Accepted Manuscript

Accepted Manuscript (Uncorrected Proof)

Title: Brain Connectivity Estimation Pitfall in Multiple Trials of EEG Data

Running title: Brain Connectivity Estimation Pitfall

Authors: Vida Mehdizadehfard^{1*}, Fanaz Ghassemi¹, Ali Fallah¹

1. Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran.

* **Corresponding Author:** Vida Mehdizadehfard, Department of Biomedical Engineering, Amirkabir University of Technology, Tehran, Iran. E-mail: mehdizadeh@aut.ac.ir

To appear in: *Basic and Clinical Neuroscience*

Received date: 2021/04/6

Revised date: 2021/06/14

Accepted date: 2021/08/21

This is a “Just Accepted” manuscript, which has been examined by the peer-review process and has been accepted for publication. A “Just Accepted” manuscript is published online shortly after its acceptance, which is prior to technical editing and formatting and author proofing. *Basic and Clinical Neuroscience* provides “Just Accepted” as an optional and free service which allows authors to make their results available to the research community as soon as possible after acceptance. After a manuscript has been technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as a published article. Please note that technical editing may introduce minor changes to the manuscript text and/or graphics which may affect the content, and all legal disclaimers that apply to the journal pertain.

Please cite this article as:

Mehdizadehfar, V., Ghassemi, F., & Fallah, A. (In Press). Brain Connectivity Estimation Pitfall in Multiple Trials of EEG Data. *Basic and Clinical Neuroscience*. Just Accepted publication Sep. 15, 2021. Doi: <http://dx.doi.org/10.32598/bcn.2021.3549.1>

DOI: <http://dx.doi.org/10.32598/bcn.2021.3549.1>

Abstract

The electroencephalography signal is well suited to calculate brain connectivity due to its high temporal resolution. When the purpose is to compute connectivity from multi-trial EEG data, confusion arises about how these trials involved in calculating the connectivity. The purpose of this paper is to study this confusing issue using simulated and experimental data. To this end, Granger causality-based connectivity measures were considered. Using simulations, two signals were generated with known AR (Auto-Regressive) coefficients and then simple MVAR (Multivariate AR) models based on different numbers of trials were extracted. For accurate estimation of the MVAR model, the data samples should be sufficient. Two Granger causality-based connectivity, GC and PDC were estimated. Estimating connectivity corresponding to small trial numbers (5 and 10 trials) resulted in an average value of connectivity that is significantly higher and also more variable over different estimates. By increasing the number of trials, the MVAR model has fitted more appropriately to the data and the connectivity values were converged. This procedure was implemented on real EEG data. The obtained results agreed well with the findings of simulated data. The results showed that the brain connectivity should calculate for each trial, and then average the connectivity values on all trials. Also, the larger the trial numbers, the MVAR model has fitted more appropriately to the data, and connectivity estimations are more reliable.

Keywords: Connectivity Analysis, Multiple trials, Auto-Regressive model, Granger causality, EEG

1. Introduction

All brain functions, including sensation, perception, cognition, and each of human action, are performed by dynamically complex neuronal networks in the brain (Omidvarnia et al., 2014). Studying these networks helps to a better understanding of the neurological development mechanisms of the brain and its related disorders. There has been a growing interest in studying the brain to identify connectivity and interactions among distributed brain regions (Bastos & Schoffelen, 2016). Several imaging techniques can be utilized to evaluate brain connectivity such as fMRI, MEG, and EEG (Cociu et al., 2017). EEG is often an appropriate technique for studying brain connectivity due to its high temporal resolution, non-invasive nature, and low-cost (Williams, Daly, & Nasuto, 2018). Two important explanations of brain connectivity have been suggested: functional and effective connectivity (non-directed and directed connectivity, respectively) (Astolfi et al., 2007). Functional connectivity is inferred based on temporal correlations between measurements of neuronal activity. In contrast, effective connectivity refers to the model of causal effects that describe the influence of a neural unit on others (Friston, 2011). So the effective connectivity demonstrates the direction of information flow in the brain.

Once the EEG signals have been recorded, the signal processing specialist is confronted with the challenge of quantifying the neural interactions and provide a reliable interpretation of the findings (Wang et al., 2014). When the number of trials/observations used to compute connectivity metrics is different, in other words, different length of data is involved in the calculation of connectivity metrics, estimated connectivity and consequently its neurophysiological interpretations are variable over different trial counts. Generally, connectivity metrics are often affected by the length of the data. A reason for this fact is that many of connectivity measures are based on the magnitude of a quantity, which always has a positive value, so the difference of measures which computed

with different data length will never be zero, and A set of human emotional faces consists of happy, sad, and anger A set of human emotional faces consists of happy, sad, and anger zero. The effect of this fact is often dependent on the data points of data: the smaller the data points, the larger the effect (Bastos & Schoffelen, 2016). For some measures like coherence, analytic estimates of this effect can be estimated and applied, but cannot be generalized to all metrics (Bokil, Purpura, Schoffelen, Thomson, & Mitra, 2007). Another suggestion to address this problem is to utilize the inferential statistical framework, based on a non-parametric/ parametric statistical test, which is not generalizable Granger causality estimates for 5, 10, 100, 500, and 1000 trials. The vertical axis represents the average GC metric ± 1 standard deviation across 100 realizations (Simulated data). The main purpose of this paper is to clarify the confusion in connectivity analysis when multiple trials of EEG data are used. The analysis pitfall that a researcher may encounter in performing connectivity analysis on real data is investigated using the simulated data and experimental data.

2. Materials and Methods

The flowchart illustrated in Figure 1 outlines the summary of the procedure in the current study. The presented procedures are evaluated using the simulated data and experimental data. In this flowchart, the essential steps are summarized. As shown in figure 1.A1, two synthetic signals are generated. Experimental EEG data are preprocessed according to figure 1.A2. The MVAR representation of signals is utilized to find out the information flow between pairs of signals and directional interactions between them as shown in figure 1.B. Finally, connectivity measure is extracted and compared in different trial numbers which are summarized in figure 1.C. All of these steps are described in detail below.

2.1 Multivariate Autoregressive Model

Directed connectivity measures can be quantified with various methods, which are divided into two groups of data-driven and model-based methods (reviewed in (Lehnertz, 2011)). Among these, multivariate autoregressive models (MVAR) have been widely utilized for biological signal analysis (Faes, Erla, & Nollo, 2012). The MVAR process is a time-frequency domain statistical tool to model directional and causal information flow between EEG electrodes. In these model-based procedures, a parametric MVAR model fits observed data time-series using some techniques like multivariate lattice algorithms, state-space models or least-squares methods (Mullen, 2010). In the last decade, an increasing number of model-based connectivity measures, closely related to Granger's definition of causality, have been proposed. Granger causality based connectivity measures were considered in the current paper.

In 1969 Granger expressed a comprehensive formalization of Granger causality (GC) (Granger & Aug, 1969). GC states that if one stochastic process $X_2(t)$ contains information in past values that permits a more accurate prediction of $X_1(t)$, then $X_2(t)$ could be called a casual to $X_1(t)$ (Blinowska, 2011). Assume $X_1(t)$ is represented by an AR model using P previous values of $X_1(t)$ with a prediction error e_{11} and A as coefficient matrix,

$$X_1(t) = \sum_{i=1}^P A_i X_1(t-i) + e_{11} \quad (1)$$

Then suppose $X_1(t)$ is represented using P previous values of $X_1(t)$ and P previous values of $X_2(t)$ with a prediction error e_{12} ,

$$X_1(t) = \sum_{i=1}^P A_i X_1(t-i) + \sum_{i=1}^P B_i X_2(t-i) + e_{12} \quad (2)$$

If the variance of e_{11} ($\delta_{11}^2(e_{11})$) is more than the variance of e_{12} ($\delta_{12}^2(e_{12})$), then it is an explanation of a causal interaction from $X_2(t)$ to $X_1(t)$.

2.2 Segmentation-based MVAR Model

The concept of segmentation-based MVAR is similar to windowing techniques. The sliding window of length W from the data is extracted and the MVAR model fitted to this data. By moving the sliding window and repeat the procedure, the MVAR coefficient matrix is obtained. Sufficient data points to fit the model is an important issue accurately. Let $\mathbf{X}(t) = x_1, \dots, x_T$ is an M -dimensional time-series EEG data of length T (M : electrodes of EEG data, T : time points per electrode). In the general case, we have M^2P coefficients to estimate (M : the number of EEG electrode, P : the order of model), which requires a minimum of M^2p data samples. Having much more than these data samples is required. When multiple trials are available, it can be assumed that each trial is a random sample from the same stochastic process and averaging of trials reduce the bias of model. So for accurate MVAR estimation, the data of all trials are used. After obtaining the frequency-domain representation of the model, several quantities can be computed related to the information flow, oscillations, and coupling in neuronal systems based on model parameters for each trial separately. In the connectivity measure of multiple trials, an essential stage is to take a vector summation operation followed by a total number of trials-normalization stage. In the comparison of calculated connectivity measures from samples with different numbers of trials, it was observed that in the condition with the smallest number of samples (low number of trials), overestimation of connectivity may occur (Friston, 2011). Therefore the larger trial numbers result in better model parameter estimation.

2.3 Granger Causality (GC)

Granger causality and Granger-based measures are susceptible to quantify bi-directional interactions. Originally, the concept of Granger causality was defined in the time domain, then the extension to the frequency domain was formulated by Geweke (Geweke, 1982). Two quantities are required to the computation of frequency domain-Granger causality: The covariance of the AR-model's residuals (Σ), and the spectral transfer matrix $H(\omega)$ (defined as $A^{-1}(\omega)$). The following fundamental identity holds: $H(\omega)\Sigma H(\omega)^* = S(\omega)$, with $S(\omega)$ being the cross-spectral density matrix for X_1 and X_2 at frequency ω . From the residuals' covariance matrix, the spectral transfer matrix and the cross-spectrum, the Granger causality in the frequency domain can be computed as follows:

$$GC_{X_2 \rightarrow X_1} = \ln\left(\frac{S_{X_1 X_1}(\omega)}{S_{X_1 X_1}(\omega) - \left(\Sigma_{X_2 X_2} - \frac{\Sigma_{X_1 X_2}^2}{\Sigma_{X_1 X_1}}\right) |H_{X_1 X_2}(\omega)|^2}\right) \quad (3)$$

Figure 1: Summary of the pipeline. (A) Generating synthetic signals. (B) Extracting the Granger causality and estimating the MVAR model of the data. (C) Calculating connectivity measure (here GC and PDC) for a different number of trials.

2.4 Partial Directed Coherence (PDC)

The PDC was defined in the following form (Baccalá & Sameshima, 2001):

$$p_{ij}(f) = \frac{A_{ij}(f)}{\sqrt{a_j^*(f)a_j(f)}} \quad (4)$$

In this equation, $A_{ij}(f)$ is the Fourier transform of MVAR model coefficients $A(t)$ and $a_j(f)$ is a column with index j of $A(f)$ matrix.

3- Results

3.1 Pipeline Validation

To simulate the effect of trial number on connectivity calculation, two simulated data were generated with known AR coefficients, a sampling rate of 400 Hz, and 500 milliseconds trial length. After extracting the Granger causality, based on 5, 10, 100, 500, and 1000 trials, simple auto-regressive models were calculated for each condition. After selecting the model parameters, the model order was tested between 1 and 20 to select the best order using AIC information criteria. The proposed model was validated using three criteria of whiteness, consistency, and stability. Finally, a model that met all three validation criteria is selected.

Two popular connectivity measures, GC and PDC, are selected to investigate in the present paper. Here, 100 simulations of a simple AR model were realized and the average and the standard deviation across realizations are considered. The simulations showed that the estimating Granger causality corresponding to small trial numbers resulted in an average value of connectivity that is significantly higher and also more variable over different estimates in comparison to conditions with higher trial numbers. By increasing the number of trials, the AR model has been fitted more

appropriately to the data and hence the Granger causality results are more reliable. Figure 2 shows the results of this simulation. A similar result is obtained for the PDC metric. As shown in Figure 3, the value of average PDC decreases with increasing the number of trials and also its variability decreases. The obtained results of these two simulations quite agree. Figure 4 and Figure 5 represent the value of GC and PDC connectivity metrics throughout the frequency band for different trial numbers respectively. As shown in these figures, the values of connectivity are lower for higher trial numbers.

Figure 2. Granger causality estimates for 5, 10, 100, 500, and 1000 trials. The vertical axis represents the average GC metric ± 1 standard deviation across 100 realizations (Simulated data).

Figure 3: Partial Directed Coherence estimates for 5, 10, 100, 500, and 1000 trials. The vertical axis represents the average PDC metric ± 1 standard deviation across 100 realizations (Simulated data).

Figure 4: GC measure for different trial number (Simulated data).

Figure 5: PDC measure for different trial number (Simulated data).

3.2 Experimental Data

A set of human emotional faces consists of happy, sad, and anger was selected from the extended Cohn-Kanade (ck+) dataset (Lucey et al., 2010). The facial expressions were presented on a computer screen for a duration of 3000 ms and immediately replaced by a black screen for 1000 ms. The task was developed using Psychtoolbox software- 3.0.14 (Brainard, Brainard, & Vision, 1997). Participants were asked to determine the emotion by pressing a key using a gamepad with three buttons corresponding to each expression. They were requested to look at the center of the screen and attempt to reduce blinks during the data acquisition. EEG signal was continuously recorded using active electrodes situated on a standard cap according to the 10-20 system using a *g.tec* amplifier and digitized at 1200 Hz. Impedances of all active electrodes were kept below 10k Ω throughout data acquisition (Mehdizadehfard, Ghassemi, Fallah, & Pouretamad, 2020).

The proposed pipeline was applied to a real EEG data. Data analysis was performed for two electrodes in frontal (F4) and occipital (O2) regions. After standard preprocessing including band-pass filtering (0.5-60 Hz), removing ocular artifact, epoch (pre-stimulus time= 500 ms, post-stimulus time=1500 ms), and removing bad epochs, GC and PDC were extracted for 5, 10, 20, and 60 trials. Estimated GC corresponding to small trial numbers resulted in an average Granger causality value that was higher over different estimates in comparison to conditions with higher trial numbers (Figure 6). As shown in Figure 7, the value of average PDC decreases with increasing the number of trials. The obtained results agree well with the findings of simulated data and the decreasing trend in the connectivity measure has the same trend in simulated data and real EEG data. Figure 8 and Figure 9 represent the value of GC and PDC connectivity between two EEG electrodes (F4 and O2) throughout the frequency band for different trial numbers respectively. As shown in these figures, the values of connectivity are lower for higher trial numbers.

Figure 6: Granger causality estimates for 5, 10, 20, and 60 trials (EEG data).

Figure 7: Partial Directed Coherence estimates for 5, 10, 20, and 60 trials (EEG data).

Figure 8: GC measure for different trial number (EEG data).

Figure 9: PDC measure for different trial number (EEG data).

4- Discussion

The purpose of the current paper is to study brain connectivity estimation pitfall related to multiple trials of EEG data. When attempting to compute connectivity from a data with many observation epochs, the question arises whether brain connectivity is calculated for each trial and then average them or for the averaged trials. When the connectivity measures were calculated on trials, it must be interpreted as a measure of dependency between averaged trials responses, rather than the underlying neural processes. Since this can be difficult to interpret, it is suggested to fit connectivity models to the ensembles of trials instead for describing information transfer. Here, the number of trials used to calculate connectivity should be considered. Estimating connectivity corresponding to small trial numbers (5 and 10 trials in our simulations) resulted in a higher average value. For a larger number of trials (100, 500 and 1000 trials in our simulations), results are more reliable. Therefore, it can be said that the larger the trial number, the better the model

parameter estimation. The analysis of connectivity metrics for different lengths of EEG data also confirmed the results of simulation data. By increasing the trial numbers, the values of connectivity measure are decreased and converged.

5- Conclusion

In this paper, we have presented a connectivity estimation pitfall when using multiple trials of EEG data. GC and PDC connectivity estimation methods, as a frequency domain demonstration of the directed relationship between pairs of signals, were applied to simulated synthetic time series. Significant decreasing in the value of connectivity measures and their variability were observed by increasing the number of trials and so the overestimation of connectivity in small trial numbers is confirmed. So for the larger the trial numbers, connectivity estimation will be more accurate and therefore result in a reliable interpretation of connectivity networks in the brain. The pitfall investigated in this paper is a general issue and it is independent of the type of task used in data recording and the method of connectivity measurement. It is hoped that this clarification will be effective in reducing the confusion of researchers and results in a proper inference.

Author contributions

Conceived and designed study: Vida Mehdizadehfar, Farnaz Ghassemi; Conducted the analysis: Vida Mehdizadehfar; Interpreted the findings: Vida Mehdizadehfar, Farnaz Ghassemi, Ali Fallah; Wrote the Manuscript: Vida Mehdizadehfar; Editing of drafts: Farnaz Ghassemi, Ali Fallah.

Conflicts of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Astolfi, L., Cincotti, F., Mattia, D., Marciani, M. G., Baccala, L. A., Fallani, F. D. V., ... Babiloni, F. (2007). Comparison of different cortical connectivity estimators for high-resolution EEG recordings. *Human Brain Mapping*, 28(2), 143–157. <https://doi.org/10.1002/hbm.20263>
- Baccalá, L. a, & Sameshima, K. (2001). Partial directed coherence: a new concept in neural structure determination. *Biological Cybernetics*, 84(6), 463–474. <https://doi.org/10.1007/PL00007990>
- Bastos, A. M., & Schoffelen, J. (2016). A Tutorial Review of Functional Connectivity Analysis Methods and Their Interpretational Pitfalls. *Frontiers in Systems Neuroscience*, 9(January), 1–23. <https://doi.org/10.3389/fnsys.2015.00175>
- Blinowska, K. J. (2011). Review of the methods of determination of directed connectivity from multichannel data. *Medical and Biological Engineering and Computing*, 49(5), 521–529. <https://doi.org/10.1007/s11517-011-0739-x>
- Bokil, H., Purpura, K., Schoffelen, J., Thomson, D., & Mitra, P. (2007). Comparing spectra and coherences for groups of unequal size. *Journal of Neuroscience*, 159, 337–345. <https://doi.org/10.1016/j.jneumeth.2006.07.011>
- Brainard, D. H., Brainard, D. H., & Vision, S. (1997). The psychophysics toolbox. *Spatial Vision*, 10, 433–436.
- Cociu, B. A., Das, S., Billeci, L., Jamal, W., Maharatna, K., Calderoni, S., ... Muratori, F. (2017). Multimodal Functional and Structural Brain Connectivity Analysis in Autism: A Preliminary Integrated Approach with EEG, fMRI and DTI. *IEEE Transactions on Cognitive and Developmental Systems*, 8920(c), 1–1. <https://doi.org/10.1109/TCDS.2017.2680408>
- Faes, L., Erila, S., & Nollo, G. (2012). Measuring connectivity in linear multivariate processes:

- Definitions, interpretation, and practical analysis. *Computational and Mathematical Methods in Medicine*, 2012. <https://doi.org/10.1155/2012/140513>
- Friston, K. J. (2011). Functional and effective connectivity: A Review. *Brain Connectivity*, 1(1), 13–36. <https://doi.org/10.1089/brain.2011.0008>
- Geweke, J. (1982). Measurement of linear dependence and feedback between multiple time series. *Journal of the American Statistical Association*, 77(378), 304–313.
- Granger, C. W. J., & Aug, N. (1969). Investigating causal relations by econometric models and cross-spectral methods. *Econometrica: Journal of the Econometric Society*, 37(3), 424–438.
- Lehnertz, K. (2011). Assessing directed interactions from neurophysiological signals - An overview Assessing directed interactions from neurophysiological signals — an overview. *PHYSIOLOGICAL MEASUREMENT*, 32(November 2011). <https://doi.org/10.1088/0967-3334/32/11/R01>
- Lucey, P., Cohn, J. F., Kanade, T., Saragih, J., Ambadar, Z., & Matthews, I. (2010). The extended cohn-kanade dataset (ck+): A complete dataset for action unit and emotion-specified expression. In *Computer Vision and Pattern Recognition Workshops (CVPRW), 2010 IEEE Computer Society Conference on* (pp. 94–101). IEEE.
- Mehdizadehfar, V., Ghassemi, F., Fallah, A., & Pouretmad, H. (2020). EEG study of facial emotion recognition in the fathers of autistic children. *Biomedical Signal Processing and Control*, 56, 101721. <https://doi.org/10.1016/j.bspc.2019.101721>
- Mullen, T. (2010). Source Information Flow Toolbox (SIFT). *Swartz Center for Computational Neuroscience*, 1–69.
- Omidvarnia, A., Azemi, G., Boashash, B., O’Toole, J. M., Colditz, P. B., Vanhatalo, S., ... Vanhatalo, S. (2014). Measuring time-varying information flow in scalp EEG signals: orthogonalized partial directed coherence. *IEEE Transactions on Biomedical Engineering*, 61(3), 680–693. <https://doi.org/10.1109/TBME.2013.2286394>
- Wang, H. E., Bénar, C. G., Pascale, P., Friston, K. J., Jirsa, V. K., Valdes-sosa, P. A., ... Bernard, C. (2014). A systematic framework for functional connectivity measures. *Frontiers in*

Neuroscience, 8(December), 405. <https://doi.org/10.3389/fnins.2014.00405>

Williams, N. J., Daly, I., & Nasuto, S. J. (2018). Markov Model-based method to analyse time-varying networks in EEG task-related data. *Frontiers in Computational Neuroscience*, 12(September), 76. <https://doi.org/10.3389/fncom.2018.00076>

Accepted Manuscript (Uncorrected Proof)