Transfer Entropy: How to Further Understand Connections among Neurons

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Abstract: Much time and effort is being put to shed some light into the mysteries of the brain, and yet so much of it still remains unknown. This is why understanding the human brain and mind is considered one of the grand challenges of the XXI Century [1]. By implementing the recently developed information measure called *Transfer Entropy*, the strength and directionality of connections among neurons can be quantified. By the means of this mathematical tool, applied to trains of neuronal activity data measured in cultured neuronal networks, the structure of a neuronal network of 1,412 neurons has been analyzed and quantified.

I. INTRODUCTION

A. Prior Discussion

The inherent vastness of the brain is what makes it the most powerful computer in existence, and therefore comprehending it in its totality is an extensive challenge. If all neurons and their corresponding connections were to be taken into account in order to study possible relationships at a neuronal level, the amount of information contained would be undoubtedly too large to handle. Hence, rather than using a direct and exhaustive approach to understand the subtleties of connections among neurons, more feasible alternatives have been explored.

As stated above, a study of the brain structure would practically be too complex to be carried out. But the structure–function relationship of the brain cannot be forgotten [2]. It would be possible to learn about neuronal connectivity by analyzing the dynamic function of the brain and extrapolating the results to its structural behavior. Using neuronal cultures, and hence dealing with a simplified system with a highly reduced number of neurons, a simpler model could be constructed by the means of numerical simulations. The analysis of the topological properties of the mapped network would yield further insight into the dynamic functional properties of the brain.

A similar approach was used by a research group from Stanford University. By studying the spontaneous activity of the blood oxygen level–dependent (BOLD) signal, a network of functional connectivity was then reconstructed [3]. This network was then used to predict the structural connectivity of several brain regions. Being able to determine a general relationship between functional and structural network at a brain level, the next step would be to learn whether this same result could be extrapolated at a more precise neuron–to–neuron level.

B. Inference of Spiking Events – *Peeling Algorithm*

Alongside the Stanford University study, in this paper the spontaneous activity of 1,412 neurons within a neuronal culture will be quantified from cellular fluorescence signals retrieved from *fluorescence imaging* experiments at Dr. Soriano's lab. The train of neuronal firings will then be inferred using the so called *peeling algorithm* developed by the Brain Research Institute at the University of Zurich [4], which will then also construct a representation of the electrical signal of the neuron. This analysis pipeline is shown in Figure 1.

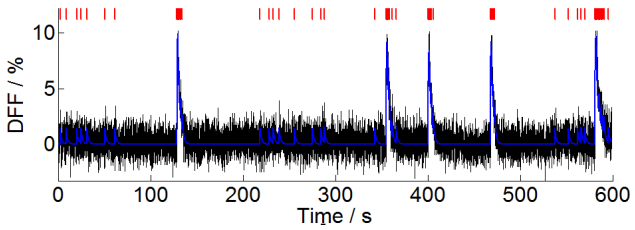

FIG. 1: **From calcium to spike trains**. Black trace: spontaneous activity recorded in a neuron using fluorescence imaging. Blue trace: reconstructed signal using the Peeling Algorithm with $A_1 = 1.5$. Red marks: reconstructed firing events (*spikes*). %DFF indicates relative change in fluorescence.

The peeling algorithm seeks for a firing pattern (*spike*) in the signal characterized by a sharp rise in fluorescence (of amplitude at least A_1) followed by a small decay. Unfortunately, experimental data is noisy and therefore adjusting well the threshold amplitude A_1 is crucial. The characteristic amplitude of a spike is not known experimentally, and therefore it has to be guessed. Low A_1 values lead to several false spike detections, and large A_1 lead to poorly represented firing trains. Hence, A_1 establishes the threshold from which any signal with greater or equal amplitude will be considered to be a spike rather than simple noise. Biologically, this spike will be understood as the neuron sending an electrical current. It is important to pay close attention to these spikes, because the relationship between an occurring spike and the activation of new spikes in other neurons due to this initial one will try to be established. Given the importance of A_1, this variable will be modified throughout this paper in order to study the sensitivity of the analysis tools to the inferred connection among neurons.

C. Mathematical Framework

In a modest attempt to get one step closer to understanding the behavior of neurons by learning how they establish connections among themselves, some mathematical tools had to be introduced in order to make the study of these connections more thorough and extensive. This is the reason why a new information measure has been recently coined, *Transfer Entropy* [7]. Before looking at its formal definition, let us analyze where it derives from.

Let x_i be a state that will occupy the process X_i with the probability distribution $p(x_i)$ and the uncertainty $log\frac{1}{p(x_i)}$, and let A be a countable set. The Shannon entropy is then defined as:

$$H(X_i) = \sum_{x_i \in A} p(x_i) log \frac{1}{p(x_i)}. \tag{1}$$

Let now $q(x_i)$ be an a priori assumption of the probability distribution of the process X_i. The Kullback entropy, which measures the error in assuming the probability distribution $q(x_i)$ over $p(x_i)$ is defined as follows:

$$K_{p|q}(X_i) = \sum_{x_i \in A} p(x_i) log \frac{p(x_i)}{q(x_i)}. \tag{2}$$

Let us now take two processes X_i and Y_i and assume a priori that they are independent, meaning that $q(x_i, y_j) = p(x_i)p(y_j)$. This particular case of Kullback entropy is known as Mutual Information.

$$M(X_i, Y_j) = \sum_{x_i \in A} \sum_{y_j \in A} p(x_i, y_j) log \frac{p(x_i, y_j)}{p(x_i)p(y_j)} \tag{3}$$

Taking X_i and Y_i to represent the behavior of two neurons, mutual information would be a very useful tool in predicting the probability of two neurons to be connected, since it quantifies the error made in assuming that they are independent. Nonetheless, if mutual information were modified to be an assymetric formula, then not only would the *strength* but also the *directionality* of the connection be quantified. Out of this necessity, another particular case of Kullback entropy was taken into consideration.

Let us take $p(x_{i+1}|x_i^{(k)}, y_j^{(l)})$ and by supposing that the state x_{i+1} of X_i depends on the k past states of $X_i^{(k)}$ but not on the l past states of $Y_j^{(l)}$, the a priori assumption becomes $p(x_{i+1}|x_i^{(k)})$. Thus, Transfer Entropy is formally defined as [6]

$$T(X_{i+1}|X_i^{(k)}, Y_j^{(l)}) = \\ = \sum_{x_{i+1}, x_i^{(k)}, y_j^{(l)}} p(x_{i+1}, x_i^{(k)}, y_j^{(l)}) log \frac{p(x_{i+1}|x_i^{(k)}, y_j^{(l)})}{p(x_{i+1}|x_i^{(k)})}. \tag{4}$$

This latter equation will be extremely useful when determining which two neurons are more likely to be connected. Due to the assymetry of Transfer Entropy, the directionality of the connections can be quantified. This quality allows to study what neuron is more likely to excite another one. Furthermore, Transfer Entropy returns the error in assuming that the behavior of neuron X_i does not depend at all on the behavior of neuron Y_i. And this makes a new and more complete analysis of neuronal cultures possible.

D. Application of Transfer Entropy

In the prior Subsection B an overview was given of how the peeling algorithm works and how it ultimately provided, depending on the value of A_1, a train of spikes structured in time. Using these spikes, the behavior of each neuron can be translated into a binary signal, where 1 designates the existence of a spike and 0 the lack of it. Thanks to the numerical translation of these signals and applying Eq. (4), the likelihood of neuron–to–neuron connectivity was calculated, which in practice is computed as the Transfer Entropy between all pairs of neurons.

By the means of the values of Transfer Entropy, and due to the newly added directionality that it provides, a directed graph can be constructed, which will represent the functional network of the neurons. From it, and by studying the topological properties of the mapped network, connections with the most relevant traits are most likely the structural, i.e. real neuron–to–neuron synaptic links, hence procuring details about the network circuitry that is not possible to easily derive by other means [5]. We remark that, in the studied neuronal cultures, one obtains the fluorescence trace for all neurons as well as their spatial location in the dish. Our goal is to derive detailed network functional maps.

II. PROGRAMMING TOOLS

The analysis of the functional network of the culture will be carried out with numerical algorithms. In the following section we explain the procedures in programming and how the obtained results were then used to create graphs representing the neuronal net.

A. Programming with MATLAB ®

All the developed programs necessary for this paper were written in MATLAB.

The first step was to write a program for the calculation of Transfer Entropy. Looking at Eq. (4), it is clear to see that the value of this measure will only be null if the quocient inside the logarithm is equal to one, but that will seldom happen. Thus, Transfer Entropy will be zero

only in isolated cases. As a result, if working with m neurons, saying that there will be around $m(m-1)$ Transfer Entropy values, which translate into the number of connections, is a reasonable hypothesis. Just working with this culture of 1,412 neurons, which cannot even be considered to be a minute sample of the brain, the number of connections rockets to 1,992,332. Since it is virtually impossible to extract any information when working with so much data, certain techniques had to be applied in order to decrease the number of connections and consider only the most relevant ones.

It is important to bear in mind that the value of Transfer Entropy on its own does not provide a direct value of the connection strength between neurons, unless it is compared to a reference. This is why after having calculated all the corresponding Transfer Entropy values a *null model* was implemented, where the binary signal of a single neuron was randomized. In this process, the number of spikes remained unmodified but their position within the signal was randomly assigned. With this new signal, the Transfer Entropy between this one neuron and the remaining ones was recalculated. This null model was repeated to consider a total of 100 surrogates, meaning that this same mechanism was repeated 100 times for each neuron. In the end, from all the 100 values calculated between the neuron x (whose binary signal was randomized) and neuron y, the value at the 90 percentile was chosen as the limit value. Finally, this value would be compared with the initial transfer entropy values prior to any randomizations, and those under the limit value would be set to zero while the rest would remain unaltered.

With this first cleansing, the less significant values of Transfer Entropy are eliminated. In other words, we take only "winning scores", i.e. those that are far from appearing by change. In a second screening stage, only the 90 percentile of this remaining Transfer Entropy values will be chosen. Only those above this value will survive this second and last cleaning. Therefore, in the end, only the upper 10% of the remaining Transfer Entropy values will be taken as "most likely good connections".

Having written all the necessary programs that will not only calculate but also clean the Transfer Entropy values, the last step was to use a program to translate these values for a graphing program to read. This final program was kindly facilitated by Dr. Javier G. Orlandi, postdoc at Dr. Soriano's group (Dept. ECM, Física).

B. Graphing with Gephi ®

The final useful values are now ready to be applied in the graphing program Gephi. In the final graphical display, each node will represent a neuron and each edge will be weighted with its corresponding Transfer Entropy value. At first, a graph with all the 1,412 culture neurons was attempted. Even though the number of edges had already decreased, it was still visually impossible to extract any information from the resulting mapped network. That it the reason why it was decided to only represent 120 random neurons. These randomly chosen neurons will be the same for all the graphs throughout this paper. Furthermore, since the neurons' positions were originally pinpointed in the culture, their coordinates were added into the program and in the final graphical representation each node (neuron) is placed equivalent to its original location within the culture.

The final graphical representation will have certain features worth mentioning. For starters, each neuron will be represented by a node whose size will be directly proportional to its degree k, understood as the sum of incoming and outgoing connections. The higher the degree, the bigger the size of the node. In addition, since graphs are directed —as a result of the directionality provided by Transfer Entropy— there will be arrows present in the graph that will depict in what direction the connection between two neurons is taking place. Moreover, both the arrows themselves and the width of the edges will vary in size, since they both will be weighted with the Transfer Entropy values. So the higher the value, the stronger the connection and also the bigger the arrows and the thicker the edges will be. Furthermore, nodes will be grouped by modularity classes, each of which will be visually represented by a particular color. Conceptually, a module is a group of neurons that connect much more among themselves than with any other neuron outside the module.

III. RESULTS AND DISCUSSION

As explained in the prior section, only 120 random neurons will be displayed. Since the position of these within the culture is known, their relative positions will be displayed in the graph in order to study whether the spatial distribution of the neurons has an impact on their connections. In addition, only the upper 10% of the Transfer Entropy values will be incorporated in the graph, i.e. the strongest 10% of the connections.

The spontaneous activity of the neurons within the culture was measured using a fluorescence imagining technique. The measures of these signals were taken along 600 seconds at a typical speed of 50 frames/s. This means that a single trace has about 3×10^4 points, and has to be down–sampled for practical analysis. To stress the importance of down–sampling, we note that in ambitious recordings in Dr. Soriano's Lab, data is acquired along 1 hour at 100 frames/s.

Hence, when expressing the binary signal train of the neurons, these 600 seconds have to be broken down into temporal bins of size T. If in the time interval described by a single bin one or more spikes take place, the relative position of this bin in the binary signal will acquire the value 1. Otherwise, its numerical value will be 0. On the other hand, the introduced threshold–amplitude A_1 establishes the threshold from which any signal with

greater or equal amplitude will be considered to be a spike rather than noise.

These two variables play a highly significant role in the final number of connection among neurons, as can be appreciated in Table 1. This is why in the next two subsections a more thorough study of these parameters will be carried out.

		A_1 (% DFF)			
		1.5	2	2.5	3
T (s)	1.5	1292	1035	759	603
	6	451	503	589	433

TABLE I: Number of neuronal connections for the 10% Transfer Entropy scores, for 120 randomly chosen neurons, and for different values (A_1, T).

A. Modifying A_1, the threshold-amplitude

In the following, the results portrayed in Figure 2 will be discussed. This same figure is attached in Appendix I at a larger scale for better visualization.

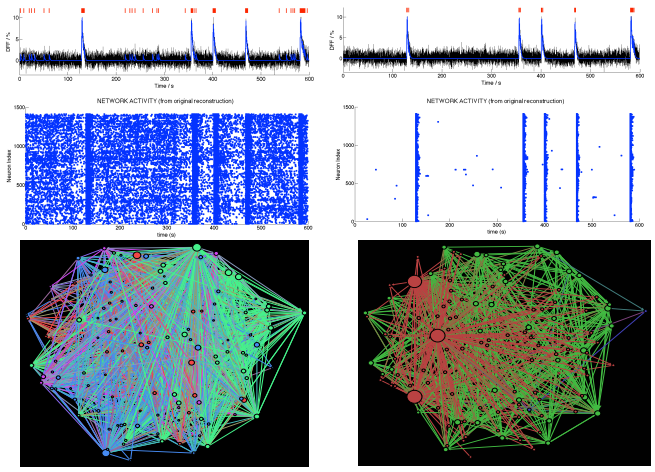

FIG. 2: **Representative results for varying** A_1. From top to bottom: Traces and spikes of a representative neuron; Raster Plots; and Connectivity Graphs. Left panels: $A_1 = 1.5$. Right panels: $A_1 = 3$. $T = 1.5$ s in all analyses.

For clarity, the electrical signal of a neuron is included in Figure 2. Two examples at different A_1 values are shown. On them, a blue line can be observed, which traces the contour of the spikes that have been calculated by the peeling algorithm working with a preassigned value of A_1. At the same time, on the top of the signals, red marks point the extracted electric impulses or firing events.

Right below this image we show the so-called *raster plots*. With the neuron index on the y–axis and the time on the x–axis, each blue dot on this plot represents the firing of a neuron, i.e., a spike. On the bottom part of the figure, the connectivity graphs are located. Graphed with the program Gephi, they provide a visual representation of the structure of the inferred neuronal networks.

Taking a closer look at the two electrical signals, which are identical since both are from the same neuron, it is clear that by the mere definition of the threshold-amplitude, the one for $A_1 = 1.5$ has much more spikes, 96, than that for $A_1 = 3$, which only has 32. When comparing the electrical signal of the neuron with the raster plot, a further relationship is spotted. The time at which the strongest peaks in the fluorescence signal take place coincide with vertical lines on the raster plot. These vertical lines mean that a very considerable amount of neurons within the culture fire coherently, at roughly the same time. This fact in turn points towards an inherent firing synchronization among the neurons in the culture.

When working with $A_1 = 3$ and analyzing the raster plot, aside from few particular spikes, most of them are concentrated in these vertical lines which represent these predominant synchronous stronger signals. On the other hand, when looking at the raster plot for $A_1 = 1.5$, even though these very same vertical lines are also present, they are harder to discern since other firings appear spread throughout the plot. These results clearly show how alterations in the threshold–amplitude parameter will radically change the final appearance of the network. When working with a high value of A_1, practically only the synchronous firings will be reported, which in turn will mean a massive loss in important information, for instance interaction between neurons outside of the coherent regime. And yet, when choosing a low value of A_1, maybe too many spikes will be dealt with, adding false interactions. Indeed, some might be part of the noise of the measure, bringing with them erroneous informations about connections between neurons to the final graphical representation.

Working with different values of A_1 therefore has an enormous effect on the final graph. Not only will there be a higher number of edges, i.e., connections when using a smaller A_1, but all other characteristics will also be modified, as can be appreciated in the networks of Figure 2. When analyzing the graph for $A_1 = 3$ (right panel in Figure 2), three nodes in red have a bigger size. These are clearly the most connected neurons within the culture. In addition, there are only two main colors in this graph, indicating that there are two main modularity classes. When comparing these characteristics to those on the graph for $A_1 = 1.5$, it can easily be seen how the three neurons that were so important in the other graph are not notorious in this case. Moreover, there is a greater range of colors present in the graph, reflecting that the neurons are broken down into more modularity classes.

Strikingly, these results portray that the modification of the threshold–amplitude also changes which neurons have a greater importance within the network, as well as how they are distributed into modularity classes.

B. Modifying T, the bin time–interval

In the following we discuss the results portrayed in Figure 3. A larger Figure 3 is attached in Appendix II for better visualization.

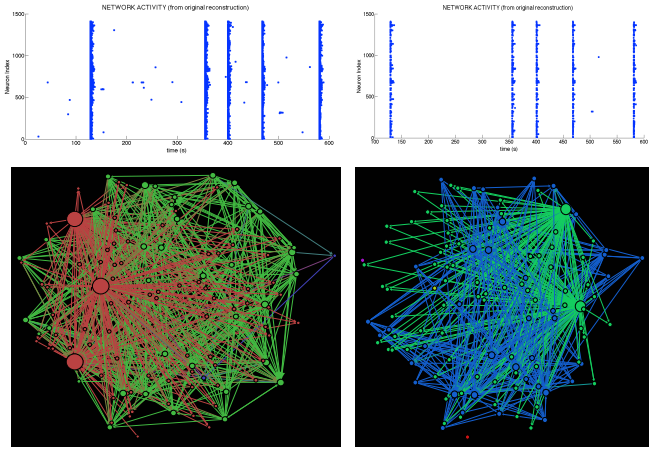

FIG. 3: **Representative results for varying** T. Data shows the raster plots and connectivity graph for $A_1 = 3$ and two different values of T. On the Left for $T = 1.5$ s; on the Right for $T = 6$ s.

Working now with the fixed value of $A_1 = 3$ and analyzing the raster plot, we see that most of the spikes are concentrated in these vertical lines which represent the predominant synchronous stronger signals, as seen previously. Yet, when assigning to T a value of 6 s, the spikes are logically even more concentrated around these main vertical lines. By increasing the value of T, it will be more likely to concentrate nearby spikes into a single one. Hence, the behavior of the connections will be even further contained in the main vertical lines, which will result in a loss of information richness. So, by choosing a larger T value and making fewer distinctions between closely occurring spikes, the final graphical representation will also be strongly altered.

Both the graphs for $T = 6$ s and $T = 1.5$ s depict two main modularity classes, even though they clearly vary from one another. More importantly, there are two highly influential neurons within the net for $T = 6$ s, located on the right hand side of the graph. Oppositely, the most important nodes for $T = 1.5$ s are situated on the left hand side. Once again, the final graphical product is also extremely altered when changing the parameter relative to the bin time–interval.

IV. CONCLUSIONS

The results clearly show that the values of A_1 and T have an incredible impact on the distribution of the neuronal firings, and thus on the final functional structure of the network. When using smaller values of A_1 and T, the raster plot exhibited a richer pattern. This richness is positive for Transfer Entropy since all the dynamic repertoire of the neurons is reflected and, in turn, their topological complexity. At the other extreme, if all traces were almost identical, the derived networks would be meaningless. Hence, in order to be sure that the functional networks is a good proxy of the structural one, first of all the optimal values of A_1 and T should be determined. Furthermore, in order to extract more subtleties from the spike trains, the measurements should last longer than 600 seconds, enabling particular signatures of each neuron to take shape.

This work indicates that unveiling the structure of the neuronal network is a very delicate issue. Since we worked with experimental data with unknown underlying circuitry, we cannot know *a priori* which parameters are better. This reflects two main aspects: (i) it is very important to pour efforts in advancing the research in reconstruction algorithms, and (ii) one needs to work in parallel with accurate simulations of neuronal networks to be able to compare the derived functional topology with the real one, and test accurately all the parameters.

Acknowledgments

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[1] "21st Century Grand Challenges." *whitehouse.gov*. N.p., n.d. Web. 15 Nov. 2015.
[2] Das TK, Abeyasingue PM, Crone JS, Sosnowski A, Laureys S, Owen AM, Soddu A (2014) Highlighting the Structure-Function Relationship of the Brain with the Ising Model and Graph Theory. BioMed Research International. Article ID 237898.
[3] Greicius MD, Superkar K, Menon V, Dougherty RF (2009) Resting-State Functional Connectivity Reflects Structural Connectivity in the Default Mode Network. Cerebral Cortex. 19:72-78.
[4] Lütcke H, Gerhard F, Zenke F, Gerstner W and Helmchen F (2013) Inference of neuronal network spike dynamics and topology from calcium imaging data. Front. Neural Circuits 7:201. doi: 10.3389/fncir.2013.00201
[5] Orlandi J, Stetter O, Soriano J, Geisel T, Battaglia D (2014) Transfer Entropy Reconstruction and Labeling of Neuronal Connections from Simulated Calcium Imaging. PLoS ONE. 9(6): e98842.
[6] Schreiber T, Kaiser A (2002) Information transfer in continuous processes. Physica D. 166: 43-62.
[7] Wibral M, Rahm B, Rieder M, Lindner M, Vicente R, Kaiser J (2010) Transfer Entropy in magnetoencephalographic data: Quantifying information flow in coritcal and cerebellar networks. Progress in Biophysics and Molecular Biology. 105: 80-97.

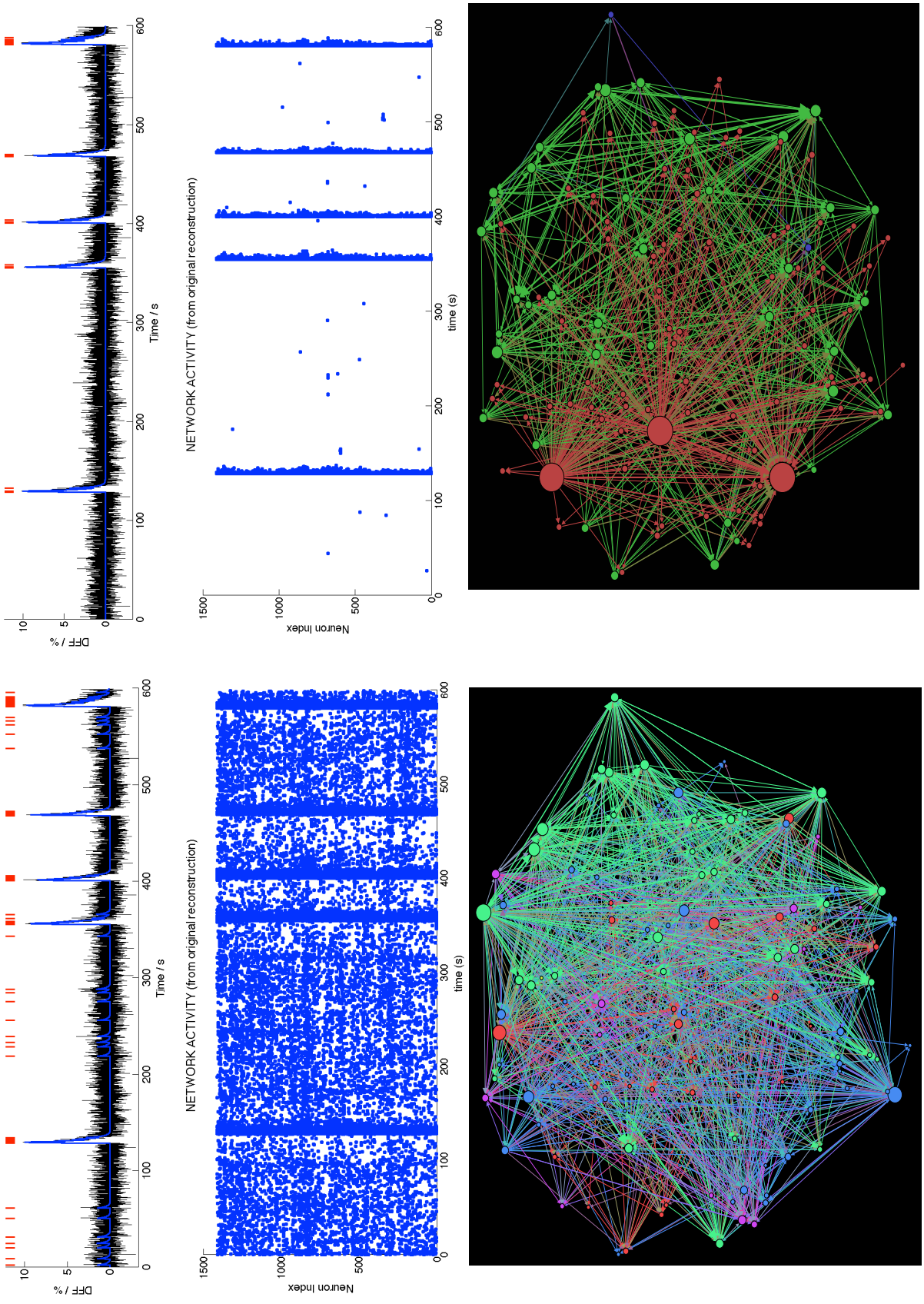

FIG. 4: **Appendix I: Figure 2 in detail**. Top: fluorescence traces and derived spike trains for a representative neuron. Center: Raster plots. Bottom: connectivity graphs. Left panels show data for $A_1 = 1.5$ %DFF, and right panels for $A_1 = 3$ %DFF. In all analyses, $T = 1.5$ s.

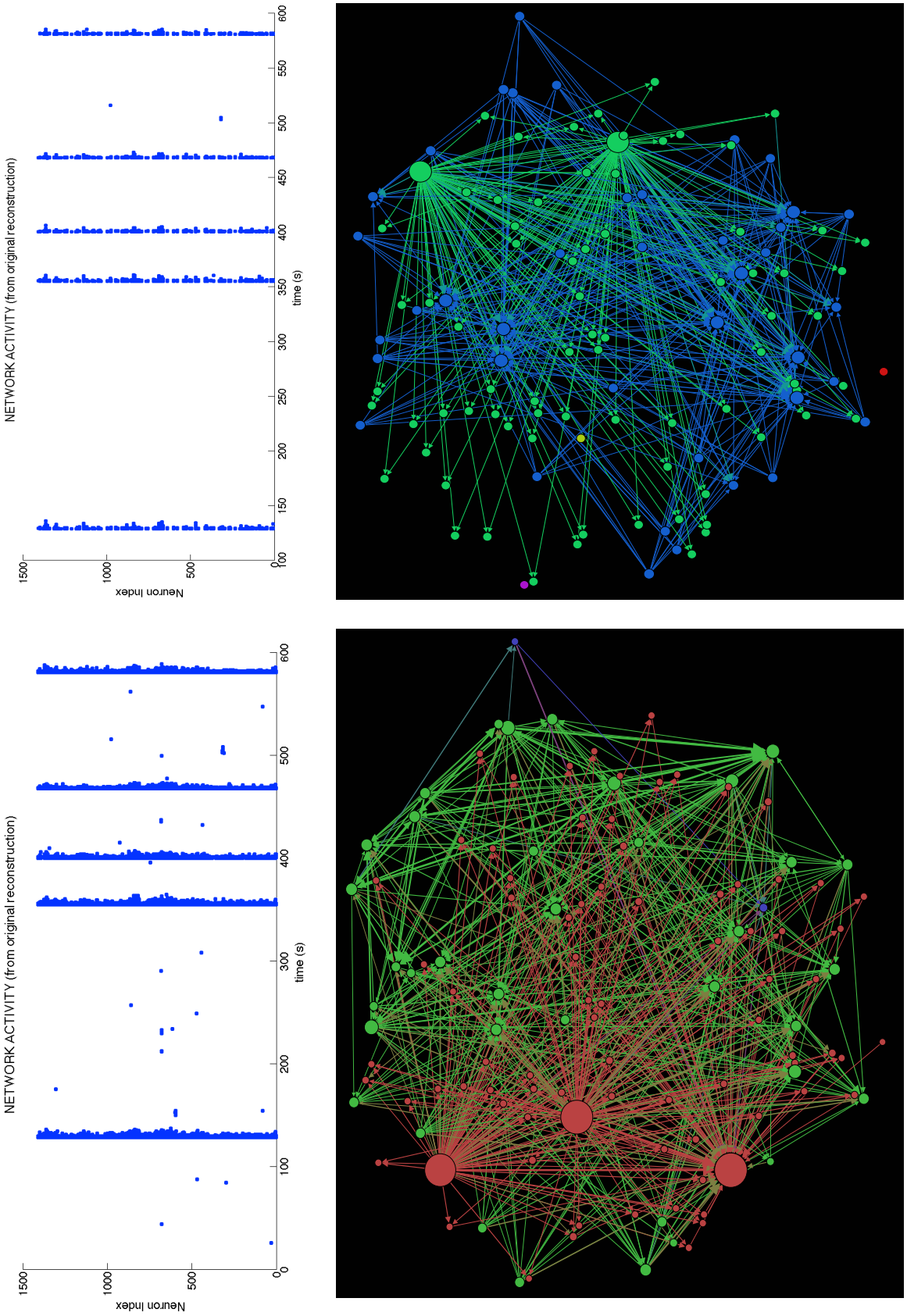

FIG. 5: **Appendix II: Figure 3 in detail**. Top: Raster plots. Bottom: connectivity graphs. Left panels show data for $T = 1.5$ s, and right panels for $T = 6$ s. In all analyses, $A_1 = 3$ %DFF.