

# NeuroCluster: A Python toolbox for nonparametric cluster-based statistical testing of neurophysiological data with respect to continuous predictors.

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## Summary

Cognitive neurophysiology offers a unique framework for studying cognitive brain-behavior relationships by relating electrophysiological signals to complex behaviors. With the advent of new technical and behavioral paradigms, researchers can design cognitive experiments that leverage both the spatiotemporal resolution of electrophysiological data and the complexity of continuous behavioral variables. Analyzing these data requires sophisticated statistical methods that can interpret multidimensional neurophysiological data and dynamic, continuous behavioral variables. Often used statistical frameworks for nonparametric, cluster-based statistical tests are specifically focused on the contrast between discrete behavioral conditions but are not suitable for assessing how continuous variables predict the occurrence of clusters in neurophysiological data. NeuroCluster is an open-source Python toolbox for analysis of two-dimensional electrophysiological data (e.g. time-frequency representations) related to multivariate and continuous behavioral variables. NeuroCluster introduces a statistical approach which uses nonparametric cluster-based permutation testing in tandem with linear regression to identify two-dimensional clusters of neurophysiological activity that significantly encodes time-varying, continuous behavioral variables. Uniquely, it also supports multivariate analyses by allowing for multiple behavioral predictors to model neural activity. NeuroCluster addresses a methodological gap in statistical approaches to relate continuous, cognitive predictors to underlying electrophysiological activity with time and frequency resolution, to determine the neurocomputational processes giving rise to complex behaviors.

## Statement of need

NeuroCluster addresses a methodological gap in cognitive and behavioral neuroscience, by providing a Python-based statistical toolbox to relate continuous predictors to two-dimensional neurophysiological activity. Continuous predictors vary over an experimental session, reflecting dynamic behaviors, underlying cognitive processes, complex movements, trial-varying experimental conditions, perceptual signals, or value-based trial outcomes (Collins & Shenhav, 2022; Hoy et al., 2021; Mathis & Mathis, 2020; ?). Standard analytical approaches for relating complex behavioral variables to neuronal activity sacrifice the complexity of neurophysiological signals by reducing the dimensionality of neuronal timeseries data (e.g., averaging across temporal, spectral, or spatial domains, or dimensionality reduction) (Crosse et al., 2016; Rey et al., 2015; Saez et al., 2018; Stokes & Spaak, 2016; ?; ?). Conversely, analysis methods

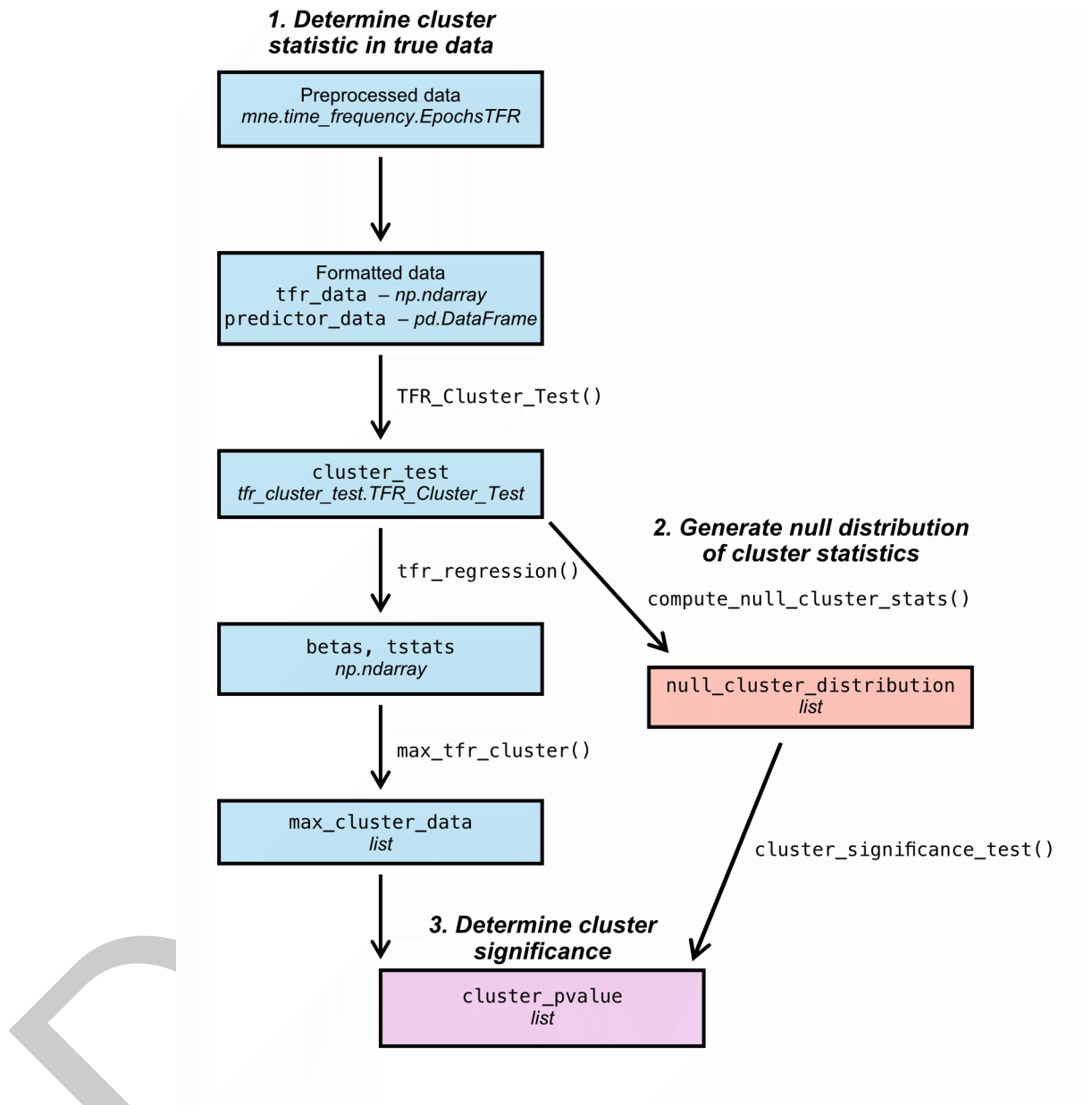
that preserve the complexity of neurophysiological data (i.e., two-dimensional timeseries) constrain behavioral predictors to discrete conditions (Domenech et al., 2020; Kosciessa et al., 2020; Maris & Oostenveld, 2007; ?; ?). Directly linking continuous experimental variables to two-dimensional physiological timeseries data offers a rigorous way to study brain-behavior relationships, by maintaining the complexity of dynamic behavior, without sacrificing the resolution of event-related neurophysiological activity.

NeuroCluster uses cluster-based permutation testing to identify significant two-dimensional clusters with respect to continuous task variables. Cluster-based nonparametric statistical testing is a standard approach to analyze two-dimensional event-related time series data, while controlling for multiple comparisons and reducing family-wise error rates (Cohen, 2014; Groppe et al., 2011; Maris, 2012; Maris & Oostenveld, 2007; Nichols & Holmes, 2002). Neurophysiological activity is typically aggregated by condition to perform a two-sample cluster-based permutation test, which tests whether the neuronal encoding patterns differ between two discrete task conditions, rather than continuous, trial-varying features (Bullmore et al., 1999; Maris & Oostenveld, 2007). While two-sample cluster-based permutation tests provide a nonparametric statistical inference tool for identifying the presence of significant clusters of activity between two conditions, they are insufficient for identifying the presence of clusters as a function of continuously varying predictors. NeuroCluster provides a solution to this analytical gap by performing linear regressions at individual points across the 2D neural matrix. This approach enables users to quantify the degree to which a continuous predictor is related to neurophysiological activity at the pixel-level and to perform analyses with multivariate behavioral data, by incorporating multiple continuous or categorical covariates in the regression models. The t-statistics corresponding to the predictor of interest from the pixel-wise regressions are thresholded by a critical t-statistic to control for the FDR, creating a binary 2D matrix (Genovese et al., 2002). The binary 2D matrix is then used to identify putative 2D clusters of activation related to the continuous predictor of interest. This process is repeated many times with the predictor of interest randomly permuted to produce a surrogate distribution of 2D clusters. Clusters that survive cluster-based permutation testing are classified as significant regions of activation with respect to the specified continuous predictor.

NeuroCluster is applicable for numerous analysis goals; the major use cases are performing an initial exploratory analysis to generate specific hypotheses, determine data-driven windows of interest, or to identify regional patterns of significant clusters within and between subjects. Future adaptations of NeuroCluster may implement mixed effects regressions, nonlinear mapping models, or group-level analysis frameworks (Bianchi et al., 2019; Ivanova et al., 2022; König et al., 2024; Yu et al., 2022). We demonstrate our approach with human intracranial local field potential data, but NeuroCluster is applicable for all types of two-dimensional neurophysiological measures (e.g., spatiotemporal clusters from EEG/MEG, cross-frequency interactions). To our knowledge, NeuroCluster presents a novel Python-based statistical software package. NeuroCluster is designed to supplement existing Python-based electrophysiological analysis toolboxes (Donoghue et al., 2020; Gramfort, 2013; Kosciessa et al., 2020; Whitten et al., 2011), particularly MNE-Python.

## NeuroCluster Documentation

NeuroCluster is accompanied by a detailed tutorial which outlines the workflow (Fig. 1) for implementing this approach with time-frequency power estimates from multi-region LFP recording.



**Figure 1:** NeuroCluster workflow. This approach involves three key steps: (1) determine cluster statistic in true data, (2) generate a null distribution of cluster statistics by permuting dataset, (3) determine significance of true cluster statistic against null distribution.

Below we outline the statistical approach implemented by NeuroCluster for performing nonparametric permutation-based cluster testing using time-frequency resolved power estimates from neural data estimated using (?) and continuous predictors (i.e., latent cognitive processes, behavior, or experimental conditions). In these example data, we are testing the hypothesis that RPEs are significantly encoded in the electrophysiological signal from a given iEEG channel time-frequency representation (TFR). The following methodological description is based on data collected from a neurosurgical epilepsy patient undergoing stereotactic EEG (sEEG) monitoring for treatment-resistant depression. During the monitoring period, the patient performed a value-based decision-making task while local field potentials (LFPs) were recorded

96 from both cortical and subcortical sites. By analyzing the patient's behavior during the task,  
97 we derived continuous variables representing hypothesized latent cognitive processes—such as  
98 the trial-by-trial computation of reward prediction errors (RPEs)—to examine their relationship  
99 with neural activity.

## 100 **1. Determine cluster statistic in true data**

101 A. Define clusters: At each time-frequency index, we perform a linear univariate (or multivariate)  
102 regression using behaviorally-derived independent variables (e.g., latent cognitive variables,  
103 behavioral measures, task conditions) to predict neuronal activity (i.e., power). The  $\beta$  coefficient  
104 represents the strength and direction of the relationship between each independent variable  
105 and the dependent variable. It is estimated from the regression model and reflects how changes  
106 in the independent variable are associated with changes in power at the specific time-frequency  
107 pair. Pixel-wise regressions are parallelized for speed. For each time-frequency pair, the  $\beta$   
108 coefficient for the regressor of interest (the independent variable of primary interest) is extracted  
109 from the regression results (Fig 2A). A t-statistic is computed for the  $\beta$  coefficient to capture  
110 how significantly different it is from zero (Fig 2B). A significance threshold is applied to the  
111 t-statistics of the  $\beta$  coefficient for the regressor of interest. If the t-statistic for a time-frequency  
112 pair exceeds the significance threshold, the pair is deemed significant. Clusters are then defined  
113 as adjacent time-frequency pairs where all pairs within the cluster have t-statistics exceeding  
114 the threshold, according to the test's desired tails (Fig 2C).

115 B. Compute cluster statistics: For each identified cluster, sum the t-statistics of all time-  
116 frequency pairs within the cluster. In a two-tailed test (the default), compute both the  
117 maximum and minimum cluster sums (Fig 2D).

## 118 **2. Generate null distribution of cluster statistics**

119 A. Permutation procedure: Labels for the behavioral predictor of interest are shuffled for the  
120 desired number of permutations.

121 B. Recalculate cluster statistic: Steps 1A/1B are repeated to define clusters and compute  
122 cluster statistics for each permuted dataset.

123 C. Construct null distribution: The cluster statistics from all permutations are compiled to  
124 create a null distribution, representing the distribution of cluster statistics under the null  
125 hypothesis (Fig 2E). The permuted TFR regressions are also parallelized at the pixel-level,  
126 while each permutation is performed sequentially. We tested many iterations of these functions  
127 with different parallelization approaches and sequential permutation-level computations with  
128 pixel-level parallelization within each TFR regression was the fastest method.

## 129 **3. Determine cluster significance**

130 A. Compare true cluster statistic to null distribution to compute p-values: The proportion of  
131 cluster statistics in the null distribution falling above (or below) the true cluster statistic(s)  
132 determines the p-value associated with the cluster(s) identified in the true data (Fig 2E).



**Figure 2:** NeuroCluster methods. A.  $\beta$  coefficients for continuous predictor of interest (RPE) predicting power in given time-frequency pair (red outline = maximum positive cluster; blue outline = maximum negative cluster). B. T-statistics corresponding with  $\beta_{\text{RPE}}$  coefficients. C. Clusters as determined using  $t$ -critical threshold. D. Maximum positive and negative clusters determined by summing  $t$ -statistics in identified clusters. E. Null distribution of cluster statistics generated by permuting dataset for predictor of interest (100 permutations; red dashed line = true cluster statistic).

#### 4. Comparison of results to existing methods.

To evaluate the advantages of NeuroCluster, we compared its results to those obtained using MNE-Python's two-sample cluster-based permutation test. This approach requires discretizing the continuous variable of interest (RPE) into distinct categories, which reduces the resolution of the behavioral predictor. Additionally, MNE-Python's implementation does not support multivariate analyses, limiting the ability to model multiple behavioral covariates simultaneously. When applying the two-sample cluster test to our data, we did not identify any significant clusters of increased or decreased activity related to RPE. In contrast, NeuroCluster successfully detected significant clusters (Fig. 2), demonstrating its ability to preserve the richness of continuous behavioral variables and reduce the likelihood of false negatives. This comparison highlights NeuroCluster as a powerful and flexible alternative to existing statistical methods for analyzing continuous brain-behavior relationships.

#### 5. Metric validation in synthetic data with known ground truth.

Thus far, we have demonstrated NeuroCluster using biological data. However, because these data are experimental, there is no definitive ground truth for the observed neural fluctuations associated with behavioral predictors. To validate the NeuroCluster method, we generated synthetic TFR data (2-200 Hz, sampling rate = 250, -1 to +1 seconds around "choice", 1 channel, 100 trials) with a known linear association between power in a specific time-frequency region and a continuous behavioral variable—in this case, the expected value of choice. Code for simulating these data is provided in the NeuroCluster repository. We then applied NeuroCluster to the synthetic dataset and, as expected, successfully identified a significant positive cluster corresponding to the known association embedded in the data (Fig. 3). This validation confirms the accuracy of NeuroCluster and provides evidence against its susceptibility to false positives.



**Figure 3:** NeuroCluster validation in synthetic data. A. Time-frequency representation (TFR) showing power differences between high ( $>0.50$ ) and low ( $<0.50$ ) expected value trials in synthetic data (1 channel, 100 trials, time-locked to “choice”). B. A significant positive cluster identified in the expected time-frequency region, consistent with the predefined association embedded in the synthetic dataset.

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## References

- Bianchi, B., Shalom, D. E., & Kamienkowski, J. E. (2019). Predicting known sentences: Neural basis of proverb reading using non-parametric statistical testing and mixed-effects models. *Frontiers in Human Neuroscience*, 13, 82. <https://doi.org/10.3389/fnhum.2019.00082>
- Bullmore, E. T., Suckling, J., Overmeyer, S., Rabe-Hesketh, S., Taylor, E., & Brammer, M. J. (1999). Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Transactions on Medical Imaging*, 18(1), 32–42. <https://doi.org/10.1109/42.750253>
- Cohen, M. X. (2014). *Analyzing neural time series data: Theory and practice*. MIT Press.
- Collins, A. G., & Shenhav, A. (2022). Advances in modeling learning and decision-making in neuroscience. *Neuropsychopharmacology*, 47(1), 104–118. <https://doi.org/10.1038/s41386-021-01126-y>
- Crosse, M. J., Di Liberto, G. M., Bednar, A., & Lalor, E. C. (2016). The multivariate temporal response function (mTRF) toolbox: A MATLAB toolbox for relating neural signals to continuous stimuli. *Frontiers in Human Neuroscience*, 10. <https://doi.org/10.3389/fnhum.2016.00604>
- Domenech, P., Rheims, S., & Koechlin, E. (2020). Neural mechanisms resolving exploitation-exploration dilemmas in the medial prefrontal cortex. *Science*, 369(6507), eabb0184. <https://doi.org/10.1126/science.abb0184>
- Donoghue, T., Haller, M., Peterson, E. J., Varma, P., Sebastian, P., Gao, R., Noto, T., Lara, A. H., Wallis, J. D., Knight, R. T., & others. (2020). Parameterizing neural power spectra into periodic and aperiodic components. *Nature Neuroscience*, 23(12), 1655–1665. <https://doi.org/10.1038/s41593-020-00744-x>
- Genovese, C. R., Lazar, N. A., & Nichols, T. (2002). Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *NeuroImage*, 15(4), 870–878. <https://doi.org/10.1006/nimg.2001.1037>



- 186 Gramfort, A. (2013). MEG and EEG data analysis with MNE-python. *Frontiers in Neuroscience*,  
187 7. <https://doi.org/10.3389/fnins.2013.00267>
- 188 Groppe, D. M., Urbach, T. P., & Kutas, M. (2011). Mass univariate analysis of event-related  
189 brain potentials/fields i: A critical tutorial review. *Psychophysiology*, 48(12), 1711–1725.  
190 <https://doi.org/10.1111/j.1469-8986.2011.01273.x>
- 191 Hoy, C. W., Steiner, S. C., & Knight, R. T. (2021). Single-trial modeling separates multiple  
192 overlapping prediction errors during reward processing in human EEG. *Communications*  
193 *Biology*, 4(1), 910. <https://doi.org/10.1038/s42003-021-02426-1>
- 194 Ivanova, A. A., Schrimpf, M., Anzellotti, S., Zaslavsky, N., Fedorenko, E., & Isik, L. (2022).  
195 Beyond linear regression: Mapping models in cognitive neuroscience should align with  
196 research goals. *Neurons, Behavior, Data Analysis, and Theory*, 1. <https://doi.org/10.51628/001c.37507>
- 198 König, S. D., Safo, S., Miller, K., Herman, A. B., & Darrow, D. P. (2024). Flexible multi-step  
199 hypothesis testing of human ECoG data using cluster-based permutation tests with GLMEs.  
200 *NeuroImage*, 290, 120557. <https://doi.org/10.1016/j.neuroimage.2024.120557>
- 201 Kosciessa, J. Q., Grandy, T. H., Garrett, D. D., & Werkle-Bergner, M. (2020). Single-trial  
202 characterization of neural rhythms: Potential and challenges. *NeuroImage*, 206, 116331.  
203 <https://doi.org/10.1016/j.neuroimage.2019.116331>
- 204 Maris, E. (2012). Statistical testing in electrophysiological studies. *Psychophysiology*, 49(4),  
205 549–565. <https://doi.org/10.1111/j.1469-8986.2011.01320.x>
- 206 Maris, E., & Oostenveld, R. (2007). Nonparametric statistical testing of EEG- and MEG-data.  
207 *Journal of Neuroscience Methods*, 164(1), 177–190. <https://doi.org/10.1016/j.jneumeth.2007.03.024>
- 209 Mathis, M. W., & Mathis, A. (2020). Deep learning tools for the measurement of animal  
210 behavior in neuroscience. *Current Opinion in Neurobiology*, 60, 1–11. <https://doi.org/10.1016/j.conb.2019.10.008>
- 212 Nichols, T. E., & Holmes, A. P. (2002). Nonparametric permutation tests for functional  
213 neuroimaging: A primer with examples. *Human Brain Mapping*, 15(1), 1–25. <https://doi.org/10.1002/hbm.1058>
- 215 Rey, H. G., Ahmadi, M., & Quiroga, R. (2015). Single trial analysis of field potentials  
216 in perception, learning and memory. *Current Opinion in Neurobiology*, 31, 148–155.  
217 <https://doi.org/10.1016/j.conb.2014.10.009>
- 218 Saez, I., Lin, J., Stolk, A., Chang, E., Parvizi, J., Schalk, G., Knight, R. T., & Hsu, M.  
219 (2018). Encoding of multiple reward-related computations in transient and sustained  
220 high-frequency activity in human OFC. *Current Biology*, 28(18), 2889–2899.e3. <https://doi.org/10.1016/j.cub.2018.07.045>
- 222 Stokes, M., & Spaak, E. (2016). The importance of single-trial analyses in cognitive neuro-  
223 science. *Trends in Cognitive Sciences*, 20(7), 483–486. <https://doi.org/10.1016/j.tics.2016.05.008>
- 225 Whitten, T. A., Hughes, A. M., Dickson, C. T., & Caplan, J. B. (2011). A better oscillation  
226 detection method robustly extracts EEG rhythms across brain state changes: The human  
227 alpha rhythm as a test case. *NeuroImage*, 54(2), 860–874. <https://doi.org/10.1016/j.neuroimage.2010.08.064>
- 229 Yu, Z., Guindani, M., Grieco, S. F., Chen, L., Holmes, T.-C., & Xu, X. (2022). Beyond t test  
230 and ANOVA: Applications of mixed-effects models for more rigorous statistical analysis in  
231 neuroscience research. *Neuron*, 110(1), 21–35. <https://doi.org/10.1016/j.neuron.2021.10.030>