Dynamic Functional Connectivity tutorial: solutions

Brainhack Tutorial Zurich
Sliding window analysis and innovation-driven co-activation pattern pipelines

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1 Sliding window analysis

1.1 Computation of connectivity time courses

Q.1: For a few regions of your choice, compare the connectivity time courses obtained with different window lengths. What happens when using very short, and very large, window lengths? Do you know of any rule as for a proper window length selection?

When the window length is short, we observe more marked fluctuations of the connectivity time courses. This may be due to two reasons: first, fewer time points enter the connectivity estimation process, which may introduce some errors, and second, there may be more sensitivity to capture fast paced real fluctuations.

Conversely, when the window length is long, we observe fewer changes. The estimated value may be more accurate, but it may also be less able to describe fast changes, because the connectivity measurement is computed over too many time points.

There is a rule of thumb to properly select window length: in order to prevent the appearance of spurious fluctuations, the window length should be larger than $\frac{1}{f_{min}}$, with f_{min} the lower frequency conserved in the preprocessed time courses.

Q.2: For a few regions of your choice, compare the connectivity time courses obtained with different step sizes. What is the influence of this parameter ?

Increasing the step size results in the computation of a lower total number of connectivity estimates, as subsequent temporal windows then show less overlap. Thus, if this value becomes too large, fluctuations in connectivity cannot be accurately resolved anymore.

Q.3: For a few regions of your choice, compare the connectivity time courses obtained using different window shapes. What shape gives the most fluctuating time courses? What about the least fluctuating ones? Why is it the case?

The most fluctuating time courses are obtained using an exponential window shape, while the least fluctuating ones are achieved with Tukey and Gaussian shapes. This is because in the exponential

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case, the very last time point of the window is given maximal weight, and so the appearance of a strong change in the time courses is translated by an abrupt switch in connectivity. Conversely, in the Tukey and Gaussian cases, the last time point is given very little importance (middle time points dominate), and so fluctuations are less marked.

Q.4: For the same time point, compare the full connectivity matrices obtained for different subjects; are they the same? Why?

The connectivity matrices obtained for different subjects at the same time point are not the same; this is because we consider a resting-state paradigm, in which intrinsic fluctuations of connectivity as well as mind wandering are uncorrelated across subjects.

Q.5: For a selected subject of your choice, compare the full connectivity matrices obtained at (1) close time points (separated by only few steps), and (2) remote time points. Do they look similar? Why?

The connectivity matrices obtained at close time points look quite similar, because they are computed on the basis of a very similar set of data points (recall that temporal windows used in the connectivity computations are strongly overlapping). Conversely, connectivity patterns at remote time points look quite different, because then, the data points entering the computations are not the same anymore.

1.2 Dynamic graph analysis and eigenconnectivities

Q.6: Provide a definition of the clustering coefficient and of the degree metrics computed here. Do they inform on local or on global brain connectivity?

The clustering coefficient (as computed on a binarized adjacency matrix) is a measure of local connectivity around a given node: it takes a value of 0 if all the neighbours of a given node are fully disconnected, and a value of 1 if all those neighbours are fully interconnected.

The degree of a node is also a measure of local connectivity: a larger value means that the considered node has a bigger number of direct neighbours in the considered graph. For a binarized adjacency matrix case, the nodal degree value is simply the number of direct neighbours, and as such, can be interpreted as a metric of *hubness*.

Q.7: For a few regions of your choice and default parameters, plot the graph metrics time courses over time; what do you observe? What conclusion can you make?

The two graph metrics show marked fluctuations throughout time, which means that local connectivity in the functional brain network undergoes very strong changes during a typical resting-state acquisition. This is in line with previous works demonstrating such variations for many graph metrics [1, 2, 3].

Q.8: Do the same for different values of edge density; what do you observe if edge density becomes

very low? What happens if it becomes very large?

If edge density becomes very low, both metrics show values very close to zero with few dynamic changes. This is because almost no more edges are retained in the assessed graph. Conversely, if edge density becomes too high, almost all edges are retained every time, and so both metrics show high values, but again, with minor fluctuations only. This highlights the importance of carefully accounting for edge density impacts in graph-based analyses.

Q.9: Assuming that subject-wise demeaning has been performed, what does an eigenconnectivity pattern represent? How can you interpret a large value for a given connection in this pattern? Do those answers change if demeaning is not performed anymore?

The eigenconnectivity patterns are the principal directions of the considered dataset. If demeaning is performed, the data at hand is fluctuations around stationary connectivity, and so, an eigen-connectivity pattern reflects a n_connectivity_pairs-dimensional direction along which there is a big variability in fluctuations around stationary connectivity (i.e.; connectivity strongly fluctuates between moments when it matches stationary connectivity, and moments when it does not).

A large value for a given connection in the pattern thus indicates that this connection strongly fluctuates around its stationary connectivity value. Note that the sign is, in itself, irrelevant: the value may be strongly negative, but still drive increased connectivity, if the weight multiplying the principal direction is negative as well. To know this, we would need to also consider the v SVD output, which contains weights for all eigenconnectivity building blocks at all sliding window time points (i.e.; their degree of expression along time).

If demeaning is not performed anymore, the interpretation changes, because then, stationary connectivity remains in the data matrix. Thus, principal directions will include a mix of dynamic and stationary influences, and the latter is expected to dominate because it typically accounts for a larger fraction of data variance.

Q.10: Generate eigenconnectivity building blocks with default parameters, and comment on what you observe (in particular concerning the first eigenconnectivity patterns). Can you recognise any resting-state network?

The very first generated eigenconnectivity pattern (which explains the largest part of data variance) appears to be quite constant across connections: this means that many connectivity fluctuations happen together in whole-brain fashion.

The second eigenconnectivity building block already displays a canonical resting-state network, the default mode network: indeed, one can see that the angular, posterior cingulate, hippocampal, parahippocampal and medial frontal areas from the atlas all show negative connectivity values with each other.

Q.11: Generate eigenconnectivity building blocks without performing subject-wise demeaning. Do the results change compared to **Q.10**? Why?

The results change, because as explained above in **Q.9**, eigenconnectivity building blocks now mostly incorporate stationary connectivity effects.

Q.12: How would you perform group comparison with the introduced analytical pipeline? Aside from the introduced variables, do you see any other metrics that could be computed, and used, for the purpose?

One could imagine to run the analytical pipeline separately on each group, and to look for differences in the obtained eigenconnectivity building blocks. This could then be followed up by more focused dFC analyses at the level of connectivity pairs exhibiting marked group differences.

Alternatively, one could run the pipeline on the whole population together, thus retrieving a common set of eigenconnectivities for all examined groups. Instead of focusing on eigenconnectivity spatial differences, it could then be insightful to look at temporal differences in their expression: for this purpose, the weights $\underline{v}$, which denote the importance of each building block at each time point, could be contrasted across groups.

Q.13: Can you provide a few limitations of the eigenconnectivity approach?

Because it relies on an SVD decomposition, the obtained eigenconnectivities are, by construction, orthogonal to each other; it may be that this constraint leads to a neurophysiologically inaccurate solution. Also, the generated patterns are difficult to interpret, as they are not sparse (every connection takes a non-null value in every eigenconnectivity case).

2 Generation of innovation-driven co-activation patterns

2.1 Frame selection process

For the answers of **Q14** to **Q.19**, please refer to the script *iCAPs_Tutorial_Brainhack_FULL.m*.

Q.14: Within the loop, extract the information about which voxels and time points show significant positive and negative innovations for the assessed subject into a matrix of size n_timepoints × n_GM_voxels. Threshold this matrix so that it contains only 0 (no innovation or negative innovation) or +1 (positive innovation) elements.

Q.15: Extract the innovation data for the assessed subject in a matrix I_pos of size n_timepoints × n_GM_voxels, thresholded so that it only keeps non-null elements for the voxels and time points with positive innovation.

Q.16: Call the function check_interconnectedness(I_pos,fHeader,mask), so that the thresholded innovation matrix that you constructed is further processed to remove its isolated positive elements. In practice, a non-null voxel must be surrounded by at least 6 non-null neighbours to be retained for further computations.

Q.17: Create a final mask of size n_timepoints $\times$ 1 denoting the frames that must be retained as moments of positive innovation by +1, and the other time points by 0. This is done by checking whether there are at least <u>n_voxels</u> voxels showing significant positive innovation.

Q.18: Use the created mask to extract the thresholded positive innovation frames, and fill <u>Data</u> with them.

Q.19: Still within the loop, perform the same process for negative innovations, so that after going through each subject, <u>Data</u> is filled with the positive and negative thresholded innovation frames retained for this subject.

Q.20: For each subject, using default parameters, compute the fraction of voxels/time points showing positive and negative innovations. What do you observe?

We observe that across subjects, the fraction of voxels/time points showing positive or negative innovation can vary a lot (between around 3 to 10%). However, the amount of positive and of negative innovations are pretty similar within the same subject.

Q.21: For each subject, using default parameters, compute the fraction of frames showing positive and negative innovations. What do you observe?

As in the previous question, we observe a large variability in the fraction of retained frames across subjects, but a high similarity in numbers for the same subject in the positive and negative innovation frame cases.

Q.22: For each subject, using default parameters, compute the fraction of frames showing both a positive and a negative innovation. What is the meaning of a joint innovation?

There is also considerable variability in the percentage of frames showing a joint positive and negative innovation across subjects: values indeed range from around 2 to up to 50% of frames! In such time points, there is at least one resting-state network increasing its activity level (positive innovation), while *at the same time*, another (or more) resting-state network(s) decrease(s) its activity level (negative innovation).

Q.23: How do the three above measures vary as a function of the <u>n_voxels</u> threshold?

If the voxel threshold is lowered, larger fractions of voxels/time points, and of frames, will be retained as showing significant innovation. This will also be the case for joint positive and negative innovation frames. Conversely, if the threshold becomes too large, there will be no more retained frames.

2.2   Generation of iCAPs

Q.24 Generate iCAPs with default parameter values; can you recognise some canonical resting-state networks?

Yes, we can recognise some resting-state networks! In particular, the first iCAPs (the ones whose changes in activity are captured most often, due to the reordering step of the iCAP generation pipeline) display, amongst other systems, the default mode (either full, including a frontal contribution, or partial, only showing posterior signal), auditory and somatomotor resting-state networks.

Q.25 Try to vary the number of clusters into which to separate innovation frames. How is the quality of retrieved iCAPs affected by using a very high value? What about a very low one?

If a very low number of clusters is selected, several different networks will be averaged together into a single map, and so the quality of the retrieved maps will be lowered. A very large number of clusters may theoretically be better to disentangle iCAP maps that only contain subtle differences, but because of the limited amount of frames at hand, past a point, the averaging will not be conducted on enough data points and the obtained maps will be noisy. Also, if a too large number of clusters is selected while there is in fact less distinct networks, a given iCAP may be separated into more than one map, which is also unwanted. Thus, one should aim at a trade-off value for the number of clusters.

Q.26 Generate iCAPs with an increasing n_voxels value, going from less than a percent to around a fifth of all gray matter voxels. How are the retrieved iCAPs influenced by this parameter? Can the use of a lower or larger value be justified from a neurophysiological point of view?

For a very liberal voxel threshold, almost all frames will be detected as showing an innovation, and so the clustering will be conducted on a set of frames that will partly be mistakenly considered as relevant (because those *bad* frames, in reality, only contain a very small, uninformative focus of innovation that may be driven by noise). This is expected to lower the quality of the retrieved maps, and indeed, obtained iCAPs then become less clearly delineated. Note that it is to partly alleviate this concern that we resort to the function check_interconnectedness, so that isolated spatial foci of innovation are removed.

For a very conservative threshold, there are fewer frames selected, and those frames have, by construction, to exhibit particularly large-scale innovation. Thus, we can expect that a larger fraction of retained time points will in fact exhibit *combined* transitions from more than one resting-state network. For this reason, the retrieved maps are then also less accurate at separately representing different networks.

Q.27 Can you provide a few possibilities for how group comparison could be performed using the TA/iCAPs pipeline? What metrics could you compare across groups?

To perform group comparison, we could imagine to run the TA/iCAPs framework on the combined population of subjects, in order to generate population-level iCAPs. Then, we could perform voxelwise statistical group comparison using subject-specific iCAP maps as data points (i.e.; the frames from each subject associated to a given iCAP are averaged together). To probe temporal features of iCAP activity levels, we could generate activity time courses by multivariate regression (that is, determine what are the best weights to assign to the iCAPs over time to retrieve voxelwise time courses as close as possible to the observed ones), and perform group comparison on features

computed from those signals (e.g., the degree of overlap between different networks, or the average level of expression of an iCAP over time).

Q.28 Do some of the maps that you find with default parameters show spatial overlap? Is this a feature that can be achieved by simpler disentanglement approaches, such as a spatial independent component analysis?

Yes, some of the maps show spatial overlap! Most strikingly, we observe the presence of many iCAPs with strong posterior cingulate and/or precuneus signal. For instance, there is both a full default mode network, and a partial one without any frontal signal.

This feature is not readily achievable by less sophisticated approaches; spatial independent component, for example, relies on spatial independence between sources (that is, between retrieved iCAPs) to solve this disentanglement problem, and spatial independence actually induces scarcely overlapped retrieved networks.

Q.29 Can you come up with a few drawbacks of the TA/iCAPs framework?

Regarding the total activation part of the framework, a first limitation is that it takes at least a few hours to solve the related optimisation problem for one subject, and so, computations for a large dataset of subjects can take considerable time. Another shortcoming is the need to partly fine tune regularisation parameters manually from a dataset to the next, as they also depend on acquisition settings and other variable features from study to study. Finally, for now, it is also required to decide on a hemodynamic response function shape to run the TA algorithm, and this shape is assumed similar across spatial locations; this is in fact a large oversimplification compared to reality, and so, may partly impede on the accuracy of obtained results.

Considering the generation of iCAPs, one may argue that the use of a k-means clustering is only justified is separate networks show innovation (i.e.; change their activity level) at separate times; indeed, as this hard clustering approach will assign every frame to only one iCAP, moments of joint transitions in activity levels will then be inaccurately accounted for in the iCAP generation process. In practice, additional analyses showed that at least 2 separate networks undergo an innovation together in around 20% of frame cases, which is a reasonably elevated number, but that this does not largely influence the generation of iCAPs.

References

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