**Fig 1: (The takeaway is that EC reveals differences FC does not)**

**A** Across-participant average FC in the LSD condition. **B** Across-participant average FC in the placebo condition. **C** Across-participant *t*-statistic values of difference between FC in LSD and placebo conditions. **D** Feature importance estimates for the FC classification model. See ’Statistical analysis’ for a detailed definition of feature importance. **E** Across-participant average EC in the LSD condition. **F** Across-participant average EC in the placebo condition. **G** Across-participant *t*-statistic values of difference between EC in LSD and placebo conditions. **H** Feature importance estimates for the EC classification model.

**Fig 2:**

**A** Bootstrap ratios (BSRs) of whole-brain EC reflecting condition differences. BSRs are the ratios of the loadings on the latent variable and the standard errors estimated from bootstrapping. The larger the magnitude of a BSR, the larger the weight (i.e., the loading on the latent variable) and the smaller the standard error (i.e., higher stability; [[55](https://www.nature.com/articles/s41386-023-01574-8#ref-CR55), [56](https://www.nature.com/articles/s41386-023-01574-8#ref-CR56)]). BSRs can be understood analogous to z-scores if bootstrap distributions are approximately normal [[57](https://www.nature.com/articles/s41386-023-01574-8#ref-CR57)]. **B** Leading Eigenvector reflecting condition differences in whole-brain EC across brain regions. **C** Brain region saliences reflecting condition differences across self-connections. **D** Brain region BSRs reflecting condition differences across self-connections.

**Fig 3:**

Across-participant t-statistic values of the difference between LSD and placebo conditions in outgoing (**A**) or incoming (**B**) thalamic connections (thresholded at *p* < 0.05, whole-brain FDR-corrected). Differences in magnitudes of connectivity are indicated by both line width and opacity. Orange lines indicate stronger connectivity under LSD. Please, see Supplement for region abbreviation key.

**Fig 4:**

**A** t-statistic of the difference between LSD and placebo conditions in self-connections. **B** Top 10 self-connections ranked by t-statistic of the difference between LSD and placebo conditions. **C** Anatomical colourmap of t-statistic of the difference between LSD and placebo conditions in self-connections. **D** Estimates of feature importance of self-connections in EC classification model. **E** Top 10 self-connections by feature importance in the EC classification model. **F** Anatomical colourmap displaying feature importance of self-connections in the EC classification model. For (**A**), (**C**): Orange and blue areas indicate stronger and weaker connectivity under LSD, respectively. For (**B**), errorbars represent the across-participant standard deviation of the differences in connectivity between conditions. In (**B**) and (**E**), abbreviations indicate the ROIs forming each connection. For (**E**), errorbars represent the across-fold standard deviation of the feature importance estimates.

**Fig 5:**

**A** Across-participant t-statistic of the difference in EC between the two directions of influence between each pair of regions, for the LSD condition. **B** Across-participant t-statistic of the difference in EC between the two directions of influence between each pair of regions, for the placebo condition. **C** Across-participant t-statistic of the difference in EC between the two directions of influence between each pair of regions, and between the LSD and placebo conditions. Differences in magnitudes of connectivity and connectivity changes are indicated in each connectogram by both line width and opacity. To maintain visibility, only the top 250 connections have been displayed.

**PFr** Prefrontal **cortex, Fr** Frontal cortex**, Ins** Insular cortex**, Tem** Temporal cortex**, Par** Parietal cortex**, Occ** Occipital cortex**, SbC** Subcortical regions**, CeB** Cerebellum**, Ver** Vermis**, Bstem** Brainstem**.**

## Region of interest label abbreviation key

|  |  |
| --- | --- |
| Abbreviation | ROI |
| AC | Cingulate Gyrus, anterior division |
| Accumbens | Accumbens |
| AG | Angular Gyrus |
| aITG | Inferior Temporal Gyrus, anterior division |
| aMTG | Middle Temporal Gyrus, anterior division |
| Amygdala | Amygdala |
| aPaHC | Parahippocampal Gyrus, anterior division |
| aSMG | Supramarginal Gyrus, anterior division |
| aSTG | Superior Temporal Gyrus, anterior division |
| aTFusC | Temporal Fusiform Cortex, anterior division |
| Brain-Stem | Brain-Stem |
| Caudate | Caudate |
| Cereb1 | Cerebellum Crus1 |
| Cereb10 | Cerebellum 10 |
| Cereb2 | Cerebellum Crus2 |
| Cereb3 | Cerebellum 3 |
| Cereb45 | Cerebellum 4 5 |
| Cereb6 | Cerebellum 6 |
| Cereb7 | Cerebellum 7b |
| Cereb8 | Cerebellum 8 |
| Cereb9 | Cerebellum 9 |
| CO | Central Opercular Cortex |
| Cuneal | Cuneal Cortex |
| FO | Frontal Operculum Cortex |
| FOrb | Frontal Orbital Cortex |
| FP | Frontal Pole |
| HG | Heschl’s Gyrus |
| Hippocampus | Hippocampus |
| IC | Insular Cortex |
| ICC | Intracalcarine Cortex |
| IFG oper | Inferior Frontal Gyrus, pars opercularis |
| IFG tri | Inferior Frontal Gyrus, pars triangularis |
| iLOC | Lateral Occipital Cortex, inferior division |
| LG | Lingual Gyrus |
| MedFC | Frontal Medial Cortex |
| MidFG | Middle Frontal Gyrus |
| OFusG | Occipital Fusiform Gyrus |
| OP | Occipital Pole |
| PaCiG | Paracingulate Gyrus |
| Pallidum | Pallidum |
| PC | Cingulate Gyrus, posterior division |
| pITG | Inferior Temporal Gyrus, posterior division |
| pMTG | Middle Temporal Gyrus, posterior division |
| PO | Parietal Operculum Cortex |
| PostCG | Postcentral Gyrus |
| PP | Planum Polare |
| pPaHC | Parahippocampal Gyrus, posterior division |
| PreCG | Precentral Gyrus |
| Precuneous | Precuneous Cortex |
| pSMG | Supramarginal Gyrus, posterior division |
| pSTG | Superior Temporal Gyrus, posterior division |
| PT | Planum Temporale |
| pTFusC | Temporal Fusiform Cortex, posterior division |
| Putamen | Putamen |
| SCC | Supracalcarine Cortex |
| SFG | Superior Frontal Gyrus |
| sLOC | Lateral Occipital Cortex, superior division |
| SMA | Juxtapositional Lobule Cortex -formerly Supplementary Motor Cortex- |
| SPL | Superior Parietal Lobule |
| SubCalC | Subcallosal Cortex |
| Thalamus | Thalamus |
| TOFusC | Temporal Occipital Fusiform Cortex |
| toITG | Inferior Temporal Gyrus, temporo-occipital part |
| toMTG | Middle Temporal Gyrus, temporo-occipital part |
| TP | Temporal Pole |
| Ver10 | Vermis 10 |
| Ver12 | Vermis 1 2 |
| Ver3 | Vermis 3 |
| Ver45 | Vermis 4 5 |
| Ver6 | Vermis 6 |
| Ver7 | Vermis 7 |
| Ver8 | Vermis 8 |
| Ver9 | Vermis 9 |

Random Forests:

Random Forest is a popular ensemble learning method that operates by constructing multiple decision trees during the training phase. The decision of the majority of the trees is then chosen by the random forest as the final decision. Each tree in the ensemble is built from a sample drawn with replacement (i.e., a bootstrap sample) from the training set.

The Random Forest algorithm introduces extra randomness when growing trees; instead of searching for the very best feature when splitting a node, it searches for the best feature among a random subset of features. This results in a greater tree diversity, which (again) trades a higher bias for a lower variance, generally yielding a better model.

Applying Random Forest to Validate Effective Connectivity FMRI Analysis

Functional Magnetic Resonance Imaging (fMRI) is a technique for mapping and understanding brain activity. Effective connectivity analysis is often used in fMRI to understand the influence that one neural system exerts over another. It's about the causality (directed causal effect) between different regions of the brain.

Random Forests could be used in this context to validate or even infer effective connectivity in a number of ways:

Feature Importance: One of the unique advantages of the Random Forest algorithm is the ability to estimate the importance of each feature in predicting the response variable. This feature can be leveraged to infer the importance of different brain regions (features) in causing a particular activity or response (target variable). The higher the feature importance, the stronger the region's influence is thought to be.

Model Validation: Random Forests can be used as a model validation technique for effective connectivity models. You can train a Random Forest on the same data used to infer connectivity, and then compare the Random Forest's predictions with those of the connectivity model. If the two sets of predictions align closely, this can be seen as a form of validation for the connectivity model.

Nonlinear Interactions: Traditional effective connectivity analysis techniques (like Structural Equation Modeling or Dynamic Causal Modeling) often make linear assumptions about the relationships between brain regions. Random Forests make no such assumptions, allowing them to capture complex, nonlinear interactions between regions.

Handling High Dimensionality: fMRI data are high-dimensional, often involving readings from thousands of voxels (volumetric pixels) for each time point. Random Forests are capable of handling such high-dimensional data without overfitting, which makes them a good fit for this application.