Recovery of neuronal networks after physical damage

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Abstract: We investigated the impact of physical damage in neuronal cultures. To do that, we recorded the evolution of the spontaneous activity in a culture before damage, just after damage and few days after it. The analysis of the data showed that the activations per minute first decreased abruptly just after the damage, to gradually recover up to strong levels in just one day. This recovery was also observed in more elaborated analysis in the context of functional connectivity and network theory. The study suggests that physical damage in neuronal circuits triggers response mechanisms that are able to restore circuit's functionality in a relatively short time period.

I. INTRODUCTION

The recovery of a neuronal network after physical damage is complex, but very important to understand how a network restores the loss functions and in which characteristic timescale. When damage is applied, a large number of connections between neurons are lost, so that new connections have to be established again [1]. It has been suggested that the neurons at the vicinity of damage activity mechanisms to help restore connectivity and activity in the neurons that got isolated [2], and thus they can carry out their functions again. When the network is recovered, not only the activity goes back to normal, but it can even increase fostered by the new connections.

Here, we investigated the functional restoration of rat cortical networks *in vitro* upon physical damage, which was inflicted by splitting the culture in two parts with a scalpel, effectually leading to two separated sub-networks that had to reconnect. The rat cortical networks were prepared in Soriano's Lab by his team. We monitored the spontaneous activity through fluorescence calcium imaging using genetically encoded Ca^{2+} sensors [3]. Since they are permanently present in the neurons, we could follow the same culture before and after the cut, thus allowing us to study the culture in detail. From the data, we quantified the activity of the culture and computed some network measures, namely the global efficiency G and the community statistic Q. These two quantities are used in the literature to measure the degree of integration in a neuronal network, and therefore they serve to characterize the impact of damage and recovery [4].

II. EXPERIMENTAL SETUP & METHODS

A. Neuronal cultures and procedure

As most of connectivity studies of neuronal networks, we used cultures, which are small living systems made of dissociated neurons grown *in vitro*. These cultures are easy to manipulate and accessible, and they allow a wide variety of preparations. All of that makes them very useful in the areas of neuronal functional development [1]. To create our cultures, we obtained neurons from Sprague-Dawley rat embryos at 18-19 days of development. The embryonic brains were dissected and the cortical neurons dissociated by pipetting. Then, neurons were suspended into a culture medium and plated onto coverslips that contained two circular discs of polydimethylsiloxane (PDMS), 6 mm in diameter each and about 1 mm thick. Neurons settled and connected on those discs.

Of the two discs, the one showing the best quality was selected for study. This occurred after a week, when the cultures had the best conditions, with a high activity. The selected culture was then recorded for 10 min in different preset times, as follows. The first recording was 15 minutes before the cut so that we had a recording of the culture without being modified. Then we made a second recording 15 minutes after the cut, thus achieving the behaviour of the neuronal network right after the damage was done. A third recording was carried out a day after the damage, so we could see how the culture was recovering. The last recordings was done two days after the damage, when new connections were starting to appear. A total of four culture realizations were explored. The obtained statistics was then averaged among realizations.

B. Calcium fluorescence imaging

We monitored spontaneous activity in our cultures through calcium fluorescence imaging (Fig. 1A), a technique to optically measure the calcium concentration in a cell, which substantially increases during neuronal activity. The technique works by taking advantage of calcium indicators, small fluorescent molecules that respond to the binding of Ca^{2+} and become fluorescent. We used the genetically encoded indicator GCAMP6s, delivered through viral infection. The advantage of GCAMP6s is that neurons express the fluorescence probe permanently and, thus, we were able to record and observe the changes in the network along different time points. Another advantage is that dead cells retain the calcium, so that one

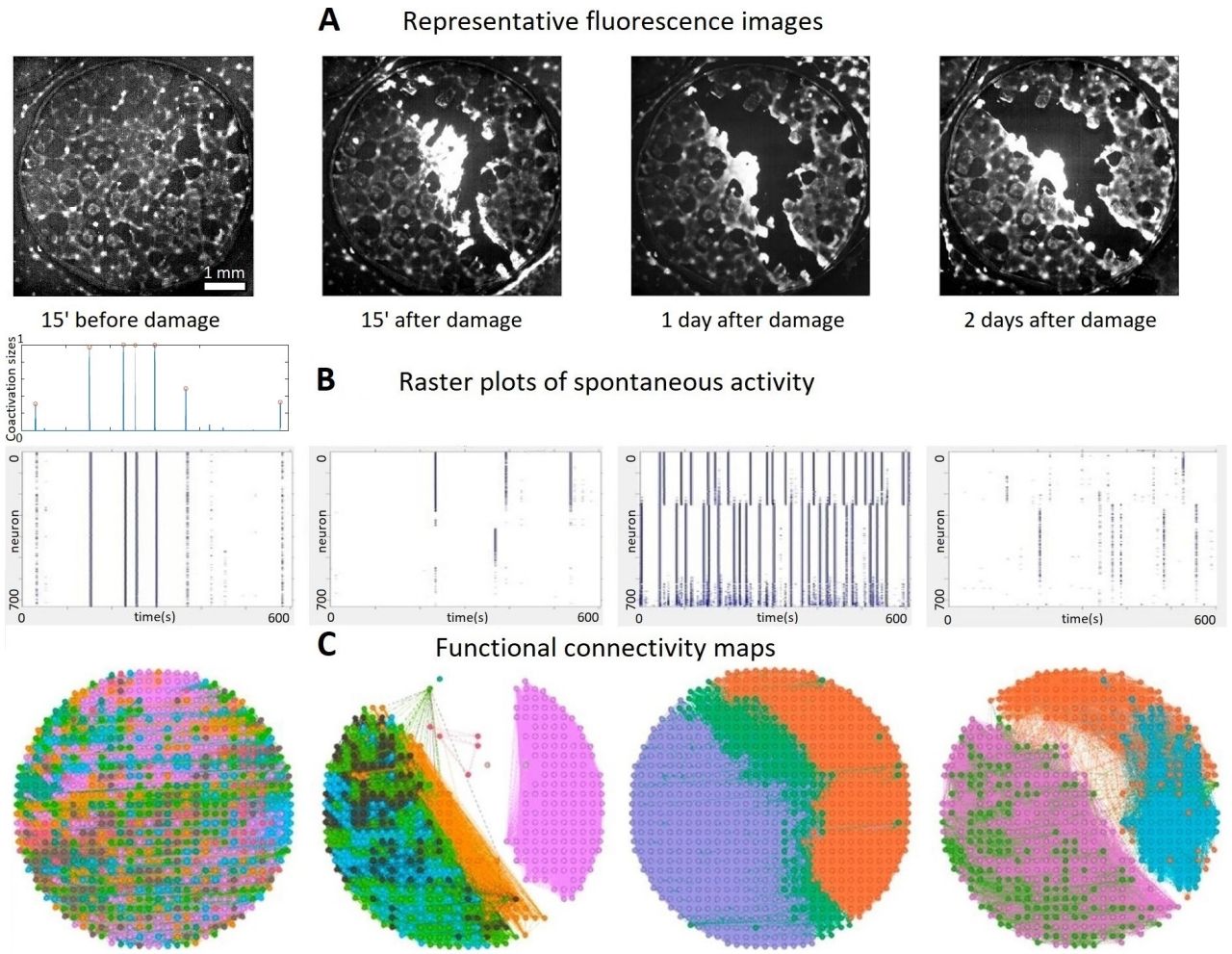

FIG. 1: **A**: Representative fluorescence images of the recordings along different time points of a culture. From left to right, snapshots of 15 minutes before and after damage, 1 day and 2 days later. **B**: Raster plots of spontaneous activity for each recording, ordered the same way as in A. For the first raster plot, the size of coactivation events is shown on the top of the raster. The raster plots illustrate that, before damage, activity corresponds to a unique region, while in the rest of the rasters activity is separated in two regions as a consequence of damage. **C**: Functional connectivity maps, ordered the same way as in A and B. From left to right, in the first one we observe that all the culture in connected. In the second one, we can clearly see a white, non-connected region where damage was made, together with an orange region of no activity. In the third one, the green part corresponds to dead neurons. In the fourth one, there are connections that are starting to appear between the previously disconnected regions.

can easily spot the affected neurons upon damage by their strong fluorescence.

Calcium is used because it moves into the neurons during the emission of action potentials, and therefore serves as indicator of activity. Calcium fluorescence data shows a typical signal characterized by a fast increase in the signal (associated with neuronal activation), followed by a slow decay (associated with the unbinding of the indicator). The fast increase can be easily detected, and therefore the data can be converted into time series of '0' (no activity) and '1' (activity, sharp increase of the signal), a representation that is known as 'raster plot' (Fig. 1B).

C. Basic data analysis

All the recordings, i.e. the four cultures with their four different time points, were processed using NETCAL [5], a MATLAB software from Soriano's Lab to manage and analyse high-speed calcium imaging experiments.

We analysed and processed all the cultures the same way: after preprocessing the recording and removing the background through the average fluorescence of the recording, we defined a circular area that approximately contained 700 *regions of interest* (ROIs). For each ROI we normalized its average fluorescence through time, and we obtained the fluorescence traces.

Through NETCAL we also processed the traces to obtain the spiking events, i.e. the '1' of our time series. This

was done by using the Schmitt trigger method, which considers two thresholds to accept or not an activation. The idea is that the fluorescence peaks should exceed a higher threshold for more than 100 ms before decaying and crossing the lower threshold.

With the spike trains, represented as binary data (1 for activity; 0 for none), we finally computed the raster plots. They served to visualize how neurons interact dynamically, and allowed us to extract simple information such as the average activity, or more advanced information such as network measures.

Having the spikes and the ROIs, we can analyse them through another software package of the Lab termed *Visualize Front*, which provides the Global Network Activity (GNA), defined as the fraction of neurons that fired together in time window of 1s. GNA data allows us to determine the size of coactivation events (top panel above the first raster plot in Fig. 1B), understood as the fraction of the network that activated together [6]. Standard healthy cultures often activate at unison and therefore the size of coactivations is close to 1.

D. Functional connectivity and network measures

With the spike trains, we calculate the functional connectivity using Pearson's correlation. Given any pair of neurons i and j, the correlation coefficients r_{ij} are:

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

where $x_i(t)$ and $x_j(t)$ are the binary time series for neurons i and j, and $\bar{x_i}$ and $\bar{x_j}$ their average values.

The values of r_{ij} were compared to a null model built by randomizing the raster plot and calculating the corresponding correlation coefficients. We obtained an average Pearson coefficient of $\langle S \rangle \simeq 0.2$ and standard deviation of $\sigma_S \simeq 0.05$. Thus, we took all values of our original r_{ij} data to be significant if they exceeded $\langle S \rangle + 2\sigma_s$. Otherwise, the observed correlations could just arise from random events. The significant functional connections were set as '1', and the rest to '0'.

We used the Software Gephi [7] in combination with the 'Brain Connectivity toolbox' [8] to represent the matrix as functional connectivity maps, coloring the neurons according to functional communities. These communities represent neurons that tend to communicate among themselves more strongly than with the rest of the network. Examples of functional connectivity maps are shown in Fig. 1C.

With the functional connectivity at hand, we use the *Global Efficiency* (G_{eff}) to understand how information is exchanged globally across the culture, This quantity measures the degree of integrability in the network [9], and is computed as:

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

where N is the total number of neurons and d_{ij} the shortest path length between neurons. Thus, the more connections exist between neurons, the smaller is d_{ij} and the higher is the efficiency in information exchange.

On the other hand, we also considered the *Community Statistic* Q to measure the density of links *within* communities compared to links *between* communities, as:

$$Q = \frac{1}{2m} \sum_{ij} \Big(A_{ij} - \frac{k_i k_j}{2m} \delta(c_i, c_j) \Big), \qquad (3)$$

where A_{ij} represents the edge weight between nodes i and j, k_i and k_j are the sum of weights of the edges attached to nodes i and j, m is the sum of all of the edge weights in the graph, δ is the Kronecker delta function and c_i and c_j are the communities of the nodes.

Q is a scalar value between 0 (the entire network is the only module) and 1 (as many modules as neurons). Typically, for $Q \gtrsim 0.3$ one can say that communities exist. Both G_{eff} and Q were computed using the 'Brain Connectivity toolbox' in Soriano's Lab software.

III. RESULTS AND DISCUSSION

A. Evolution of average activity

Fig. 1A illustrates the sequence of action in our damage experiments. Although we used four different cultures, we provide examples for only a representative one. For each culture, we measured activity 15 min before damage, 15 min after day and then waited for the culture to recover, measuring again 1 day and 2 days after damage. As seen in Fig. 1B, the culture was split in two parts upon damage (left and right), with activity dropping substantially to recover afterwards. Interestingly, activity in all experiments tended to overshoot during recovery to stabilize afterwards.

The average activity of the culture was quantified by counting the number of spontaneous collective activations (vertical bars in the raster plots) that were present in 10 minute. So if our average activity is 1, that means that the culture has fired ten times in our ten minutes recording. As the neuronal network changed upon damage, the number of firing sequences changed too, making it a good parameter to observe how the network is evolving through time. We carried out this activity analysis separately for the left and the right parts of the culture that formed after the cut, allowing us to quantify how the different parts evolved, as shown in Fig. 2.

We observe that average activity drastically decays after damage, because the neuronal network is damaged and stops its activity. However, the average activity from the recording of a day right after the damage is very high. This overshoot is very clear in Fig. 1B. We hypothesize that here connections between neurons are being build again, restoring the function of the network.

As we can see in Fig. 2, both left and right regions have a similar behaviour, with an average activity below two

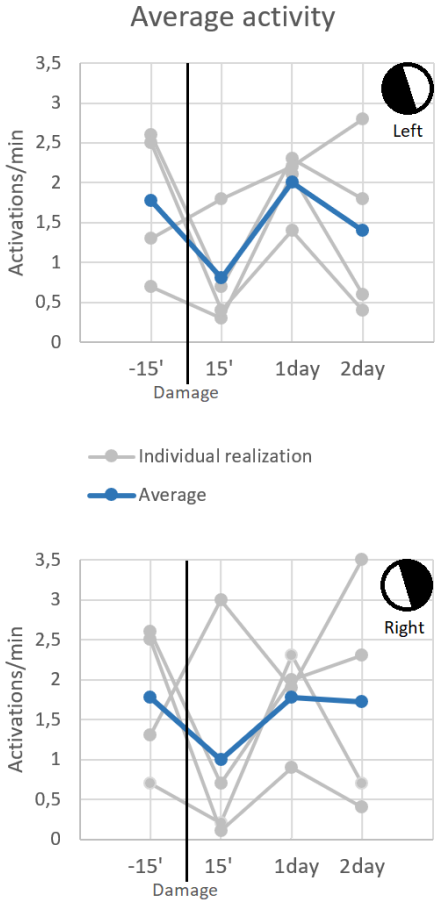

FIG. 2: Average activity of the neuronal network. The grey dots are individual realizations of each of the four cultures in every recording (15 minutes before the damage and 15 minutes, one and two days after damage). The blue dots are the average of the four cultures. The top graph shows the average activity for left region; the bottom graph for the right region.

activations per minute at first, then decreasing abruptly to one activation per minute or even less and, a day after damage, increasing their average activity to be as high as before damage, or even more. For the last recording (two days after the damage), the activity decreases slightly again. We argue that new connections that form between the two sides help regulating the system and it gradually returns to pre-damage activity levels.

B. Global network activity

The global network activity (GNA), i.e. the fraction of the network activating together within a 1s window, allows us to quantify the distribution of co-activation sizes. As said before, when the networks fires together before damage, the GNA values are high and therefore coactivation sizes are close to 1, i.e. the entire network. Upon damage and fragmentation, the coactivation sizes should substantially drop.

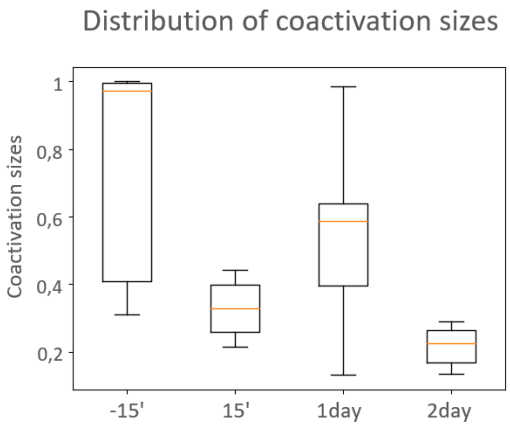

FIG. 3: Evolution of the global network activity. The box plots are the distribution of coactivation sizes for only the representative culture of Fig. 1, and for 15 minutes before the damage, 15 minutes after it, and one and two days later.

The distribution of coactivation sizes was represented as box plots. We can observe in Fig. 3 that, before damage, the average coactivation sizes (orange horizontal bar) is the highest because the network fired together. After the damage, the co-activation sizes decayed abruptly since the neuronal network was separated in two regions, so each one fired alone and, even though some connections between them were starting to appear, the culture still fired in two very differentiated regions.

In Fig. 3, the left and right regions of the culture have been evaluated together. Thus, the centre-right box plot has big 'whiskers' (the lines extending from the boxes). The regions could have been separated but, letting it this way, we can illustrate that if the only available data are the coactivation sizes (and not the representative fluorescence images as in Fig. 1A), we could observe that a bizarre behaviour emerged and use it to signal the possible presence of damage in the network.

C. Evolution of global efficiency and connectivity statistic Q

The global efficiency (G_{eff}) provides a quantification of the communication between neurons, giving us the information of how connected the neuronal network is.

As observed in Fig. 4A, the global efficiency decays abruptly right after the damage is done, but it increases to similar values to the ones before the damage within a day. That means that even though the neurons stopped exchanging information right after the damage, they changed their connections and made the neuronal network well-connected, despite the fact that the culture was not fully recovered.

As said before, the community statistic Q measures the density of links inside communities compared to links between communities, letting us see if the communities are strong ($Q \gtrsim 0.3$) or not.

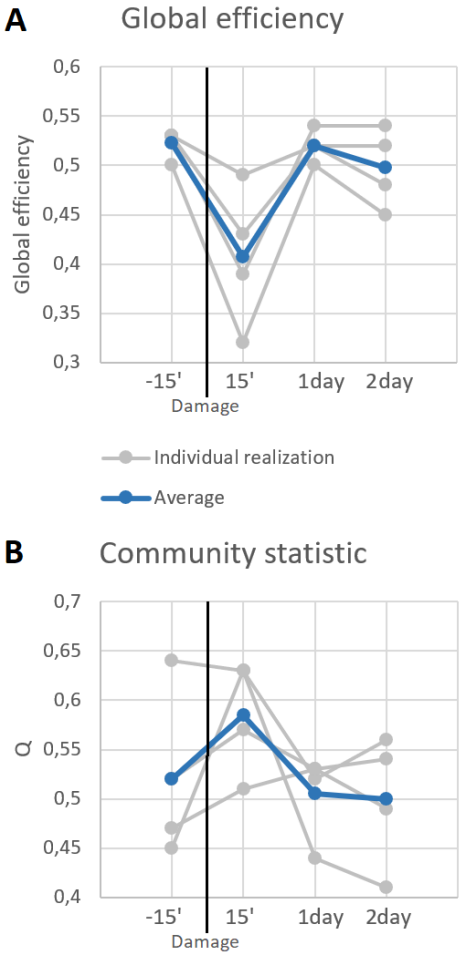

FIG. 4: The grey dots are the individual realization of each of the four cultures in every recording (15 minutes before and after damage, and one and two days after it). The blue dots are the average of the four cultures. **A**: Global efficiency of the neuronal network. **B**: Community statistic Q.

As observed in Fig. 4B, the community statistic Q before the damage is low, but it increases abruptly in just 15 minutes after the damage, in accordance with the loss of connections and the emergence of strongly isolated regions that are the left and right parts. Long after damage (1 and 2 days), Q has substantially decayed to values lower than before damage, indicating that, on average, despite the important loss of neurons, the network has created or reinforced the overall connectivity to make the network stronger than before.

IV. CONCLUSIONS

The recovery of a neuronal network after physical damage is an interesting approach to help us to understand how neuronal circuits gradually restore their function, and the time-scales needed.

Right after the damage, all the functions changed abruptly to a low average activity, a weakly connected neuronal network (G_{eff}), and a fragmentation of the system in communities (high Q), together with an incapacity for the neurons to fire synchronously (low co-activation sizes). But with some time, the neuronal network responded to the damage as a global, and, even though the two regions were still very differentiated, new connections started to appear thanks to the big increase of the average activity, making the neuronal network to be well-connected again and the community statistic to acquire lower values than before the damage. The latter indicates that, indeed, the network established news connections or reinforced existing ones. In the context of the brain, this means that damaged circuits can gradually recover and carry out again impaired functionalities.

In conclusion, physical damage in a neuronal network forces it to reorganize its connections between neurons to recover the functions of the network as a global.

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