the higher its fragmentation. This figure has been provided by Dr.Soriano to illustrate the relation between different magnitudes.

The final magnitude is the Modularity Q, a scalar that measures the ratio between the number of links inside communities and the links between communities. It was calculated using the Louvain method, a complex algorithm that consists of an iterative method formulated in two steps. In the first one, each neuron has a community assigned. For each node i we consider j neighbours and evaluate the change of modularity that would take place by setting i to be part of the community of j. The neuron i is then placed in the community which provides the maximum gain of Q. If there is no gain achievable, i stays in its original community. The second step consists in building a network whose nodes are the communities found during the first step, and the process is repeated until there is no further gain of Q. Namely, the algorithm assigns neurons to modules until Q converges to its maximum value, until there is no possibility for increasing modularity by moving one entity to the neighbouring one. Note that when moving nodes, Q changes, so in different runs of the program Q may vary slightly. Note that if $Q \simeq 0$, the network forms a huge only community, whereas if $Q \simeq 1$, there are as many communities as neurons [2].

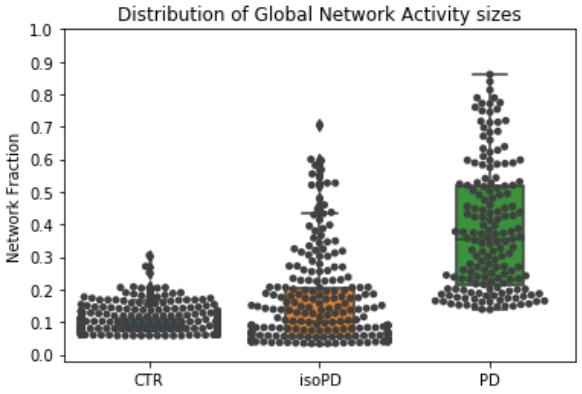

FIG. 5: GNA sizes. CTR and isoPD cultures reveal a moderate size of collective activations, while PD tends to show high sizes. Each boxplot contains data from 3 cultures. Error bars are standard deviation.

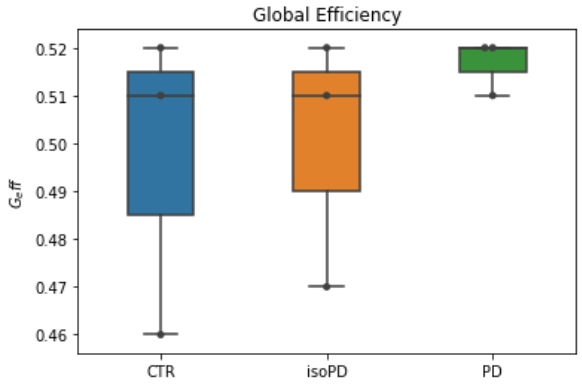

FIG. 6: Distribution of G_{eff}. PD peaks towards high values. Control and isoPD are more balanced.Each boxplot contains data from 3 cultures. Error bars are standard deviation.

IV. RESULTS

A. GNA Sizes

It can be seen in the raster plot of Fig. 2 that for the control culture there is a balance between steady vertical lines and single dots. However, for the PD culture, there are mostly steady lines. IsoPD presents a balance between sole and synchronous activations; it is an intermediate form, with a behaviour closer to controls.

The distribution of GNA sizes is represented as boxplots in Fig. 5. The distribution shifts towards high values for the PD culture, as one may expect from the raster plots, whereas control goes more towards small sizes. IsoPD is in between both.

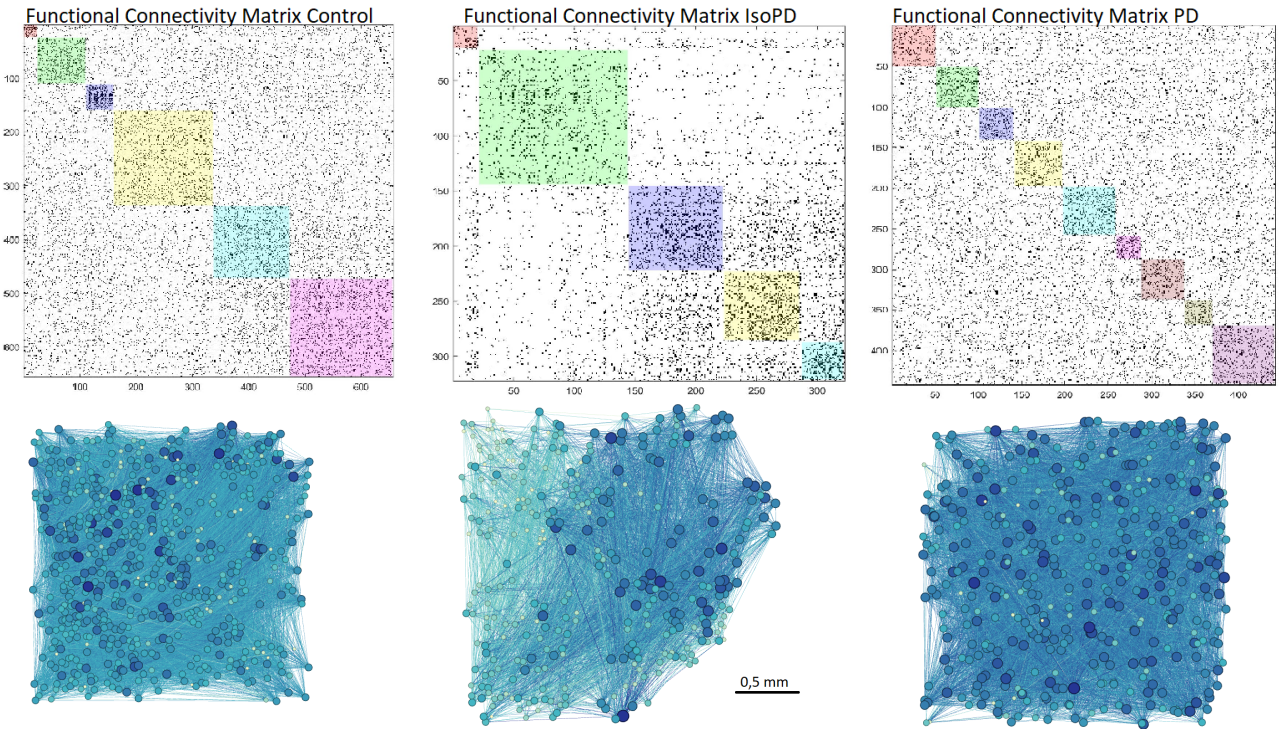

FIG. 7: a) Correlation matrix: The correlation matrixes above show the different modules present in the networks, and manifest the segregated behaviour of the PD culture. In the control and isoPD cultures, there is a balance between big communities and small ones. b)Gephi images: nodes and links are colour coded according to the strength and weight: the darker the colour, the stronger the bond. The size of the nodes reflects their importance in terms of the number of connections. Links are undirected since the directionality of projections cannot be discerned.[3] Gephi program provides a measure of the activity, revealing that anomalies in the PD culture depend on the behaviour of its activity rather than on its strength.

B. Global Efficiency

Results are shown in Fig. 6. The PD culture is highly synchronous, with neurons showing collective behaviour and that are deeply connected. Thus, PD cultures have an excess of integration and higher G_{eff} than isoPD and control cultures. However, there is strong variability for isoPDs and controls. This variability comes from the fact that sometimes cultures do not behave similarly. For controls and isoPD some neurons are connected globally, but others have more localized connections; whereas in PD the global efficiency values are very high in all cultures.

C. Modularity and network structure

Fig.7 shows the adjacency matrices and the network functional maps for the three conditions. Modules, highlighted in colors, allow for efficient exchange of information among brain regions, and promote their functional specialization by creating boundaries that restrict the spread of information across the entire network [3].

To quantify differences, Fig. 8 compares the modularity values, Q. For PD, there is a global sharing of information since there is no functional specialization anymore. It presents lower Q, so that the neurons which regulate the collective dynamics are dead, and there is no balance between the local and global computation.

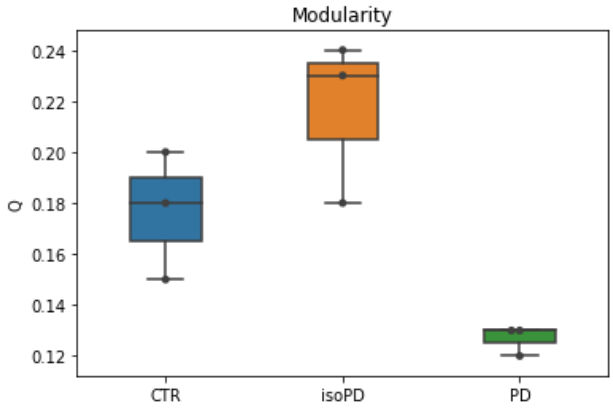

FIG. 8: Modularity values. For PD, Q is significantly lower, indicating a failure in forming clear communities. CTR and isoPD show higher Q, which indicates a capacity for segregation and specialization. Each boxplot contains data from 3 cultures. Error bars are standard deviation.

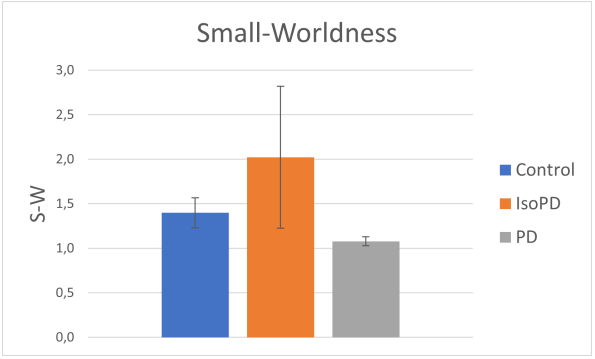

FIG. 9: Average Small–worldness for each set of cultures. Error bars are standard deviation, 3 values per condition.

D. Student's Test

It is a statistical quantification that calculates the value for the magnitude p for normal distributions and establishes that, if comparing two sets of data p is lower than 0.05, non-correlation can be affirmed, and statistical difference is achieved. For the modularity Q and small–worldness data, these are the results:
-Control and isoPD: $p > 0.05$ (non–significant).
-Iso and PD: $p < 0.01$.
-Control and PD: $p < 0.001$.

For values under 0.001, the difference is very significant. This condition is proven to be true for control and PD.

E. Small–Worldness

Small–worldness is the ratio between the clustering coefficient and the average path length:

$$c_i^{sw} = \frac{2E_i}{k_i(k_i-1)} = \frac{Number\ of\ triangles}{Number\ of\ paths\ of\ length\ 2}. \quad (3)$$

The path length is related to the number of topological steps needed to go from one neuron to another. The brain, among with other natural systems, is small–world. The presence of a small–world organization indicates a balance between segregation and global integration [3]. In the equation, E_i is the number of edges between the neighbours of i. The clustering coefficient of the network C^{sw} is then the mean of c_i^{sw} over all nodes [8].

The PD culture loses this small–world property because this balance no longer exists. $C^{sw}_{\mathrm{PD}} \simeq 1$,due to the fact that there are many more connections, the system is more integrated than it should: the neurons communicate a lot more between them, and that is why they shoot together more frequently. If all of the neurons are connected, the system is not efficient, because many connections are needed. For a normal system, the average path length is low —there is no energy wasted in making unnecessary connections— and the clustering coefficient is high.

V. CONCLUSIONS

Parkinson is still quite an unknown disease, and although analysis in network theory can be performed to discover more about its behaviour, not everything comes out as expected. The studied magnitudes provide an insight into how a culture with Parkinson behaves in an understandable manner, but some aspects are still in the darkness, and due to their complexity, it is complicated to study them. This difficulty resides in the fact that the brain is a dynamic system, and it is alive. Since it is not an static system, disposed at perfect conditions in order to be studied, some aspects contradict the intuition. However, it has been proven that there are several differences between a healthy culture and an ill one, which may be useful for the diagnosis and for developing new therapies.

Acknowledgments

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