Article

How Functional Connectivity Measures Affect the Outcomes of MST Neuronal Network Characteristics in Patients with Schizophrenia Compared to Healthy Controls

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Abstract: Background: Modern computational solutions enabling evaluation of the global neuronal network arrangement seem to be particularly valuable for research on neuronal disconnection in schizophrenia. However, a vast number of algorithms used in these analyzes may be an uncontrolled source of results' inconsistency. **Objective:** The aim of our study was to verify whether the comparison of schizophrenia patients with healthy controls, in terms of indexes describing the organization of the neural network, will give analogous results when these parameters are calculated using two different functional connectivity measures. **Methods:** Resting-state EEG recordings from schizophrenia patients and healthy controls were collected. Based on these data, Minimum Spanning Tree (MST) graphs were computed two times using two different functional connectivity measures (phase lag index, PLI, and phase locking value, PLV). **Results:** Two series of between-group comparisons regarding MST parameters calculated on the basis of PLI or PLV gave contradictory results, in many cases the values of a given MST index based on PLI were higher in patients, and the results based on PLV were lower in patients than in the controls. Additionally, within the patients group, selected network measures were significantly different when calculated from PLI or PLV. **Conclusions:** The selection of FC measures significantly affects the parameters of MST-based neural networks and might be a source of disagreement between the results of network studies on schizophrenia.

Keywords: functional connectivity; schizophrenia; EEG; neuronal networks; PLI; PLV; MST

1. Introduction

Psychiatric diseases and milder forms of psychological health disturbances become one of the key social and health challenges around the world. According to a recent report on the prevalence of mental health problems in the US (2020), in 2019 about 51.5 million American adults (20.7% of the population) suffered from any kind of mental illness, and 13.1 million adults were diagnosed with serious mental illness (SMI), which mainly includes severe diseases that significantly hinder or prevent independent functioning, education and paid work. In this context, it is of vital importance, that in both of mentioned populations the young adults aged 18-25 represents the highest age-related prevalence of psychological health problems [1]. Schizophrenia is one of the most burdening mental illness with clinical onset occurring in adolescence or early adulthood. Disease development has a commonly slow and hidden course with a long-lasting increment of the so-called negative syndromes, i.e. affective blunting, social isolation, loss of interests and initiative. The usually second phase relates to exacerbation of more or less explicate psychotic symptoms such as auditory verbal hallucinations and delusions which might be described as incorrect judgments of reality and other people's behaviour,

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i.e. in a form of persecutory or grandeur beliefs [2,3]. This well-known psychiatric psychopathology is accompanied by the presence of cognitive dysfunctions affecting patients abilities to comprehend complex language communication, maintain focused attention, plan, solve everyday problems and generally regulate goal-directed behaviours [4,5].

Despite the existence of numerous theoretical approaches to understanding the aetiology of schizophrenia, one may say that currently, a substantial part of neuroscientists acknowledges schizophrenia as a brain connectivity disorder given the vast body of evidence documenting neuronal miswiring and disturbances in the organization of functional integration at the level of synapses, groups of neurons, hemispheres and the so-called large-scale neuronal networks [6,7]. The disconnection hypothesis suggests that individual symptoms might be explained with reference to abnormalities regarding mechanisms granting optimal coordination of a given group of neuronal structures, and on the other hand, it enables understanding the complexity of the psychosis' clinical picture as a result of multi-layered disruptions in the organization of the whole brain, and not only damage or hypofunction encompassing individual cortical areas or subcortical structures [8,9].

Undisputed progress in research on schizophrenia conducted with the application of functional connectivity (FC) measures and network theory solutions caused a noticeable advance in modern comprehension of the disease as a systemic pathology of the nervous system, but on the other hand, rapid introduction of many computational methods used in the reconstruction of the neural network gave rise to some methodological confusion related to the multiplicity of mathematical algorithms of a similar function used in parallel. This peculiar excess of algorithms and computational designs may be one of the sources of heterogeneity of the obtained results, especially since the very process of network reconstruction is multi-phase and involves the use of various signal processing techniques at different stages [10-12]. Finding the optimal computational tool to establish unique features of schizophrenia-related patterns of functional connectivity and large-scale network configuration seems to be a more and more important goal in clinical neuroscience, especially considering still unmet goal to elaborate differential diagnosis methods based on objective, biological markers [13].

Considering the above, we have performed analyses aimed at illustrating the extent to which the selection of just two different functional connectivity indicators could affect the obtained results regarding the comparison of the structure of the global neural network in the sample of patients diagnosed with schizophrenia compared to a demographically similar control group. Analyses of global neural network configuration carried out with the use of graph theory – here with application of the Minimum Spanning Tree [14], provide many characteristics describing the organization of brain activity, and above all, they inform whether a given network is dominated by mechanisms of integration or selection [15] and whether a network process the information according to the principle of reduced wiring costs and efficiency economy [16]. Network research in schizophrenia, including those using graph theory and its characteristics, such as path length, clustering coefficient, and small-worldness, is particularly important because it captures the activity of the whole brain as an organized, or disorganized system [17]. However, we postulate, the computational complexity of these analyses and potentially unhampered freedom of choice regarding applied functional connectivity measures may make them susceptible to volatility and eventually result in low outcomes reproducibility.

Therefore, we assume that our study can generate two main findings: establishing whether and to what extent the final graph theory network parameters show independence from the input data (FC measures), in terms of differentiating schizophrenia patients from healthy controls, and if it proves that these graph-theory indicators are independent, it will be possible to determine which FC parameter differentiates patients and controls to the most extent, which may guide future research. By the independence of network parameters from the input data, we mean that the direction of differentiation be-

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tween patients and controls will be the same and that these two groups will be differentiated by the same network parameters, no matter on what FC measure they were calculated. Also, the lack of significant intra-group differences in the MST parameters calculated on the basis of two different FC measures was considered an indicator of the non-susceptibility of these algorithms to input factors. However, if it turns out that the graph-theory parameters, calculated based on two different FC measures will give completely different results in terms of differences and similarities between the two groups, then it will indicate that they are more dependent on the specificity of included FC measures, and in consequence, that the selection of a specific FC algorithm actually creates the final results regarding the specificity of the whole-brain network architecture in patients with schizophrenia compared with healthy controls.

2. Materials and Methods

2.1. Participants

The study included a group of patients diagnosed with schizophrenia according to DSM-5 classification, aged 20-35, with at least 10 years of education. The following exclusion criteria were taken into account: prior diagnosis of intellectual disability, psychoactive substances addiction, structural abnormalities of the brain or other MRI-indicators of its acquired damage (e.g. post-traumatic or vascular changes), comorbidity of neurological diagnoses, taking benzodiazepines and antiepileptic drugs, more than three psychotic episodes requiring hospitalization, pronounced features of the metabolic syndrome. Patients included in the study group had to be treated only with atypical antipsychotics. The patients came from the 1st Psychiatry Department of the Medical University of Lublin. After completing the clinical group and determining its basic demographic characteristics, healthy individuals were selected to form a control group using the pairwise selection method. The exclusion criteria in the control group were: diagnosis of mental and neurological diseases and disorders, head injuries and concussions, taking medications that may affect the EEG recording (e.g. hypnotics, benzodiazepines). Subjects did not receive remuneration for participating in the study. All participants gave their written consent to the study, and the research project was positively assessed by the local ethics committee.

2.2. EEG recordings acquisition

First, for each participant, 15 minutes of resting-state EEG (eyes closed) data were recorded with 19-scalp position, electro-cap (Electro-Cap International Inc., Ohio, USA) and Ag/AgCl disk electrode. Electrodes were distributed according to the 10-20 International system (Fp1, Fp2, F3, F4, C3, C4, P3, P4, O1, O2, A1, A2, F7, F8, T3, T4, T5, T6, Fz, Pz and, Cz). Darning the acquisition, subjects sit in the well-lit and quiet room. The electrode impedances were kept below 5 k and the data was filtered from 0.5 Hz to 70 Hz (with active notch filter set at 50 Hz) when the sampling rate was 512 Hz. The data was exported into ASCII format after recording. Post-processing procedure were made in the EEGLAB [18] program, which is a Matlab toolbox. In the first step of post-processing procedure, signal was filtered with the band pass filter 0.5Hz – 45Hz (second-order Butterworth filter). Secondly, the reference was changed offline into the averaged. Thirdly, from the processed signal, 75 eight-second long epochs (4096 samples) without artifacts were extracted for each patient by a clinical neurophysiologist. Lastly, EEG signals were divided into six frequency bands using finite impulse response filters: the delta (0.5 - 4Hz), theta (4-8 Hz), low alpha (8-10 Hz), high alpha (10 - 12 Hz), beta (13 -30 Hz) and gamma (30 – 45 Hz).

2.3. Functional connectivity indicators: PLI and PLV

The first algorithm used to measure the functional connectivity strength was the phase lag index (PLI). The PLI is a phase synchronization metric based on the asymmetry

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of the distribution of phase differences between two signals, which may be calculated using the analytical signal based on the Hilbert transform. When compared to other synchronization detection approaches, such as synchronization likelihood or phase coherence, the PLI is less influenced by the influence of a shared source because zero-lag synchronization has been excluded from the analysis. According to Stam and coworkers "The phase lag index is based upon the idea that the existence of consistent, nonzero phase lag between two time series cannot be explained by volume conduction from a single strong source and therefore, renders true interactions between the underlying systems rather likely" [19, pp. 1179]. The PLI is obtained from the phase difference $\Delta\phi(t_k), k = 1...N$ of time series by means of:

$$PLI = \left|\left\langle sign[\Delta\phi(t_k)]\right\rangle\right|$$

Where the $\Delta\phi$ is the phase difference and $\langle ... \rangle$ denotes the average time t. The PLI quantifies the relative phase distribution's asymmetry which indicates to the likelihood that the phase difference $\Delta\phi$ will be in the interval $-\pi < \Delta\phi < 0$ is different from the likelihood that it will be in the interval $0 < \Delta\phi < \pi$. The range of PLI values is between 0 and 1, where zero value indicates no coupling or coupling with phase difference centered around 0 (mod π), and 1 values indicate perfect phase locking at a value of $\Delta\phi$ different from 0 (mod π). The stronger nonzero phase locking means that the PLI will be larger [19].

The second algorithm applied to evaluate functional connectivity was phase locking value (PLV) [20]. PLV quantifies similar synchronization tendencies in the EEG signals. The advantage of PLV is that it can measure the phase component separately from the amplitude component for a particular frequency range. PLV assess the latencies at which phase synchrony occurs or modest phase variation occurs across trials in the situation of recurrent stimuli. The PLV computing process incorporates the instantaneous phase difference between signals in the chosen frequency band in order to establish the phase synchronization of two EEG signals. The synchronization measure PLV, at time instant t is defined as:

$$PLV = 1N|\sum n=1N exp\{j(\Delta\phi(t,n))\}$$

Where N is the total number of trials and $\Delta\phi(t,n)=\phi1(t,n)-\phi2(t,n)$ is the instantaneous phase difference between the signals.

PLV is used in the majority of EEG research to assess the inter-trial variability of phase at time t. PLV is close to one if there is no substantial phase change between trials; otherwise, it is or zero. A PLV value of zero indicates that the phase difference between the two signals is not synced, while a PLV value of one shows that the signals are totally synchronized [21].

2.4. Global neuronal network reconstruction: application of the Minimum Spanning Tree

The functional connectivity matrixes created by the PLI and PLV algorithms for each frequency band were converted into graphs. Every graph consisted of nodes (ie, EEG electrodes) and edges (ie, functional connectivity values between each pair of electrodes gathered from PLI/PLV). By applying Kruskal's algorithm on each PLI/PLV adjacency matrixes, calculated for each frequency band epoch, acquired from each participant, the Minimum Spanning Tree (MST) graph has been constructed. The first step in this process is to sort the edge weights from least to greatest. Once all the nodes are separated, the algorithm begins reconnecting them starting with the node that has the greatest weight. The algorithm then continues to add the connection with the next greatest weight, and so on, until all of the nodes are linked. In contrast, if a new connection with the node during the adding method causes a cycle or loop, the connection will be refused, and the next edge will be rated by the weight value [15]. Various graph metrics can be established

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throughout the MST computation process. However, in order to keep the computational methodology in line with the original MST basis, we generated results describing 8 parameters presented in the official guide and MST computation instructions developed by Cornelius J. Stam's team (http://home.kpn.nl/stam7883/brainwave.html) [22]. In accordance with the work of van Dellen and co-workers [14], the most straightforward MST parameters are diameter and leaf number/leaf fraction. Furthermore, these variables enable to classify the networks as integration, or segregation-dominated. When the network topology express increased diameter and decreased leaf number, it's so called a line-like network topology and the second network type is the one with a low diameter and a high leaf number, then it is called star-like network in which integrative processes dominates [15]. Leaf fraction can be defined as the number of nodes on the tree with degree = 1 and can be calculated from: Lf = L/m, where L is the leaf number. The leaf number range starts from 2 (typical of a line-like topology) and its maximum value is equal to: m = N1 (a star-like topology). The leaf number is associated with the tree diameter (d) a parameter which can be defined as the largest distance between any two nodes. Additionally, apart from those mentioned global MST metrics, one can also describe the optimal tree topology with the function called tree hierarchy. Tree hierarchy pictures the transfer of information from one node to another in the shortest path, assuming that there is no overload in the central node of the tree. Additional MST metrics might be evaluated, and these are Kappa, R, Teff, ASP and Mean. Parameter R is a derivative of the Pearson correlation coefficient, indicating the assortativity level, i.e. the feature of the network consisting in the fact that high-degree nodes should be connected with nodes having the same magnitude. However, in more chaotically constructed networks, high-degree nodes can be directly connected with low-degree nodes. The assortativity ranges from -1 to +1, and negative values are typical more for networks where the magnitude of connected nodes is substantially different [23]. Kappa is also called the degree of divergence, measures the broadness of degree distribution. Decreased value of Kappa indicates a decreased number of highly connected nodes called "hubs" [14,15,23]. Teff is defined as: 1 - diameter/(N - leaf number + 1), this measure indicates how close the diameter, for a given N and leaf number, is to its lowest possible value. Teff ranges between 0 and 1. ASP is an abbreviation meaning the average shortest path computed for the whole MST. This is not normalized index. And, Mean of the MST is the value of mean weight of all edges constituting the MST graph.

2.5. Statistical analyses

Demographic variables were compared between groups with Student's *t*-test or, for nominative variables (e.g. sex), with χ^2 test. Due to the fact that the subjects from two groups were matched in pairs and the potential differences in variables such as age, gender and education were controlled, the comparison of MST parameters calculated on the basis of both PLI and PLV between the two groups was carried out using two-tailed *t*-test. Cohen's d has been applied as an effect size indicator. In the intra-group analysis of SCH patients, a within-subjects ANOVA was used, where the above parameters were summarized in two different versions, one based on PLI and the other based on PLV. The partial eta square (η_p^2) was used as an indicator of the effect size.

3. Results

3.1. Demographic and clinical characteristics of the studied groups

After applying inclusion and exclusion criteria, collected groups consisted 20 patients with first-episode schizophrenia, 10 women and 10 men, aged 20.20. Healthy controls group also consisted 10 men and 10 woman aged 20.10. As shown in Table 1, groups did not differed in terms of age, sex and education.

Table 1. Demographic and clinical characteristics of the studied groups.

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	HC n = 20 M (SD)	SCH n = 20 M (SD)	p
Age (years)	21.10 (1.80)	21.20 (1.96)	0.867[a]
Male / female	10 / 10	10 / 10	0.999[b]
Education (years)	13.10 (1.29)	12.90 (1.16)	0.610[a]
Duration of illness (months)		19.50 (4.96)	
Duration of untreated psychosis (months)		5.60 (3.10)	
Risperidone equivalents		4.82 (0.93)	
PANSS[c] Positive subscale		14.20 (3.52)	
PANSS Negative subscale		17.40 (4.89)	
PANSS General subscale		32.60 (10.93)	
PANSS total		64.20 (10.18)	

Note. [a] two-tailed *t* test, [b] χ^2 test (df = 1), [c] Positive and Negative Syndrome Scale [25].

All SCH patients were treated with atypical antipsychotics, the majority of them (60%) with Olanzapine, the average dose expressed as risperidone equivalent reached 4.82 ± 0.93 units. According to exclusion criteria, none of the patients was taking benzodiazepines nor anticonvulsants, 4 patients (20%) were additionally treated with SSRI antidepressants (sertraline), and 9 (45%) were taking chlorprothixene for sporadic insomnia at a single dose not exceeding 30 mg. The duration of illness in clinical group was about 11 months and the duration of untreated psychosis about 4 months.

3.2. Between-groups comparison of MST outcomes calculated on the basis of PLI and PLV

Since SCH patients and individuals from HC group did not differed in terms of demographic variables, comparisons of MST-related network measures based on PLI or PLV indicators of functional connectivity were computed directly with application of two-tailed Student's *t* test. Table 2 contains results of this comparisons with Cohen's d used as an effect size indicator.

Table 2. Comparisons of the studied groups regarding MST-related network measures based on PLI or PLV indexes of functional connectivity in all included frequencies.

Frequency	Network measure	PLI / PLV	HC		SCH		t	p	d
			M	SD	M	SD			
Delta	Kappa	PLI	2.819	0.394	2.902	0.382	-0.677	0.502	0.21
		PLV	2.294	0.134	2.216	0.076	2.244	**0.030**	0.71
	R	PLI	-0.321	0.225	-0.349	0.147	0.454	0.652	0.15
		PLV	-0.174	0.211	-0.072	0.146	-1.780	0.082	0.56
	Diameter	PLI	0.422	0.077	0.410	0.077	0.455	0.651	0.16
		PLV	0.563	0.085	0.575	0.060	-0.483	0.631	0.16
	Leaf	PLI	0.558	0.102	0.583	0.077	-0.867	0.391	0.28
		PLV	0.394	0.071	0.361	0.052	1.677	0.101	0.53
	Hierarchy	PLI	0.385	0.072	0.396	0.049	-0.546	0.587	0.18
		PLV	0.298	0.054	0.276	0.042	1.417	0.164	0.45
	Teff	PLI	0.229	0.095	0.219	0.104	0.297	0.767	0.10
		PLV	0.212	0.091	0.232	0.075	-0.740	0.463	0.24
	ASP	PLI	3.591	0.424	3.517	0.399	0.568	0.572	0.18
		PLV	4.412	0.383	4.469	0.237	-0.562	0.576	0.18
	Mean	PLI	0.272	0.045	0.253	0.059	1.157	0.254	0.36
		PLV	0.639	0.046	0.823	0.031	-14.650	**< 0.0001**	4.69

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Theta	Kappa	PLI	3.055	0.765	3.319	1.068	-0.897	0.375	0.28
		PLV	2.336	0.150	2.247	0.105	2.162	**0.036**	0.69
	R	PLI	-0.438	0.154	-0.459	0.164	0.414	0.681	0.13
		PLV	-0.273	0.191	-0.152	0.178	-2.081	**0.044**	0.66
	Diameter	PLI	0.424	0.117	0.405	0.098	0.566	0.574	0.18
		PLV	0.591	0.118	0.594	0.074	-0.091	0.927	0.03
	Leaf	PLI	0.586	0.119	0.616	0.118	-0.816	0.419	0.25
		PLV	0.405	0.076	0.374	0.059	1.409	0.166	0.46
	Hierarchy	PLI	0.398	0.067	0.404	0.065	-0.275	0.784	0.09
		PLV	0.309	0.050	0.297	0.047	0.769	0.446	0.25
	Teff	PLI	0.189	0.111	0.173	0.112	0.457	0.650	0.14
		PLV	0.165	0.109	0.193	0.065	-0.994	0.326	0.31
	ASP	PLI	3.602	0.640	3.467	0.617	0.679	0.500	0.21
		PLV	4.522	0.596	4.556	0.424	-0.211	0.833	0.07
	Mean	PLI	0.341	0.113	0.339	0.125	0.031	0.974	0.02
		PLV	0.721	0.093	0.824	0.037	-4.571	**< 0.0001**	1.46
low-Alpha	Kappa	PLI	2.886	0.479	3.066	0.820	-0.849	0.401	0.27
		PLV	2.333	0.115	2.222	0.082	3.503	**0.001**	1.11
	R	PLI	-0.342	0.187	-0.431	0.162	1.602	0.117	0.51
		PLV	-0.344	0.153	-0.143	0.242	-3.127	**0.003**	0.99
	Diameter	PLI	0.466	0.081	0.441	0.104	0.840	0.406	0.27
		PLV	0.580	0.107	0.600	0.077	-0.658	0.514	0.21
	Leaf	PLI	0.538	0.067	0.597	0.110	-2.009	0.051	0.65
		PLV	0.422	0.075	0.358	0.055	3.058	**0.004**	0.97
	Hierarchy	PLI	0.360	0.058	0.405	0.061	-2.381	**0.022**	0.76
		PLV	0.321	0.062	0.281	0.047	2.278	**0.028**	0.73
	Teff	PLI	0.184	0.104	0.136	0.115	1.380	0.175	0.44
		PLV	0.160	0.097	0.202	0.089	-1.392	0.171	0.45
	ASP	PLI	3.705	0.443	3.602	0.525	0.673	0.504	0.21
		PLV	4.443	0.466	4.638	0.360	-1.472	0.149	0.47
	Mean	PLI	0.308	0.033	0.326	0.077	-0.938	0.354	0.30
		PLV	0.623	0.079	0.801	0.041	-8.937	**< 0.0001**	2.83
high-Alpha	Kappa	PLI	2.741	0.518	2.949	0.638	-1.132	0.264	0.36
		PLV	2.341	0.144	2.241	0.111	2.450	**0.018**	0.78
	R	PLI	-0.341	0.154	-0.392	0.146	1.062	0.294	0.34
		PLV	-0.261	0.236	-0.231	0.135	-0.487	0.628	0.16
	Diameter	PLI	0.458	0.078	0.422	0.088	1.363	0.180	0.43
		PLV	0.561	0.082	0.616	0.101	-1.896	0.065	0.60
	Leaf	PLI	0.533	0.083	0.588	0.108	-1.808	0.078	0.57
		PLV	0.419	0.083	0.374	0.076	1.767	0.085	0.57
	Hierarchy	PLI	0.370	0.056	0.402	0.074	-1.545	0.130	0.49
		PLV	0.324	0.066	0.293	0.065	1.527	0.134	0.47
	Teff	PLI	0.205	0.079	0.187	0.095	0.627	0.534	0.21
		PLV	0.185	0.101	0.161	0.113	0.716	0.477	0.22
	ASP	PLI	3.800	0.426	3.556	0.478	1.695	0.098	0.54
		PLV	4.400	0.408	4.713	0.461	-2.276	**0.028**	0.72
	Mean	PLI	0.321	0.051	0.316	0.054	0.328	0.744	0.10
		PLV	0.607	0.074	0.788	0.055	-8.664	**< 0.0001**	2.78
Beta	Kappa	PLI	2.616	0.313	2.841	0.512	-1.674	0.102	0.53
		PLV	2.352	0.130	2.299	0.120	1.331	0.190	0.42

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	Overall in case of 23 variables	R	PLI	-0.308	0.167	-0.363	0.172	1.021	0.313	0.32
			PLV	-0.238	0.195	-0.191	0.210	-0.720	0.475	0.23
		Diameter	PLI	0.469	0.073	0.438	0.067	1.372	0.177	0.44
			PLV	0.536	0.104	0.550	0.053	-0.533	0.596	0.17
		Leaf	PLI	0.525	0.075	0.555	0.069	-1.327	0.192	0.42
			PLV	0.424	0.065	0.413	0.079	0.480	0.633	0.15
		Hierarchy	PLI	0.369	0.047	0.377	0.051	-0.531	0.598	0.16
			PLV	0.313	0.055	0.324	0.069	-0.540	0.591	0.18
		Teff	PLI	0.200	0.052	0.207	0.096	-0.271	0.787	0.09
			PLV	0.220	0.121	0.205	0.087	0.435	0.665	0.14
		ASP	PLI	3.857	0.441	3.624	0.393	1.765	0.085	0.56
			PLV	4.284	0.504	4.369	0.302	-0.645	0.522	0.20
		Mean	PLI	0.188	0.034	0.161	0.039	2.270	**0.028**	0.74
			PLV	0.542	0.081	0.781	0.061	-10.429	**< 0.0001**	3.33
Gamma		Kappa	PLI	2.686	0.402	3.755	1.652	-2.812	**0.007**	0.89
			PLV	2.366	0.137	2.261	0.136	2.435	**0.019**	0.77
		R	PLI	-0.320	0.226	-0.491	0.180	2.646	**0.011**	0.84
			PLV	-0.276	0.196	-0.222	0.155	-0.958	0.344	0.31
		Diameter	PLI	0.477	0.085	0.366	0.099	3.797	**0.001**	1.20
			PLV	0.527	0.089	0.619	0.127	-2.627	**0.012**	0.84
		Leaf	PLI	0.533	0.089	0.663	0.127	-3.743	**0.001**	1.19
			PLV	0.433	0.075	0.380	0.090	2.001	0.052	0.64
		Hierarchy	PLI	0.384	0.061	0.430	0.063	-2.287	**0.027**	0.74
			PLV	0.313	0.057	0.296	0.059	0.931	0.357	0.29
		Teff	PLI	0.171	0.096	0.167	0.104	0.127	0.899	0.04
			PLV	0.220	0.114	0.157	0.099	1.857	0.070	0.59
		ASP	PLI	3.864	0.441	3.199	0.591	4.030	**0.0001**	1.28
			PLV	4.260	0.441	4.663	0.657	-2.273	**0.028**	0.72
		Mean	PLI	0.221	0.037	0.144	0.028	7.321	**< 0.0001**	2.35
			PLV	0.553	0.081	0.842	0.057	-12.936	**< 0.0001**	4.13

. *Note.* Bold font indicates statistically significant between-group differences at the level of $p < 0.05$.

Overall, in case of 23 variables the between-group difference reached the threshold of $p < 0.05$. Among these variables 15 were based on PLV and 8 on PLI. Considering the effect sizes variable "Mean" differentiated the groups to the most extend. The largest intergroup differences concerned the following variables: PLV-based Mean MST in delta frequency (d = 4.69). PLV-based Mean in gamma frequency (d = 4.13). PLV-based Mean in beta frequency (d = 3.33). PLV-based Mean in low-alpha frequency (d = 2.83). PLV-based Mean in high-alpha frequency (d = 2.78) and PLI-based Mean in gamma frequency (d = 2.35). It is worth noticing. that in some pairs of MST results. values based on PLV were significantly higher in SCH than in controls. while the same MST variables based on PLI in the same frequency presented the opposite difference. i.e. values were lower in SCH patients than in controls. For example. in gamma band Mean MST based on PLI in groups (SCH *versus* HC) were: 0.144 *versus* 0.221. while for PLV-based measure: 0.842 *versus* 0.553. Besides MST Mean. the other network metrics which differentiated the groups to the most extend (with d > 1) were: PLI-based ASP (d = 1.28). PLI-based Diameter (d = 1.20). PLI-based Leaf (1.19). all in the gamma. and PLV-based Kappa in low-alpha band (d = 1.11). As in the case of MST Mean. also in some of these variables. there could be observed the opposite directions of difference in measures based on PLI and PLV indicators (e.g. ASP in gamma band).

3.3. Within-SCH group comparisons of PLI and PLV-based MST indicators

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Another step of analysis considered within-group evaluation regarding possible discrepancies between MST variables based on PLI or PLV indexes of connectivity strength in SCH patients. We performed within-subjects ANOVA (MST indicators as dependent variables and PLI *versus* PLV as an independent) with Bonferroni *post hoc* test's significance level corrected for multiple testing separately for each frequency. According to diagrams displayed in Figure 1 (a – f), in the majority of cases MST indicators such as Kappa. ASP and Mean were significantly different depending on whether they were calculated on the basis of PLI or PLV markers, although all come from the same SCH individuals. Mentioned differences reached the level of significance corrected for the number of multiple tests. Interaction effects (FC indicator x MST value) in all analyzed frequencies were significant ($p < 0.0001$, $\eta_p^2 > 0.4$), also after correction for multiple comparisons. In detail, there were significant within-group differences in all six analyzed frequencies regarding mentioned indicators. Additionally, in the delta band, the R also was significantly different for computations based on PLI or PLV (*post hoc* $p < 0.0001$). In all included frequencies values of Kappa were higher for PLI compared with PLV, while values of ASP and Mean were significantly higher for PLV compared with PLI. Except for the delta band, the values of R, Diameter, Leaf, Hierarchy and Teff in all other bands took an analogous range (all *post hoc* $p > 0.05$), whether they were computed with an application of PLI or PLV.

4. Discussion

The disconnection theory of schizophrenia assuming that the disease's etiopathogenesis is grounded on abnormal patterns of synchronizations between a given set of brain areas is one of the major research trends in neuroscientific studies on this illness [26]. On the other hand, this conceptual orientation is strongly associated with rapidly developing methodological and computational advancement of the functional connectivity (FC) and neuronal networks analysis research paradigms. Although the vast part of FC research uses fMRI and assess levels of BOLD co-activation between brain regions occurring in common time interval [27], a number of FC research using EEG and MEG is still growing [28]. EEG-related FC measures can be, with some simplification allowed, divided into those based on the amplitude of the signal and those based on the phase of oscillatory activity. In general, amplitudes correlations give FC arrangements similar to those obtained from the fMRI, however, these indexes suffer from the susceptibility to volume conduction disturbances. One way to circumvent this limitation was to design FC indicators relying on correlations between the voltage fluctuations ongoing with time delay [29]. Due to the problems related to the influence of volume conduction and signal noise on the FC parameters, more and more various computational methods were developed to estimate the strength of the relationship between distinct aspects of the EEG signals and the interactions between the reconstructed signal sources. For example Wang with co-workers [30] indicated that there is about 42 methods, while Bakhshayesh et al. [31] showed that 26 types of FC-algorithms might be applied to analyze synchronizations between non-stationary, non-linear signal, such as this coming from EEG recordings. Unfortunately, only recently have research and computational analyzes begun to evaluate which of these methods give rise to relatively repetitive, closely related to phenotypic characteristics connection patterns that show significant inter-individual and modest intra-individual variance [29,32,33].

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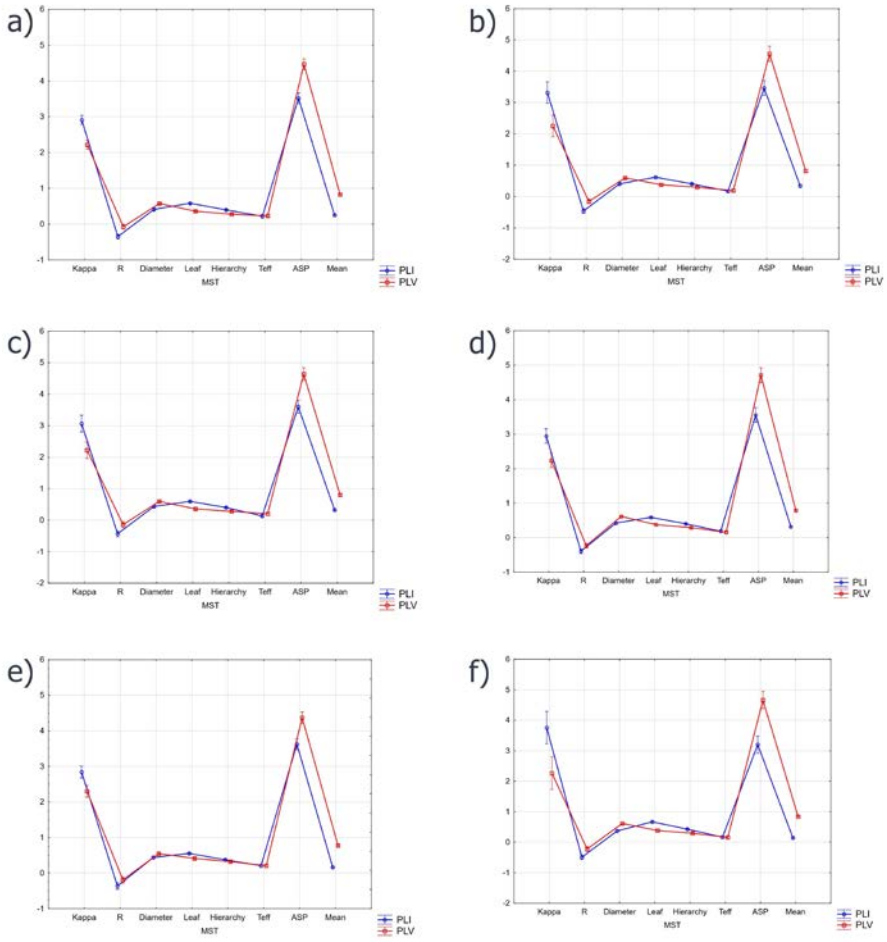

Figure 1. Within-subjects comparison of MST indicators (Kappa, R, Diameter, Leaf, Hierarchy, Teff, ASP, Mean) based on PLI (blue curve) and PLV (red curve) FC-strength indicators in the SCH group. The vertical bars represent 0.95 Confidence Intervals. Comparisons were computed selectively for individual frequencies: a) delta, b) theta, c) low-alpha, d) high-alpha, e) beta, f) gamma.

Despite some advances in assessing FC measures in terms of their validity and reproducibility, according to our knowledge, the recognition of how different FC-indexes may affect the scope and direction of differences between schizophrenia patients and healthy controls regarding the parameters of the global neural networks configuration described in the language of the graph theory has not yet been carried out. The answer to this question seems important taking into account the significant range of studies outcomes regarding the specificity of the organization of neural networks in schizophrenia and the observed heterogeneity of results in this area [7]. This heterogeneity is observed even within a set of studies using the same computational solutions to obtained global neural network arrangement typology, such as a Minimum Spanning Tree [15]. In a current extensive review aimed to find the repeatable constellation of graph-theory networks reconstructions based on MST authors indicated, that regarding the adult psychiatric population, especially schizophrenia, the results are indisputably conflicting. However, the problem of using FC input gained from different connectivity computations was not considered as a possibly important origin of inconsistency [34]. To verify if two different types of FC indicators might generate different or even contradictory results on the extent to which the global neural network of schizophrenia patients differs from the healthy controls, we conducted two identical computational analyses, one with PLI as a synchronization measure, and second with PLV. These two algorithms belong to the same group of methods assessing the FC strength, i.e. methods based on phase lag.

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According to Li et al. [35], PLV is not fully resistant to the volume conduction problem leading to increased amount of spurious synchronizations coded as genuine connections, while PLI, although alleviates this limitation by excluding zero phase diversity, is less effective in managing with resistance against noise. On the other hand, Rizkallah with co-workers [36] documented, that the network created on the basis of PLV used as an FC measure applied to EEG recordings was more similar to the fMRI network arrangement, compared with a PLI-based network. Our results indicated that MST-measures based on PLV indexes of synchronization differentiated schizophrenia patients from controls almost twice as often as in case of PLI. The power of groups differentiation applies primarily to the index 'Mean' based on PLV. Consequently, in all frequencies, this value was higher in patients, what suggests that their network is built with edges of higher overall weight, sometime interpreted as the cost (e.g. the 'energy') needed to transfer the information between nodes or the length of the average edge [22].

In our opinion, the most important result of the current study is that if the groups were significantly differentiated by the same MST parameter calculated based on PLI and PLV, then the result of this comparison was the opposite. i.e. the given MST parameter was once higher in the clinical group compared to the healthy controls and in the case of calculating the network marker with another FC index, lower in the clinical group. Such results apply to global network parameters in the gamma band, especially with regard to Kappa, Diameter, ASP and Mean. This seems to suggest that the outcomes of whole-brain network analyzes in schizophrenia are susceptible to applied FC-indicators and depending on the applied synchronization measures, completely different values can be obtained, informing about the extent to which the organization of the neural network of schizophrenia patients differs from that of controls. None of the MST indicators showed a consistently similar difference between patients and controls, whether it was calculated using connectivity strength values computed using PLI or PLV. The inconsistency of some MST indicators, here understood as their dependence on the included FC-measures, is also seen regarding intra-subject comparisons. In detail, it appeared that indexes such as Kappa. ASP and Mean were significantly different within the patients group depending whether they were computed using PLI or PLV indexes of functional connectivity. It is worth noting, that Mean and Kappa were also inconsistent regarding inter-groups comparisons.

Summing up, our results indicate that MST-based measures of whole-brain network arrangement encapsulated in the language of graph-theory are susceptible to entered indexes of functional connectivity, and at least in the case of comparing patients and controls, contradictory MST values might be obtained depending on what exact FC algorithm was used. The above finding seems to be particularly important for schizophrenia research, on the one hand, because the network and connectivity analyzes fit into the current understanding of the disease as a consequence of neuronal disconnection, on the other hand, the majority of already classic, yet still valid conceptual propositions suggested that schizophrenia is a disorder resulting not from pathology limited to narrowed, strictly localized cerebral dysfunction, but from a general, systemic brain disorder that to a varying degree affects various areas and mechanisms granting their mutual modulations [37,38].

Despite these important settlements, some limitations of our study and proposal for further investigations should be addressed. First of all, it might be not undoubtedly stated, that presented outcomes are specific to schizophrenia. It is possible, that the majority of detailed results are just secondary to applied computational solutions and analyzed discrepancies regarding MST results dependent on the included FC indexes would appear also when another clinical group would be compared with healthy controls. Secondly, our study does not enable to recognize whether MST graph-theory network characteristics are more or less susceptible to various FC measures comparing with more conventional approaches analyzing such network features like small-worldness and clustering coefficient [15,24]. Therefore, our analysis should not be considered as a clear indication of what type of network organization measures to choose in future research,

rather as a study showing that the results of graph-theory network analyzes depend on the built-in FC parameters. Nevertheless, some clear conclusions seems to emerge. We postulate that it is necessary to critically evaluate these network arrangement computational indicators which show marked intra-subject variability or are prone to exhibit such variability when even modest input design conditions are entered. Mathematically correct development of a given algorithm does not necessarily mean that it accurately depicts some aspect of the living brain functioning. Additionally, it seems indispensable to conduct a large-scale analysis of many FC and neuronal network indicators, having various mathematical and theoretical background, and verify which of them bring similar results e.g. in a form or between-group differences and which simultaneously have minimized intra-subject variance. It may also turn out that future research on disruptions in the whole-brain organization in diseases such as schizophrenia will gain a greater level of results reproducibility and consistency, when more effort will be invested in critical analysis and selection of the already available computational possibilities in terms of their accuracy and reliability, than on the further multiplication of mathematical solutions without verifying their compliance with the specificity of the activity of the biological evolution effect, which is the brain.

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